Posterior amygdala regulates sexual and aggressive behaviors in male mice

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Sexual and aggressive behaviors are fundamental to animal survival and reproduction. The medial preoptic nucleus (MPN) and ventrolateral part of the ventromedial hypothalamus (VMHvl) are essential regions for male sexual and aggressive behaviors, respectively. While key inhibitory inputs to the VMHvl and MPN have been identified, the extrahypothalamic excitatory inputs essential for social behaviors remain elusive. Here we identify estrogen receptor alpha (Esr1)-expressing cells in the posterior amygdala (PA) as a main source of excitatory inputs to the hypothalamus and key mediators for mating and fighting in male mice. We find two largely distinct PA subpopulations that differ in connectivity, gene expression, in vivo responses and social behavior relevance. MPN-projecting PA

Esr1+ cells are activated during mating and are necessary and sufficient for male sexual behaviors, while VMHvl-projecting PA

Esr1+ cells are excited during intermale aggression and promote attacks. These findings place the PA as a key node in both male aggression and reproduction circuits.

Are the excitatory inputs to the MPN and VMHvl important for social behaviors? An early study demonstrated that 1-glutamate infusion into the hypothalamus can elicit attack in cats[1]. Microdialysis in the MPN revealed a gradual increase of glutamate over the course of male sexual behaviors[2]. The infusion of glutamate uptake inhibitors into the MPN facilitated copulations[3]. These early findings suggest that excitatory glutamatergic inputs to the medial hypothalamus are essential for both sexual and aggressive behaviors, but what are the sources of these excitatory inputs? Anatomical tracing revealed the PA, also known as the amygdala-lol hippocampal area[4], as one candidate source. The PA is considered to be a part of the cortical-like amygdalar complex[5] and is largely glutamatergic (http://mouse.brain-map.org/experiment/show/70436317/). Moreover, similarly to the MPN and VMHvl7,29,30, the PA is enriched for Esr1 expression (http://mouse.brain-map.org/experiment/show/79591677/; ref. 31). Estrogen has a profound impact on social behaviors7; thus, regions with abundant estrogen receptors are likely to be relevant for social behaviors. Indeed, expression of c-Fos, a surrogate of neural activity, increased in the PA after male aggression and sexual behaviors8,13, although the function of the PA and in vivo responses remain largely unknown. Here, we use the MPN and VMHvl as our entry points to examine the functions of Esr1+ cells in the PA (PA

Esr1+) in controlling sexual and aggressive behaviors in male mice.

Results

Male PA cells that project to MPN and VMHvl are largely distinct. Because the MPN and VMHvl contribute to different social behaviors, we hypothesized that PA neurons that project to the MPN and VMHvl are likely non-identical cells and play differential roles in male sexual and aggressive behaviors. To test this, we first injected a retrograde tracer into the MPN or VMHvl of male mice and found that labeled cells distributed differently within the PA. PA cells that projected to the MPN (PA

M) were abundant in the anterior dorsomedial part of the PA (Fig. 1a,b,c), whereas VMHvl


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the anteroposterior axis of the PA after CtB injections into the MPN (red line) and VMHvl (blue line). and the percentage of esr1
the total PA population (k 3 animals). Viral strategy for dual-retrograde labeling of PAesr1
both PAMPN and PAVMHvl cells preferentially overlapped with Esr1
throughout the anteroposterior axis (Fig. 1c–e). Additionally, the numbers of PAesr1
were modified from previous work50 (Extended Data Figs. 1 and 10; see Supplementary table 1 for detailed statistics).

Figs. 1 | Largely distinct subpopulations of PAesr1+ neurons project to the MPN and VMHvl. a, c. Examples showing CtB injection sites (green) in the MPN and VMHvl; scale bars, 200 µm. LV, lateral ventricles; ac, anterior commissure; 3V, third ventricle. b, d, Images are representative of Esr1 expression (red) and CtB labeling (green) in the anterior (top row; bregma –2.30 mm) and posterior (bottom row; bregma –2.75 mm) PA after CtB injections into the MPN (a) and VMHvl (b). Right, magnified views of the white boxed areas; scale bars, 200 µm (left) and 20 µm (right). e. The numbers of CtB+ cells along the anteroposterior axis of the PA after CtB injections into the MPN (red line) and VMHvl (blue line). f. The percentage of CtB+ cells expressing Esr1 and the percentage of Esr1+ cells in the total PA population. In e and f, cells from VMHvl-injected mice (n = 3) and MPN-injected mice (n = 3) were used. Two-tailed unpaired t-test. *P < 0.05 and ****P < 0.0001. g, Experimental schematics for dual labeling of PAMMPN and PAVMHvl cells with Alexa Fluor 555–CtB and Alexa Fluor 647–CtB. h, i, Images are representative of MPN- (red) and VMHvl- (green) projecting cells in the anterior (h) and posterior (i) parts of the PA; right, magnified views of the white boxed areas; scale bars, 200 µm (left) and 20 µm (right). j. The percentage of CtB+ and overlapping cells in the total PA population (n = 3 animals). k, Viral strategy for dual-retrograde labeling of PAesr1+MPN and PAesr1+VMHvl cells. l, m, Images are representative of EYFP+ (green; from VMHvl) and mCherry+ (red; from MPN) labeled cells in the anterior (l) and posterior (m) PA. Bottom: magnified views of the white boxed areas; scale bars, 200 µm (top) and 20 µm (bottom). n, Schematics showing the four subregions (DL, VL, DM and VM) in the anterior (left) and posterior (right) PA where the cell counts were obtained. o. The numbers of PAMMPN and PAVMHvl cells labeled with retrograde tracer in each of the four subregions across the anterior and posterior PA (n = 3 animals). Images in a–d, h, i, l and m are representative of n = 3 animals. Statistical significance was determined using two-way ANOVA with Bonferroni’s multiple-comparisons test; ****P < 0.0001. All data in e, f, j and o are expressed as the mean ± s.e.m. Brain atlas images in a–c and g were modified from previous work35 (Extended Data Figs. 1 and 10; see Supplementary Table 1 for detailed statistics).

projections (PAMMPN) were concentrated in the ventrolateral PA throughout the anteroposterior axis (Fig. 1c–e). Additionally, both PAMMPN and PAVMHvl cells preferentially overlapped with Esr1+ cells: nearly all PAMMPN cells expressed Esr1 (95.5% ± 1.1%, n = 3 animals), while approximately half of PAVMHvl cells contained Esr1 (55.6% ± 2.5%, n = 3 animals; Fig. 1f).
Fig. 2 | PA<sup>Esr1</sup> cells provide monosynaptic excitatory inputs to MPN<sup>Esr1</sup> and VMHvl<sup>Esr1</sup> cells. a. Schematics for the channelrhodopsin (ChR2)-assisted circuit mapping and example images showing ChR2-EYFP expression in PA<sup>Esr1</sup> cells (top) and ChR2-EYFP fibers from PA and mCherry<sup>+</sup> cells in the MPN and VMHvl (bottom). Insets: magnified views of the boxes that contain mCherry-expressing cells (red) and biocytin-filled recorded cells (white). Scale bars, 200 µm (top) and 20 µm (insets). Brain atlas images are modified from Paxinos and Franklin<sup>50</sup>. Histological images are representative of n = 10 animals. b, i. Pie charts showing light-evoked synaptic responses in MPN<sup>Esr1</sup> (b) and VMHvl<sup>Esr1</sup> (i) cells. Bottom: example oEPSC and oIPSC traces evoked by 0.5-ms blue-light pulses (blue vertical lines) and expanded views of the boxed areas. c–e. The amplitude (c), decay time (d) and latency (e) of oEPSCs (black) and oIPSCs (red) of MPN<sup>Esr1</sup> cells; n = 25 and n = 19 cells, respectively, from ten animals. f. The example oEPSC and oIPSC traces before and after CNQX bath application. g, h. The oEPSCs (g) and oIPSCs (h) were abolished after bath application of CNQX; n = 6 cells from six animals. j–o. Characterization of light-evoked oEPSCs and oIPSCs in VMHvl<sup>Esr1</sup> cells; n = 23 and n = 20 cells from ten animals in j–l and n = 6 cells from six animals in n and o. All data in c–e and j–l are presented as the mean ± s.e.m. Statistical significance was determined using a two-tailed unpaired t-test (e and k). Mann–Whitney test (c, d, j and l) and Wilcoxon matched-pairs signed-rank test (g, h, n and o); *P < 0.05 and ****P < 0.0001 (Extended Data Fig. 2; see Supplementary Table 1 for detailed statistics).

To compare PA<sup>MPN</sup> and PA<sup>VMHvl</sup> cells in the same animals, we injected Alexa Fluor 647-conjugated cholera toxin subunit B (CTB) into the VMHvl and Alexa Fluor 555–CTB into the MPN and found that PA<sup>VMHvl</sup> and PA<sup>MPN</sup> cells were largely distinct at the single-cell level. Among all PA cells, 16.7% ± 1.5% of cells were labeled in retrograde from the MPN, 22.2% ± 3.0% of cells were from the VMHvl and only 3.3% ± 0.7% of cells were labeled from both regions (n = 3 animals). The dual-labeled cells were largely located in the middle zone where PA<sup>MPN</sup> and PA<sup>VMHvl</sup> cells converge (Fig. 1g–j). To further visualize MPN-projecting PA<sup>Esr1+</sup> (PA<sup>Esr1+MPN</sup>) cells and VMHvl-projecting PA<sup>Esr1+</sup> (PA<sup>Esr1+VMHvl</sup>) cells, we injected a retrograde herpes simplex virus (HSV) encoding a red fluorescent protein with Cre-dependent expression (HSV-heF1α-LSIL-mCherry) into the MPN and HSV-heF1α-LSIL-EYFP encoding a yellow fluorescent protein with Cre-dependent expression into the VMHvl of Esr1-2A-cre male mice (Fig. 1k). Consistent with CTB labeling, PA<sup>Esr1+MPN</sup> and PA<sup>Esr1+VMHvl</sup> cells showed a largely distinct spatial distribution (Fig. 1l–o) and overlapped minimally at the single-cell level (PA<sup>Esr1+MPN</sup>/total labeled cells, 54.3% ± 0.2%; PA<sup>Esr1+VMHvl</sup>/total cells, 39.6% ± 0.8%; overlapping/total cells, 5.1% ± 1.8%)
Male PAEs1 cells provide monosynaptic excitatory inputs to MPNEs1 and VMHvlEs1 cells. Consistent with previous reports, we found that the PA consists of mainly excitatory neurons (92.3%, n = 2 mice) using male Vglut1 (Slc17a7)-ires-cre × Ai6 (Zs Green reporter mouse) and Vgat (Slc32a1)-ires-cre × Ai6 mice (Extended Data Fig. 2a–c). Histological analysis revealed that approximately 50% of PA cells expressed Esr1 and over 95% of PAEs1 cells were glutamatergic (Extended Data Fig. 2d).

We next examined whether PAEs1 cells provided direct synaptic inputs to MNPs and VMHvl cells, especially the Esr1 cells relating to social behavior. We injected AAV-Ef1α-DIO-ChR2-EYFP into the PA and AAV-hSyn-DIO-mCherry into the MPN or VMHvl of male Esr1-2A-cre mice (Fig. 2a). Three weeks later, we performed whole-cell voltage-clamp recordings from mCherry+ cells in the MPN or VMHvl while stimulating PA terminals using 0.5 ms blue-light pulses (Fig. 2b). We observed optogenetically evoked excitatory postsynaptic currents (oEPSCs) and inhibitory postsynaptic currents (oIPSCs) in the majority of recorded cells in the MPN (53.1%; Fig. 2b–e). oEPSCs featured a short latency (2.24 ± 0.18 ms; n = 25 cells), whereas oIPSCs displayed significantly longer latencies (8.58 ± 1.21 ms; n = 19 cells), suggesting that the oEPSCs were likely monosynaptic, while oIPSCs were polysynaptic (Fig. 2e). Consistent with this hypothesis, bath application of 6-cyano-7-nitroquinolinine-2,3-dione (CNQX), an AMPA-type glutamate receptor blocker, abolished both oEPSCs and oIPSCs (Fig. 2f–h).

Similarly to Esr1+ cells in the MPN, two-thirds of VMHvlEs1+ cells showed short-latency oEPSCs (2.06 ± 0.20 ms; n = 23 cells) followed by long-latency oIPSCs (10.43 ± 1.40 ms; n = 20 cells) upon 0.5 ms of light stimulation (Fig. 2i–j). CNQX application abolished both oEPSCs and oIPSCs (Fig. 2m–o). Altogether, these results suggest that PAEs1+ cells provide direct excitatory and indirect inhibitory inputs to both MPN and VMHvlEs1+ cells.

Male PA subregions that project to the MPN and VMHvl display differential molecular phenotypes. Given the spatial segregation between MPNPs and VMHvlEs1 cells, we wondered whether these two populations also differ molecularly. As a first clue, a higher percentage of MPNPs cells overlapped with Esr1 expression than was seen with PAVMHvl+ cells (Fig. 1f). To address this question more systematically, we injected red and green retrobeads into the MPN and VMHvl of male C57BL/6J mice, respectively, used laser capture microdissection (LCM) to collect fluorescence-intense PA subregions, as well as the adjacent posterior part of the basomedial amygdala (BMAp), and then performed RNA sequencing (RNA-seq; Fig. 3a). Principal-component analysis of RNA-seq results revealed that samples from the same PA subregion clustered together and apart from other regions (Fig. 3b). We found 694 genes, including Esr1, with enriched expression in the PA compared to the BMAp (Fig. 3c and Supplementary Table 2). Additionally, 172 genes were found to have a higher level of expression (fold change > 1.5) in the MPN-projecting subregion of the PA than in the VMHvl-projecting subregion, and 215 genes showed the opposite pattern (Fig. 3e and Supplementary Table 2). Then, we performed in situ hybridization to visualize mRNA expression of several candidate genes enriched in the PA. Consistent with the RNA-seq data, Pde11a, Prokr2 and Zic2 were expressed at high levels in the entire PA but not in the neighboring BMAp (Fig. 3d). Chrna7, Npy2r and Nnat transcripts were predominantly localized to the MPN-projecting dorosomedial part of the PA, whereas Etv1, Calb2 and Dlk1 transcripts were largely confined to the VMHvl-projecting ventrolateral part of the PA (Fig. 3f).

Double in situ hybridization for Chrna7 and Dlk1 revealed their largely non-overlapping expression in PA (Dlk1 positive, 27.9 ± 2.5 cells per section; Chrna7 positive, 71.8 ± 2.8 cells per section; double positive, 2.0 ± 0.3 cells per section; n = 4 sections from two animals). Lastly, we combined retrograde tracing and in situ hybridization and confirmed that over 90% of PAVMHvl+ cells expressed Chrna7, whereas over 80% of PAVMHvl+ cells expressed Dlk1 (Fig. 3g–i). Altogether, these data suggest that two largely distinct PA subpopulations with different gene expression and hypothalamic projection patterns exist in male mice.

PAEs1-MPN and PAEs1-VMHvl cells respond differentially during male sexual behaviors and aggression. To understand whether Esr1 marks the social behavior-specific population in the PA, we performed double staining of c-Fos and Esr1 in animals that experienced mating and fighting. Approximately 74% and 67% of c-Fos+ cells induced by mating and fighting contained Esr1, respectively, significantly higher than the outcome expected by chance (~50%; Extended Data Fig. 3a–c). Additionally, c-Fos+ cells induced by mating and fighting distributed to different subregions of the PA; while mating-induced c-Fos+ cells were concentrated in the anterior half of the PA, fighting-induced c-Fos+ cells were distributed evenly along the anteroposterior axis of the PA (Extended Data Fig. 3f). As mating- and fighting-induced c-Fos expression patterns aligned with MPNPs and VMHvl retrograde labeling patterns in the PA, respectively, we next investigated the potential differential responses of PAEs1-MPN and PAEs1-VMHvl cells in male sexual and aggressive behaviors.

We performed fiber photometric recording in male Esr1-2A-cre mice by injecting HSV-hEf1α-LSL-GaMP6f into the MPN or VMHvl and implanted an optic fiber above the PA (Fig. 4a,b and Extended Data Fig. 4; refs. 7,18). Histological analysis revealed that...
the majority of GCaMP6f cells overlapped with Esr1 expression (93.5% ± 1.0% cells, n = 3 animals; Fig. 4c). Consistent with the fact that a higher percentage of PAoMPN cells expressed Esr1, we observed a higher number of GCaMP6f cells in the PA in animals with virus injected into the MPN than in the VMHvl (PAoMPN: 310 ± 31 cells per animal; PAoMPN+VMHvl: 171 ± 13 cells per animal; n = 5 animals each).

We found that PAoMPN and PAoVMHvl cells showed complementary response patterns during male–male and male–female encounters (Fig. 4d–z). PAoMPN and PASVMHvl cells showed responses to male than females, during both entrance and investigation (Fig. 4q–l). Complementary responses in PAoMPN and PASVMHvl cells persisted throughout the consummatory phase of social behaviors: PAoMPN cells showed stronger signals during male mating than attacking, while PAoVMHvl cells showed significantly higher responses during attacking than mating (Fig. 4j–n, u–z). We noticed that increased Ca2+ upon female introduction than male introduction, as well as higher responses during female investigation than male investigation (Fig. 4f–i, z). By contrast, PAoMPN and PASVMHvl cells showed higher responses to males than females, during both entrance and investigation (Fig. 4q–l). Complementary response patterns in PAoMPN and PASVMHvl cells persisted throughout the consummatory phase of social behaviors: PAoMPN cells showed stronger signals during male mating than attacking, while PAoVMHvl cells showed significantly higher responses during attacking than mating (Fig. 4j–n, u–z). We noticed that increased Ca2+ in PAoMPN cells do not simply reflect movement initiation as no increases were observed when recorded males initiated movements without subsequent mounting attempts (Extended Data Fig. 5a–e). Importantly, increases in PAoMPN Ca2+ do not simply reflect movement initiation, as no increases were observed when recorded males initiated movements without subsequent mounting attempts (Extended Data Fig. 5a–e). PAoMPN cells continued to increase activity as sexual behaviors advanced (Fig. 4j–n, u–z). In contrast, PAoVMHvl cells showed the highest response during fighting against male intruders (0.18 ± 0.03, n = 5 animals) and only showed moderately increased activity during the advanced stages of sexual behaviors (Fig. 4u–z). In contrast to PAoMPN cells, PAoVMHvl cells showed no consistent increase in activity when the male initiated mounting (Extended Data Fig. 5f–j). The investigation of a plastic object elicited no detectable change in the activity of either PAoMPN or PAoVMHvl cells (Fig. 4z and Extended Data Fig. 6a–d). In three control animals that expressed GFP in PAoVMHvl cells, we observed little change in fluorescence during any social behaviors (Extended Data Fig. 6e–q). Altogether, these data show that the male PAoVMHvl preferentially sends matting-related signals to the MPN and aggression-related signals to the VMHvl.

Inhibition of male PAoMPN and PAoVMHvl cells causes largely distinct deficits in social behaviors. Next, we investigated whether PAoMPN and PAoVMHvl cells were necessary for male sexual and aggressive behaviors. We evaluated the effect of bilateral injection of a retrograde HSV expressing Flippase (Flp) in a Cre-dependent manner into either the MPN or VMHvl and AAV-Elf1α-DIO-hM4Di-mCherry into the PA of male Esr1-2A-cre mice (Fig. 5a,b).

As expected, the vast majority of hM4DI-mCherry-expressing cells were positive for Esr1 expression (86.9% ± 0.6%, n = 3 animals; Fig. 5c) and were located in the PA (88.0% ± 2.4% in PAoMPN+VMHvl cells and 91.3% ± 3.8% in PAoMPN+VMHvl cells; n = 8 animals each; Extended Data Fig. 4b).

Three weeks after virus injection, we screened for animals that showed consistent sexual and aggressive behaviors before injecting clozapine-N-oxide (CNO) and saline on separate days. In males expressing hM4DI in PAoMPN cells, CNO nearly abolished sexual behaviors but did not affect aggressive behaviors (Fig. 5d–m). On the day of saline injection, all tested males showed sexual behaviors toward a receptive female and achieved deep thrust within the 10-min test period. Strikingly, on days of CNO injection, five of eight males did not attempt to mount, and none achieved deep thrust (Fig. 5e–j). In contrast, aggressive behaviors, including attack duration and latency to attack, were not affected (Fig. 5l,m). Investigatory behaviors toward the female increased, possibly as a result of the decrease in sexual behaviors, while the duration of male investigation remained unchanged (Fig. 5d,k). In a separate experiment, we examined changes in male sexual behaviors after hM4DI-mediated inactivation of an unselected population of PAoVMHvl cells and observed similarly striking impairments in sexual behaviors (Extended Data Fig. 7).

Inactivating male PAoVMHvl cells caused different behavioral deficits. While CNO injection did not reduce mounting attempts or shallow thrust, it decreased the duration of deep thrust and increased its latency (Fig. 5o–q), consistent with the reported role of the VMHvl in intromission1. Furthermore, PAoVMHvl cell inactivation decreased aggression, as indicated by a significant reduction in attack duration (Fig. 5v,w). Investigations toward males and females were unchanged after PAoVMHvl cell inactivation (Fig. 5n,a). Altogether, PAoVMHvl cells are indispensable for all aspects of male sexual behaviors, while PAoMPN cells mainly mediate male aggression but also play a minor role in advanced stages of sexual behaviors.

Pharmacogenetic activation of male PAoMPN and PAoVMHvl neurons promotes sexual and aggressive behaviors. To test whether PAoMPN and PAoVMHvl cells were sufficient to drive male sexual and aggressive behaviors, respectively, we bilaterally injected HSV-hElf1α-LS1L-Flp into either the MPN or VMHvl and AAV-Elf1α-DIO-hM3Dq-mCherry into the PA in male Esr1-2A-cre mice (Fig. 6a,b). Histological analysis revealed that the vast majority of hM3Dq-mCherry-expressing cells were positive for Esr1 (89.1% ± 7.0%, n = 3 animals; Fig. 6c) and confined in the PA (88.2% ± 2.8% in PAoMPN+VMHvl cells, n = 8 animals; 94.0% ± 2.0% in PAoMPN+VMHvl cells, n = 6 animals; Extended Data Fig. 4c). All tested males were naive, to minimize spontaneous aggression and sexual behaviors.

Fig. 4 | Different response patterns of PAoMPN and PAoVMHvl cells during social behaviors. a, Experimental schematics. b, Images are representative of fiber tracks (yellow lines) and GCaMP6f expression (green) in PAoMPN and PAoVMHvl cells; blue, DAPI; scale bar, 200 μm. c, Images are representative of the overlap of expression of GCaMP6f (green) and Esr1 (red) in the PA; scale bar, 20 μm. The percentage of GCaMP6f neurons expressing Esr1 is shown on the chart. Data are presented as the mean ± s.e.m. from n = 3 animals. d, Representative Ca2+ traces of PAoMPN cells during interaction with a female (d) and male (e) intruder. The colored shading marks behavioral episodic. e, n, Peri-event histograms (PETHs) of the Ca2+ signal of PAoMPN cells aligned to the introduction of the intruder and onset of various social behaviors. The lines represent data from individual animals (gray) and the population average (colored); n = 7 animals, 5 of which showed attack. o, p, Representative Ca2+ traces of PAoVMHvl+VMHvl neurons during interaction with a female (o) and male (p) intruder. q, y, PETHs of the Ca2+ signal of PAoVMHvl+VMHvl cells aligned to intruder introduction and onset of various social behaviors. z, Peak Ca2+ signal of PAoMPN+VMHvl and PAoVMHvl+VMHvl cells during the introduction and investigation of social and non-social stimuli and during various stages of sexual behaviors and attack; n = 7 animals for PAoMPN group, 5 of which showed attack; 5 animals for PAoVMHvl+VMHvl group. All data in z are presented as the mean ± s.e.m. Statistical significance was determined using one-way ANOVA with Tukey’s multiple-comparison test; *P < 0.05, **P < 0.01 and ***P < 0.001 (Extended Data Figs. 3–6; see Supplementary Table 1 for detailed statistics).
**Figure 1:** Responses of PAESr1+MPN and PAESr1+VMHvl neurons to female intruder introduction. (a) Schematic of an EoP cell with a representation of the rewards channel in the anterior PAG (PA) and the social reward circuit.

(b) Representative GCaMP6f images of PAESr1+MPN and PAESr1+VMHvl cells. Scale bar, 20 μm.

(c) Bar graph showing the percentage of PAESr1+MPN and PAESr1+VMHvl cells expressing GCaMP6f in response to sniffing of a female intruder.

**Figure 2:** Time course of neuronal activity in PAESr1+MPN and PAESr1+VMHvl cells following female and male intruder introduction. (d) Female intruder introduction.

(e) Male intruder introduction.

(f) Introduction of female intruder (n = 7).

(g) Introduction of male intruder (n = 7).

(h) Sniff female (n = 7).

(i) Sniff male (n = 7).

(j) Attempt mount (n = 7).

(k) Shallowly thrust (n = 7).

(l) Deeply thrust (n = 7).

(m) Ejaculate (n = 7).

(n) Attack (n = 5).

**Figure 3:** Time course of neuronal activity in PAESr1+MPN and PAESr1+VMHvl cells following female and male intruder introduction. (o) Female intruder introduction.

(p) Male intruder introduction.

(q) Introduction of female intruder (n = 5).

(r) Introduction of male intruder (n = 5).

(s) Sniff female (n = 5).

(t) Sniff male (n = 5).

(u) Attempt mount (n = 5).

(v) Shallowly thrust (n = 5).

(w) Deeply thrust (n = 5).

(x) Ejaculate (n = 5).

(y) Attack (n = 5).

**Figure 4:** Summary of behavioral responses. (z) Bar graph showing the mean ± SEM of the behavioral response of PAESr1+MPN and PAESr1+VMHvl cells to intruder introduction, investigation, and action.

**Note:** The figures illustrate the neural activity patterns in response to different social interactions, highlighting the role of the anterior PAG and VMHvl in social reward processing.
During testing, we sequentially introduced a non-receptive adult female and a group-housed non-aggressive male into the home cage of the test male mice. In males expressing hM3Dq in PA
\( \text{Esr1}^{+}\text{-MPN} \) cells, intraperitoneal injection of low-dose CNO (0.1 mg kg\(^{-1}\)) promoted female-directed sexual behaviors without altering aggressive or investigatory behaviors toward either males or females (Fig. 6d–q).

Following saline injection, four of the eight males showed mounting attempts and only two of the eight males achieved a brief shallow thrust. In contrast, after CNO injection, all males attempted to mount, often repeatedly, and all achieved shallow thrust despite the uncooperative behaviors of the non-receptive females (Fig. 6e–h).

Interestingly, when 0.5 mg kg\(^{-1}\) CNO was administered, which induces a higher level of neural activation than 0.1 mg kg\(^{-1}\) CNO, we observed a mixture of sexual and aggressive behaviors toward females: all males showed sexual behaviors and half of those also attacked females after several mounting attempts (Extended Data Fig. 8a–g). Importantly, the aggressive behavior appeared to be specifically directed toward females as male-directed aggression did not change, suggesting that PA
\( \text{Esr1}^{+}\text{-MPN} \) cells do not promote natural intermale aggression (Extended Data Fig. 8a,h,m).

By contrast, activating PA
\( \text{Esr1}^{+}\text{-VMHvl} \) cells did not affect sexual or investigatory behaviors toward males or females at either CNO concentration (Fig. 6r–w and Extended Data Fig. 8n–z). However, administering CNO but not saline promoted aggression in a dose-dependent manner. While only two of the eight males attacked males after saline injection, five of the eight males attacked males and two males attacked females following injection with 0.1 mg kg\(^{-1}\) CNO (Extended Data Fig. 8a,t,y,z). At 0.5 mg kg\(^{-1}\) CNO, all animals attacked males and six of the eight males also attacked females (Fig. 6w,x,d,e), strongly suggesting that PA
\( \text{Esr1}^{+}\text{-VMHvl} \) cells promote aggressive behaviors.

We also activated PA
\( \text{Esr1}^{+}\text{-VMHvl} \) and PA
\( \text{Esr1}^{+}\text{-MPN} \) cells optogenetically (20 Hz, 20 ms, 0.5–7.5 mW). While activation of PA
\( \text{Esr1}^{+}\text{-VMHvl} \) cells elicited stimulation-locked attack, we did not observe any increase in sexual behaviors upon optogenetic activation of PA
\( \text{Esr1}^{+}\text{-MPN} \) cells (Extended Data Fig. 9). In fact, these animals showed low levels of sexual behaviors during the entire testing period, suggesting that strong and synchronous exogenous stimulation of PA
\( \text{Esr1}^{+}\text{-MPN} \) cells is insufficient to promote sexual behaviors. Taking these findings together, activation of PA
\( \text{Esr1}^{+}\text{-VMHvl} \) neurons elicits aggression, whereas enhancing the natural responses of PA
\( \text{Esr1}^{+}\text{-MPN} \) cells promotes male sexual behaviors toward females.

Connectivity of male PA
\( \text{Esr1}^{+}\text{-MPN} \) and PA
\( \text{Esr1}^{+}\text{-VMHvl} \) neurons. To achieve a comprehensive understanding of the connectivity of PA
\( \text{Esr1}^{+}\text{-MPN} \) and PA
\( \text{Esr1}^{+}\text{-VMHvl} \) cells, we injected retrograde HSV-hEfi1-LS1L-Flp into either the MPN or VMHvl and AAVs encoding TVA receptors and a rabies glycoprotein with Fp-dependent expression into the PA of male Esrl-2A-cre mice. After 2 weeks, EnvA-pseudotyped, glycoprotein-deleted rabies virus (EnvA-RVΔG-eGFP) was injected into the PA (Fig. 7f). After 7 d, we examined inputs (eGFP+ cells) to PA
\( \text{Esr1}^{+}\text{-MPN} \) and PA
\( \text{Esr1}^{+}\text{-VMHvl} \) starter cells (eGFP+mCherry+ cells) across the entire brain (Fig. 7g–l). The hippocampus represented the largest input to both PA
\( \text{Esr1}^{+}\text{-MPN} \) and PA
\( \text{Esr1}^{+}\text{-VMHvl} \) cells, and approximately half of all cells labeled in retrograde, regardless of the PA starter cell population, were located in the hippocampus, including 30% in CA1 and 20% in the vSub (Fig. 7h,i,l). Several other regions, including the medial septum, paraventricular thalamus, posterolateral and posteromedial parts of cortical amygdala (CoApl and CoApn) and BMAp, also provided moderate inputs to both PA
\( \text{Esr1}^{+}\text{-MPN} \) and PA
\( \text{Esr1}^{+}\text{-VMHvl} \) cells. Interestingly, PMs and MeApd, two regions that are known to project to the PA, appeared to target the PA
\( \text{Esr1}^{+}\text{-MPN} \) subpopulation preferentially
\( \text{Esr1}^{+}\text{-VMHvl} \), whereas PA
\( \text{Esr1}^{+}\text{-VMHvl} \) cells received relatively strong inputs from regions processing volatile olfactory information, including the piriform cortex (Pir) and piriform–amygdalar area (PAA; Fig. 7h,i,l). Altogether, PA
\( \text{Esr1}^{+}\text{-MPN} \) and PA
\( \text{Esr1}^{+}\text{-VMHvl} \) cells are both well positioned to integrate olfactory, contextual, experiential and emotional state-related information and serve as important players in the male sexual and aggressive circuit, respectively.

Discussion

In this study, we identified the PA as a key node in both the aggression and mating circuits in male mice. Specifically, we found two...
largely distinct PA subpopulations that project to the MPN and VMHvl, and each serves important social functions in male mice. Among PA cells that project to the MPN or VMHvl, less than 10% of cells project to both regions. PAEsr1+MPN neurons gradually increase activity as sexual behaviors advance and are necessary and sufficient for the behaviors. By contrast, PAEsr1 VMHvl cells play only a minor role in sexual behaviors and are excited mainly during intermale aggression and promote attack. Cell-type-specific tracing revealed...
that PA_Esr1+MPN and PA_Esr1+VMHvl cells receive rich inputs from hippo-campal regions and project densely to the medial hypothalamus, as well as brain regions along the vomeronasal pathway.

**PA neurons play important roles in male social and aggressive behaviors.** Nearly three decades ago, Canteras et al. performed classical anterograde tracing from the PA, revealing its dense projection to the medial hypothalamus, and accordingly proposed a role for the PA in social behaviors. Despite this early insight, the functional role of the PA in male social behaviors has not yet been examined. Recently, Zha et al. reported that Vglut1-expressing cells in the PA that project to the VMHvl can bidirectionally modulate male aggression. This finding is largely consistent with our results, which show a key role of PA_Esr1+VMHvl cells in male aggression. However, PA_Esr1+VMHvl cells are likely to be more aggression specific than PA_Vglut1+VMHvl cells, as PA_Esr1+VMHvl but not PA_Vglut1+VMHvl cells showed a consistent increase in Ca²⁺ levels during male investigation and attack. Indeed, while over 95% of Esr1+ cells express Vglut1, less than half of Vglut1+ cells express Esr1 (Extended Data Fig. 2).

Our results further demonstrate an indispensable role of Esr1+ PA neurons in male sexual behaviors: inhibiting PA_Esr1+ or PA_Esr1+MPN cells abolishes virtually all aspects of sexual behaviors. The central role of the PA in sexual behavior is particularly striking considering the relatively inconsistent behavioral deficit caused by inhibiting other major upstream regions of the MPN, including the MeA, BNSTpr and VMHvl. Neither the killing of aromatase-expressing cells nor acute inhibition of GABAergic cells (the major population) within the MeA decreases sexual behaviors in male mice. Lesioning of the BNST causes only minor impairments in sexual behaviors, such as increased latency to ejaculation, although a recent study showed that inactivation or ablation of aromatase-expressing neurons in the BNSTpr reduced intromission and ejaculation. In addition, killing VMHvl progesterone (PR)-expressing cells only slightly decreases the number of intromissions. In contrast, we show that inactivation of PA_Esr1+MPN cells compromises the entire sequence of male sexual behaviors, from mounting initiation to ejaculation.

In comparison to the clear behavior changes elicited by optogenetic activation of PA_Esr1+VMHvl cells, optogenetic stimulation of PA_Esr1+MPN cells failed to elicit sexual behaviors. This could be because mating is a multistage, sequential process and the MPN contains cells with diverse response patterns during sexual behaviors: some MPN cells are excited during the pursuit of females and mounting initiation but are inhibited during intromission, while other cells are activated only during advanced stages of sexual behaviors. Nonselective, synchronous activation of PA_Esr1+MPN afferents likely recruits MPN cells with diverse functions and thus fails to drive a coherent behavioral motor program. Additionally, unlike VMHvl cells, which are mainly glutamatergic, MPN cells are primarily GABAergic and form extensive local synapses. Thus, activation of functionally diverse MPN cells by the PA may lead to a general suppression of activity and, consequently, a lack of sexual behaviors altogether. Unlike optogenetic activation that directly evokes action potentials, hM3Dq activation increases the excitability of cells by engaging endogenous GABA signaling. Importantly, hM3Dq-mediated neural activation is dependent on ligand concentration. A previous study using mice expressing hM3Dq in cortical pyramidal cells found that 0.1 mg kg⁻¹ CNO increased locomotion, while 0.5 mg kg⁻¹ CNO elicited seizures, suggesting that CNO can be titrated to elevate neural activity within a physiological range. It is therefore likely that the endogenous synaptically driven spiking pattern of PA neurons, which in turn contribute to the diverse response patterns of MPN cells, is essential for generating complex behavioral sequences such as mating.

**PA-enriched genes and their relevance to social behaviors.** Mating- and aggression-related PA subregions show clear differences in gene expression patterns. Differentially expressed genes include transcription factors (Etv1 and Zic2), G-protein-coupled receptors (Npy2r and Prokr2), ion channels (Chrn7) and calcium-binding proteins (Calb2), among others. One of the PA-enriched genes, Zic2, has been implicated previously in aggression: mice with morphologic mutations in Zic2 showed decreased intermale aggression and social dominance. Interestingly, the authors also noted a high abundance of Zic2 in the PA and a decreased cell density in the area in mutant mice. Furthermore, wild-type mice show strong acetylcholinesterase (AChE) staining in the PA, whereas AChE levels are low in mutant animals. The strong AChE staining in the PA is consistent with our finding that Chrn7 is highly expressed in the PA. Our retrograde tracing revealed that the PA receives a moderate input from the medial septum, a region containing abundant cholinergic cells, suggesting the PA as a potential site for cholinergic modulation of aggression.

**Sexually dimorphic PA.** What is the function of the PA in females? Anatomical studies in rodents revealed that the PA exists in both males and females with comparable volume, morphology and cell density. However, several lines of evidence suggest that the PA is likely to play sexually dimorphic roles in social behaviors. First, the MPN serves differential functions between males and females. While the MPN is the central region for male sexual behavior, its primary function in females is to mediate maternal behaviors. Second, VMHvl function is sexually dimorphic. While the VMHvl is key to male aggression and only plays a minor role in male sexual behaviors, it is indispensable for both female sexual behaviors and aggression. In females, the VMHvl appears to contain a molecularly distinct compartment specialized for female sexual behaviors, which is absent in males. Third, the PA shows a
sexually dimorphic projection pattern. While both male and female PA cells project to the MPN, VMHvl, BNStpr and MeApd, female PA cells seem to lack a strong projection to the AVPV\(^{29}\). Taking these findings together, we speculate that female PA cells could be relevant for various social behaviors, including maternal, sexual and aggressive behaviors. The organizations of those behavior-relevant populations and their downstream circuits are likely to differ from those in males. Future studies focusing on the female PA will help elucidate the sexual dimorphism of social behavior circuits.

The placement of the PA in social behavior circuits in males. In 2000, Larry Swanson proposed a basic wiring diagram for controlling motivated behaviors based on extensive developmental and neuroanatomical evidence\(^{14}\). In his model, the forebrain exerts ‘top–down’ voluntary control on various motivated behaviors through a set of triple descending projections to the ‘behavioral control column’ in the upper brainstem\(^{15}\). The rostral part of the behavioral control column is for ingestive and social behaviors and contains several hypothalamic nuclei, including the MPN and...
Fig. 7 | Inputs and outputs of PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}MPN and PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}VMHvl cells. a, The viral strategy to trace the outputs of PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}MPN and PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}VMHvl cells. b, Pie charts showing the distributions of all eYFP-expressing cells; n = 3 animals in each group. c, The density of the terminal fields was measured as the normalized average pixel intensity in major downstream regions of PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}MPN and PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}VMHvl cells; n = 3 animals in each group. d, Images are representative of the fibers coming from PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}MPN (d) and PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}VMHvl (e) cells. PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}MPN cells project densely to the VMHvl and PMv; scale bars, 200 µm. f, The viral strategy for labeling the direct upstream cells of PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}MPN or PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}VMHvl populations. FLeXFrt, Flp-dependent expression; G, rabies glycoprotein; tC, tVA–mCherry fusion. g, Pie charts showing the distributions of all starter cells; n = 3 animals in each group. h, Distributions of the cells upstream of PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}MPN and PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}VMHvl cells; n = 3 animals in each group. i, Images show the starter cells in the PA (yellow) and neighboring PA neurons that project to the starter cells (green); insets, enlarged view of the boxed areas; scale bars, 200 µm and 20 µm (insets). j, Images are representative of the rabies-labeled cells in various brain regions, presumably upstream of PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}MPN (j) and PA\textsuperscript{Esrt1-2A-Cre}\textsuperscript{+}VMHvl (l) cell populations; scale bars, 200 µm. Images in d, e and i–l are from n = 3 animals. All data in g and h are presented as the mean ± s.e.m. Statistical significance was determined using two-way ANOVA with Bonferroni’s multiple-comparison test; *P < 0.05 and **P < 0.01 (Extended Data Fig. 10; see Supplementary Table 1 for detailed statistics).
VMHvl. The caudal part of the column is for general exploratory and foraging behaviors and contains the mammillary nucleus, the substantia nigra (SNr) and the ventral tegmental area. In his view, the overall pattern of motivational behavior circuitry originates in the cortical map, with its regionalization into functionally distinct areas, and then sequentially involves the striatum and pallidum followed by the brainstem. The cortical projection is primarily glutamatergic, whereas the striatal and pallidal projections are primarily GABAergic. A familiar example of the triple projection is the isocortical–dorsal striatopallidal system in which (1) cortical regions send direct glutamatergic projections to the SNr and collateral and topographic projections to the dorsal striatum; (2) the dorsal striatum sends GABAergic projections to the SNr with a collateral projection to the pallidum; and (3) the pallidum then sends GABAergic projections to the SNr with a collateral to the thalamus, which in turn projects to the cortex (Extended Data Fig. 10). Importantly, in this grand scheme, each and all of the anatomical structures in the cerebral hemisphere belong to the prototypical cortex, striatum or pallidum.

According to this view, we propose that the PA along with the heavily interconnected vSub constitutes the ‘social cortex’ and mediates top–down regulation of the hypothalamic behavioral control column. Similarly to the classical isocortical–dorsal striatopallidal system, the PA/vSub, MeA and BNST provide a triple descending projection to the column (Extended Data Fig. 10). First, the PA/vSub (cortex) sends excitatory projections directly to the column (MPN and VMHvl), as well as collaterals to the MeA (rostrocaudal striatum)16. The MeA then sends inhibitory projections to the same part of the behavioral column (MPN and VMHvl), as well as collaterals to the BNST (rostrocaudal pallidum). Lastly, the BNST provides disinhibitory projections to the column. In this model, the PA/vSub occupies a similar position in the social behavior circuit as the motor cortex in the voluntary motor circuit and determines the timing of the behavior initiation. Indeed, PA cells increase activity when an animal makes a first move toward a female to initiate mounting. Suppressing PA activation blocks the initiation of sexual behaviors altogether.

The discovery of PA as a key node for social behaviors supports the existence of a universal diagram underlying cortical control of movement. The PA is potentially a specialized ‘cortex’ to control a set of stereotypical movements constituting innate social behaviors. This study offers a new framework for understanding how social behaviors are organized and initiated at the circuit level and opens new avenues for studying the neural generation of innate behaviors.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-020-0675-x.

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Methods
Mice. All procedures were approved by the NYULMC Institutional Animal Care and Use Committee (IACUC) in compliance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. Adult male mice aged 12–18 weeks were used for all studies. Mice were housed in temperature-controlled rooms with 40–60% humidity under a 12-h light–dark cycle (dark cycle, 10 p.m. to 10 a.m.), with feed and water available ad libitum. Animals used for CTB retrograde tracing and RNA-seq experiments were 14–to 16-week-old wild-type C57BL/6 mice purchased from Charles River. Male EnvA-G-deleted rabies-eGFP was injected into the PA of the same side. One week later, 600 nl of HSV-heLa-LS1L-Flp was injected into the MPN (VMHvl) and 100 nl of AAVDJ-Ef1-βDIO-hM4Di-mCherry into the PA. Test animals that showed correct bilateral targeting of the PA were included in the final analysis (n=5 of 9).

For pharmacogenetic activation of PAEsr1 cells, 100 nl of AAV1-hSyn-DIO-hM4Di-mCherry was injected bilaterally into the PA. Control animals were injected bilaterally with 100 nl of AAV2-hSyn-DIO-hM4Di-mCherry into the PA. Test animals that showed correct bilateral targeting of the PA were included in the final analysis (n=5 of 10).

For pharmacogenetic inhibition of PAEsr1 cells, 100 nl of HSV-heLa-LS1L-Flp was injected into the MPN (VMHvl) and 200 nl of AAVDJ-hSyn-Con/h-chr2(H134R)-EYFP was injected into the PA bilaterally. Control animals were bilaterally injected with 130 nl of HSV-heLa-LS1L-Flp into the MPN (VMHvl) and 100 nl of AAVDJ-Ef1-βDIO-hM4Di-mCherry into the PA. Test animals that showed correct bilateral targeting in the MPN and correct bilateral virus expression in the PA (n=8 of 10) and correct bilateral targetting in the VMHvl and correct bilateral virus expression in the PA (n=8 of 10) were included in the final analysis.

For pharmacogenetic activation of PAEsr1 or PAEvm1 cells, 100 nl of HSV-heLa-LS1L-Flp was injected into the MPN (VMHvl) while 120 nl of HSV-heLa-ls1l-EYFP was injected into the ipsilateral PA. Control animals were bilaterally injected with 130 nl of HSV-heLa-LS1L-Flp into the MPN (VMHvl) and 100 nl of AAVDJ-Ef1-βDIO-hM4Di-mCherry into the PA bilaterally. Then, a 200-μm optic fiber assembly (Thorlabs, FT2000EM, CFL230) was implanted 200 μm above each side of the PA during the same surgery. Test animals that showed correct bilateral targeting in the MPN, correct bilateral expression of ChR2-EYFP in the PA and correct bilateral fiber placements in the PA were included in the final analysis (n=5 of 10). Test animals that showed correct bilateral targeting in the VMHvl, correct bilateral expression of ChR2-EYFP in the PA (>100 positive cells in all sections) and bilateral fiber placements in the PA were included in the final analysis (n=5 of 16).

Immunochemistry. Immunofluorescence staining proceeded as previously described. Briefly, mice were perfused transcardially with 1× PBS followed by 4% PFA. Brains were postfixed in 4% PFA for 2–3 h at 4°C followed by 48 h in 15% sucrose, embedded in OCT mounting medium, frozen on dry ice, cut to 50–70-μm sections using a cryostat (Leica Biosystems) and collected in PBS using a 12-Well plate. For antibody staining, brain sections were washed with PBS three times and blocked in PBS-T (0.3% Tween 20 and 100× in 1× PBS) with 10% normal donkey serum at room temperature. Sections were then incubated in primary antibody diluted in blocking solution (donkey anti-rabbit, donkey anti-goat, AlexaFluor 488 or 594, 1:500; Thermo Fisher, A11055, R37118 and R37119) with DAPI (1:20,000; Thermo Fisher, D1306) or NeuroTrace 435/455 Blue Fluorescent Nissl Stain (1:200; Thermo Fisher, N21479) for 2–3 h at room temperature. Sections were then washed with PBS three times, mounted on Superfrost slides (Fisher Scientific, 12-550-15) and coverslipped for imaging using a confocal microscope (LSM 510 or 700 microscope) and/or a virtual slide scanner (Olympus, VS120).

Fluorescence in situ hybridization. Extracted brains were frozen on dry ice, and 12-μm coronal brain sections were collected using a cryostat (Leica Biosystems). For all genes except Pde11a, we performed RNAscope labeling following the manufacturer’s protocol (Advanced Cell Diagnostics). For Alexa Fluor 555–CTB–labeled tissue, the incubation time for protease solution IV was shortened from 30 min to 30 s to preserve Alexa Fluor 555–CTB expression. For Pde11a, RNA probes were generated from the pSPT19 vector (Sigma) using primers reported on https://www.brain-map.org/. Specifically, pSPT19 plasmid was cut with EcoRI and the antisense strand was transcribed using T7 RNA polymerase with digoxigenin-labeled UTP (DIG RNA labeling kit; Sigma-Aldrich, 11715025910; ref. 53), amplified with biotin-conjugated tyramide (PerkinElmer,
Cell counting and axon-terminal quantification. To analyze labeled cells, >20 confocal or epifluorescence images were acquired, and cells were counted manually using ImageJ. Cells that were not entirely contained within a given region of interest were excluded from analyses. For counting DAPI, Nissl, CTb*, Fo* and Esr1+ cells, >20 fluorescence confocal images were acquired. For counting GCAmP6e, EYFP and mCherry in dual-retrograde labeling, photomicroscopy experiments, >20 fluorescence images were acquired with a virtual slide scanner (Olympus, VS120). For Fig. 7h, the PAE in each brain section was divided into four quadrants along the short and long axes. Then, labeled cells in each quadrant were counted, and the sum from all sections (6–7 sections, 50 μm per section, ranging from bregma −2.15 mm to −2.90 mm) was calculated and averaged across animals. The total number of area counts (VMHvl, vSub, and vAmy) was then calculated by subtracting the intensity (F(avg)) of each area from the baseline (F(max)). The normalized mean was calculated as 

\[ \text{Normalized Mean} = \frac{F_{\text{avg}} - F_{\text{max}}}{F_{\text{max}}} \]

for each animal, normalized by the average intensity of each region across all the analyzed regions. The normalized F(avg) was then used for calculating the average terminal field intensity across animals.

In vitro electrophysiological recordings. For 1S2a-pre males, recordings were typically obtained from 4–6 animals. AAV2-hSyn-DIO-mCherry or AAV2-knd2ad-DIO-EYFP was injected into the MPN and VMHvl. Three weeks after infection, extracellular recordings were performed with tetrodes, and cell recordings were performed using an intracellular electrode. Standard whole-cell voltage clamp recordings were performed using glass electrodes (2–4 MΩ) containing 135 mM CsMeSO4, 10 mM HEPES, 1 mM EGTA, 3.3 mM QX-314 chloride salt, 4 mM Mg-ATP, 0.3 mM Na-GTP and 8 mM sodium phosphocreatine (pH 7.3 adjusted with CsOH). Series resistance monitored during the session with a female intruder, a group-housed non-aggressive male BALB/c mouse was placed in the home cage of the test male mouse (‘resident’) until the resident reached ejaculation. After the session with a female intruder, a group-housed non-aggressive male BALB/c or C57BL/6N mouse was placed in the home cage for 10 min and then an object was introduced into the home cage of the recorded male for 5 min. Each stimulus was presented with 5 min in between. To analyze the recording data, the MATLAB function ‘findchangepts’ was used to identify points where the oEPSC was mediated by the AMPA receptor and the oIPSC was polysynaptic and dependent on the oEPSC. When the male began to rapidly thrust against the female’s rear; and ‘deeply thrust’, the male extended his forelimbs to grasp and mount the female’s flanks; ‘shallowly thrust’, the male thrust against the female’s rear without any extension of the forelimbs. Sniffing was defined by nose contact to the foreign object. Mouse behavior was manually annotated frame by frame by an experimenter who was not blinded to the group assignment of the animals. Attack events in a randomly selected set of videos were annotated using DeepLabCut21 and Simba22, and good agreement between human and machine annotations was observed. During annotation, the neural responses were not available to the experimenter.

Fiber photometry. The test animals were screened for both aggressive and sexual behaviors before the surgery and randomly assigned to experimental and control groups. Fiber photometry was performed as described previously23. Briefly, a 390 Hz temporal sine wave (300 μm; LED light, M470F1; LED driver, LEDD1B; Thorlabs) was bandpass filtered (passing band, 472 ± 15 nm; FF02-470/20, Semrock) and delivered to the brain to excite GCAmP6e. The emission lights traveled back through the same optic fiber, were band pass filtered (passing bands, 535 ± 25 nm; FF01-535/505, Semrock), passed through an adjustable zooming lens (SMINR01, Thorlabs) and a bandpass filter (780 nm; Smart Silicon Photoreceiver, Newport, 2151) and were recorded using a real-time processor (RZ5, TDT). The envelope of the 390 Hz signals reflected the intensity of GCAmP6e and was extracted in real time using a custom TDT OpenEx program. The signal was low-pass filtered with a cutoff frequency of 5 Hz. During recording, a security receiver in the signal path to the CA1 was first applied to obtain the instantaneous baseline signal. The instantaneous ΔF/ΔF0 value was calculated as 

\[ \frac{F_{\text{atom}} - F_{\text{base}}}{F_{\text{base}}} \]

The PETH of a given behavior was constructed by aligning the ΔF/ΔF0 signal to the onset of the behavior. The ΔF/ΔF0 values during the attempt mount and shallow-thrust episodes were calculated based on trials that were not followed by a deep thrust episode. The peak ΔF/ΔF0 values for each behavior episode were calculated as the maximum ΔF/ΔF0 of the PETHs during each behavioral episode minus the average ΔF/ΔF0 in the duration-matched period before the behavior onset. The resulting values for all episodes of the same behavior type were then averaged for each animal and plotted in Fig. 8a. The location of the animal was tracked using a custom software (https://github.com/pdollar/toolbox23). The velocity of the animal was calculated as the displacement of the animal’s location between two adjacent frames. The change points in velocity were determined using the MATLAb function ‘findchangepts’. Only change points after 2 s of immobility (velocity < 2 pixels per frame) were included for analysis. We separated the change points based on whether a mounting attempt occurred within 3 s and calculated the velocity change to Ca2+ signal in Ca2+ signal activity. If less than 1 s to 1 s of the change points either followed by or not followed by sexual behaviors.

Pharmacogenetic neural silencing. The test animals were screened for both aggressive and sexual behaviors before the surgery and randomly assigned to experimental and control groups. For PAEsr1 activation experiments, test animals were naive (no previous sexual experience or tests for aggression) and were group housed before the virus injection and singly housed after surgery. Animals were assigned to test and control groups randomly. Three weeks after surgery, test males were intraperitoneally injected with saline and CNO (0.1 or 0.5 mg kg−1; Sigma, C8832) on separate days. At 30 min after saline or CNO injection, a sexually receptive female C57BL/6N mouse was placed in the home cage of the test mouse, and the test mouse was allowed to freely interact with the female for 10 min or until the male showed 15 mounting attempts or 10 instances of intromission, whichever duration was shorter. For PAEsr1 activation experiments, males were screened for aggressive and sexual behaviors both before and 2 weeks after surgery. During aggression screening, a group-housed BALB/c male mouse was introduced into the home cage of the test mouse, and only animals that achieved intromission during the 10-min testing period were included in the final test. Three weeks after the injection, mice were intraperitoneally injected with saline or CNO (5 mg kg−1; Sigma, C8832) on alternate days. At 30 min after saline or CNO injection, a sexually receptive female C57BL/6N mouse was placed in the home cage of the test mouse, and the test mouse was allowed to freely interact with the female for 10 min or until the male showed 15 mounting attempts or 10 instances of intromission, whichever duration was shorter. For PAEsr1 and PAEsr1+VHm activation experiments, test animals were naive (no previous sexual experience or tests for aggression) and were group housed before the virus injection and singly housed after surgery. Animals were assigned to test and control groups randomly. Three weeks after surgery, test males were intraperitoneally injected with saline and CNO (0.1 or 0.5 mg kg−1; Sigma, C8832) on separate days. At 30 min after saline or CNO injection, a sexually receptive female C57BL/6N mouse was placed in the home cage of the test mouse, and the test mouse was allowed to freely interact with the female for 10 min or until the male showed 15 mounting attempts or 10 instances of intromission, whichever duration was shorter. For PAEsr1 and PAEsr1+VHm activation experiments, males were screened for aggressive and sexual behaviors both before and 2 weeks after surgery. During aggression screening, a group-housed BALB/c male mouse was introduced into the home cage of the test mouse, and only animals that achieved intromission during the 10-min testing period were included in the final test. Three weeks after surgery, sexual and aggressive behaviors were tested after CNO and saline injection. During each test, the test mouse first encountered a receptive female C57BL/6N mouse, and only animals that showed a minimum of five attacks during the 10-min testing period were included in the final test. Three weeks after the injection, mice were intraperitoneally injected with saline or CNO (5 mg kg−1; Sigma, C8832) on alternate days. At 30 min after saline or CNO injection, a sexually receptive female C57BL/6N mouse was placed in the home cage of the test mouse, and the test mouse was allowed to freely interact with the female for 10 min or until the male showed 15 mounting attempts or 10 instances of intromission, whichever duration was shorter. For PAEsr1 and PAEsr1+VHm activation experiments, males were screened for aggressive and sexual behaviors both before and 2 weeks after surgery. During aggression screening, a group-housed BALB/c male mouse was introduced into the home cage of the test mouse, and only animals that achieved intromission during the 10-min testing period were included in the final test. Three weeks after surgery, sexual and aggressive behaviors were tested after CNO and saline injection. During each test, the test mouse first encountered a receptive female, for a maximum of 10 min (see above), and then, after the female was removed for 3 min, the test mouse encountered a non-aggressive male BALB/c mouse for 10 min.
injection, a non-receptive female and then a non-aggressive BALB/c male mouse were introduced to the home cage of the test mouse, each for 10 min with 2 min in between. No test mouse achieved deep thrust in this experiment as the female was non-receptive.

Intersectional optogenetic behavioral experiments. Test animals were naive (no previous sexual experience or tests for aggression) and were group housed before the virus injection and singly housed after surgery. Animals were assigned to test and control groups randomly. After 3 weeks of viral incubation, on the test days, two 200-μm multimode optical fibers (Thorlabs, FT200EMT) were connected with bilaterally implanted ceramic ferrules (Thorlabs, CFC230-10) using a match sleeve (Thorlabs, ADAL1). Randomly selected group-housed adult BALB/c male mice, castrated BALB/c male mice and C57BL/6N female mice were introduced into the home cage of the test mouse. Blue light (473 nm, Shanghai Dream Laser) was delivered through the fiber bilaterally in 20-ms pulses at 20 Hz with an intensity of 0.5–7.5 mW for 60 s using OpenEx (TDT). The light intensity was measured at the fiber end during pulsing using an optical power meter (Thorlabs, PM100D). A sham stimulation period (0 mW) for 60–80 s was interleaved with the real 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 in the same session.

Subpopulation-specific input and output mapping. To investigate downstream targets of PA and BMAp or PAMPN and PAVMHvl populations was performed using the limma R package60 with normalized gene reads through the open-source software iDEP61. Ge, S. X., Son, E. W. & Yao, R. iDEP: an integrated web application for discovery rate (FDR) threshold of <0.05 and a fold change >1.5 between PA and BMAP populations, and subpopulation-specific genes in the PA were selected with an FDR <0.07 and a fold change of >1.5 between PA and BMAP populations.

Statistics. All statistical analyses for histological, in vivo and in vitro recording and behavioral experiments were performed using MATLAB2018a (Mathworks) and brains were collected, flash frozen in chilled 2-methylbutane (approximately −80°C) within 5 min and stored at −80°C until sectioning. Coronal sections (20 μm) were obtained using a cryostat and mounted on PEM membrane slides (Leica Microsystems, 1150189). The slides were pretreated with 100% ethanol for 1 min and air dried. PA+ and PA−/− subpopulations were laser dissected based on the retrograde labeling using an LMD6000 fluorescence microscope (Leica). The RNA samples and cDNA libraries were prepared with a PicoPure RNA isolation kit (Thermo Fisher, KIT0204) and SMARTer Stranded Total RNA-seq Kit (Clontech, 635006) as previously described29. The cDNA libraries were sequenced with an Illumina HiSeq 4000 using high-output mode to achieve greater depth of coverage. Sequencing reads were aligned to the mouse genomes (build mm10/GRCm38) using the splice-aware STAR aligner27, and then the counts for each gene were generated using featureCounts28. Differential gene expression analysis between the PA and BMAp or PA+ and PA−/− populations was performed using the limma R package29 with normalized gene reads through the open-source software iDEP (http://ge-lab.org/idep/). We selected whole PA region-enriched genes with a false discovery rate (FDR) threshold of <0.05 and a fold change >1.5 between PA and BMAP populations, and subpopulation-specific genes in the PA were selected with an FDR <0.07 and a fold change of >1.5 between PA and BMAP populations.

Data availability
We have uploaded the RNA-seq data from this manuscript to the Gene Expression Omnibus (GEO) under accession number GSE151798. All other data that support the findings of this study are available from the corresponding authors upon request.

Code availability
MATLAB code ScanImage is available at https://github.com/bernardosabatin/SablabScanImage. MATLAB codes for behavioral annotation and tracking are available at https://github.com/poller/toolbox/. Custom TDT OpenEx programs and MATLAB codes for fiber photometry data analysis will be provided upon request to the corresponding authors.

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Author contributions
D.L. conceived the project, designed experiments, analyzed data and wrote the paper. T.Y. co-designed and performed most experiments, analyzed data and co-wrote the paper. D.W. and S.C.S. performed slice recording and analyzed data. N.X.T. supervised the slice recording experiment, analyzed data and edited the paper. B.L. generated AAV-Ef1α-idiO-hM3Dq-Cherry and AAV-Ef1α-idiO-hM3Dq-M-Cherry. Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | PA^Esr1^+ cells provide the largest inputs to the medial hypothalamus among all the Esr1^+ cells in the amygdala, related to Fig. 1. 

**a.** The retrogradely labeled Esr1^+ cells in the amygdala after HSV injection into VMHvl (green) and MPN (red). Scale bar: 200 μm. Images are representative of n = 3 mice. 

**b, c.** The distributions of mCherry^+ (MPN projecting, b) and EYFP^+ cells (VMHvl projecting, c) after injecting HSV-hEf1α-LSIL-mCherry into the MPN and HSV-hEf1α-LSIL-EYFP into the VMHvl of Esr1-2A-Cre male mice. Data in b and c are presented as mean ± s.e.m. PA: posterior amygdala; MeApd: medial amygdala posterior dorsal subdivision; MeApv: medial amygdala posterior ventral subdivision; MeAad: medial amygdala anterior dorsal subdivision; MeAav: medial amygdala anterior ventral subdivision; CoAp: cortical amygdala posterolateral part; CoApm: cortical amygdala posteromedial part; CoAa: cortical amygdala anterior part; BLAa: basolateral amygdala anterior part; BLAp: basolateral amygdala posterior part; BMAp: basomedial amygdala posterior part; PAA: piriform-amygdalar area.
Extended Data Fig. 2 | Characterization of the neurotransmitter type of PA^esr1+ neurons, related to Fig. 2. a and b, Overlay between Vglut1 (a) or Vgat (b) (green) and Esr1 (red) in the PA from bregma level −2.00 to −2.90 mm. Vglut1 and Vgat are visualized using Vgat-ires-Cre × Ai6 and Vglut1-ires-Cre × Ai6 lines, respectively. Right showing the enlarged view of the boxed area. Scale bars: 200 μm (bottom) and 20 μm (upper right). c, The percentage of Vglut1+ and Esr1+ cells in the total neuronal populations in PA, the percentage of PA^esr1+ cells that are glutamatergic or GABAergic, and the percentage of glutamatergic cells expressing Esr1 and the percentage of GABAergic cells expressing Esr1. n = 2 animals for each group. Data in c are presented as mean.
Extended Data Fig. 3 | Topographical Fos expression patterns in the PA after mating and fighting, related to Fig. 4. a–c, Representative images showing the expression of c-Fos (green) and Esr1 (red) in the PA at bregma level −2.30 mm (left) and −2.75 mm (right) after handling (a) (n = 3 animals), mating (b) (n = 3 animals) or fighting (c) (n = 4 animals). Right showing the enlarged views of the boxed areas. Scale bars: 200 μm (left) and 20 μm (right). d, The number of Fos+ neurons in the PA increased significantly after mating and fighting. One-way ANOVA with Tukey’s multiple comparison test. e, Majority of Fos+ cells induced by mating or fighting express Esr1 in the PA. Red and blue dashed lines mark the percentage of Esr1+ cells in the total PA population. Two-tailed unpaired t-test. f, The number of Fos+ neurons expressing Esr1 in the PA along the anterior-posterior axis after handling control (gray), mating (red) or fighting (blue). All data in d, e, and f are presented as mean ± s.e.m. *p < 0.05, **p < 0.001. For detailed statistics information, see Supplementary Table 1.
Extended Data Fig. 4 | Virus injection and expression sites and optic fiber placements for recording and functional manipulation experiments, related to Figs. 4, 5 and 6. a–c. Coronal brain sections at the bregma level of MPN, VMHvl and PA showing the virus injection or expression sites (dots) and optic fiber placements (lines) in fiber photometry and pharmacogenetics experiments. Each dot or line represents one animal. Injections and recording sites in each animal are unilateral in (a) and bilateral in (b, c). Brain atlas images are modified from ref. 10.
Extended Data Fig. 5 | PA\textsuperscript{Esr1+MPN} cells but not PA\textsuperscript{Esr1+VMHvl} cells increase activity during initiation of sexual behaviors, related to Fig. 4. a, Representative \textsuperscript{Ca}\textsuperscript{2+} trace (black) of PA\textsuperscript{Esr1+} cells and the test animal’s movement velocity (red) during interactions with a female mouse. Vertical lines mark the computer detected velocity changing points that are either followed by mounting attempts within 3 s (magenta) or not (blue). Gray shades mark the manually annotated attempted mount (dark gray), shallow-thrust (median gray) and deep-thrust (light gray). b, \textsuperscript{Ca}\textsuperscript{2+} signal aligned to the onset of movement initiation, either followed by sexual behaviors (left) or not (right). c, Bar graphs showing that the slope of the \textsuperscript{Ca}\textsuperscript{2+} signal is significantly positive between 0 and 1 s (shades in b) after movement initiation only if the animal later initiated mounting. The average latency from movement initiation to manually annotated mounting initiation is 1.3 s. d, Velocity of the animal aligned to the automatically detected velocity changing points, either followed by sexual behaviors (left) or not (right). e, Bar graphs showing the velocity change from \(-1–0\) s to \(0–1\) s in (d) does not differ between trials followed by sexual behaviors and those not. n = 5 animals in b–e, f–j. PA\textsuperscript{Esr1+VMHvl} cells showed no increase in \textsuperscript{Ca}\textsuperscript{2+} activity during the movement initiation regardless whether it is followed by attempted mounting or not. Plots are organized in parallel to those shown in a–e. n = 6 animals in g–j. All data in c, e, h and j are presented as mean ± s.e.m. One sample two-tailed t-test in c and h. Two-tailed paired t-test in e and j. *p < 0.05. For detailed statistics information, see Supplementary Table 1.
Extended Data Fig. 6 | No change in PA<sup>Ear1+MPN</sup> and PA<sup>Ear1+VMHvl</sup> Ca<sup>2+</sup> signal during non-social interaction and no change in fluorescence signal during social behaviors in GFP control animals, related to Fig. 4. a and c, Representative traces showing the GCaMP6 signal of PA<sup>Ear1+MPN</sup> (a) and PA<sup>Ear1+VMHvl</sup> (c) cells during object interaction. Gray shades mark sniffing object episodes. (b and d) PETHs of Ca<sup>2+</sup> signal (ΔF/F) of PA<sup>Ear1+MPN</sup> (b) and PA<sup>Ear1+VMHvl</sup> cells (d) aligned to sniffing object. n = 7 (PA<sup>Ear1+MPN</sup>) and 5 (PA<sup>Ear1+VMHvl</sup>) animals. e, A representative image showing GFP (green) expression in the PA<sup>Ear1+</sup> cells. Blue: DAPI. Scale bar: 200 µm. Yellow dashed lines indicate the optic fiber location. f, g, Representative Ca<sup>2+</sup> traces during interaction with a female (f) and a male intruder (g) introduced into the home cage of the recording mouse. Colored shades mark the behavioral episodes. h, The peak ΔF/F during various social behaviors of all animals. n = 3 animals. All data in h are presented as mean ± s.e.m. One-way ANOVA with post-hoc Tukey’s test. p > 0.05. i-q, PETHs of fluorescence signals aligned to intruder introduction (i, j), sniffing female (k), sniffing male (l), attempted mounting (m), shallow-thrust (n), deep-thrust (o), ejaculation (p), and attack (q). Gray and bold color lines indicate results from individual animals and the population average, respectively. Vertical dashed blue lines indicate time 0. For detailed statistics information, see Supplementary Table 1.
Extended Data Fig. 7 | hM4Di mediated PAsr1 cell inactivation impaired male mouse sexual behaviors. Related to Fig. 5. a, c, d. CNO injection prolonged the latencies to attempt mount (a), shallow thrust (c) and deep thrust (e) in PAsr1-hM4Di but not mCherry expressing animals. b, d, f. The durations of shallow thrust (d) and deep thrust (f) significantly decreased after CNO injection in test but not control groups. Note that each test lasts 10 min. For animals that did not show the relevant behaviors within the testing period, the latency will be set at 600 s. n = 5 animals in a-f. All data in a-f are presented as mean ± s.e.m. Two-way ANOVA with repeated measures followed by Bonferroni multiple comparison test. *p < 0.05; **p < 0.01. For detailed statistics information, see Supplementary Table 1.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | hM3Dq mediated activation of PA\textsuperscript{Esr1+MPN} and PA\textsuperscript{Esr1+VMHvl} promotes sexual and aggressive behaviors in naïve male mice in a CNO dose dependent manner, related to Fig. 6. a, Representative raster plots showing the behaviors towards male and female intruders after saline, 0.1 mg/kg CNO and 0.5 mg/kg CNO i.p. injection in PA\textsuperscript{Esr1+MPN} hM3Dq expressing animals. Scale bar: 60 s. b–g, Both 0.1 mg/kg and 0.5 mg/kg CNO shortened the latency to attempted mount, shallow thrust and increased the duration of attempted mount and shallow thrust while only 0.5 mg/kg promoted female-directed aggression. h–m, CNO injection into PA\textsuperscript{Esr1+MPN} hM3Dq expressing animals did not promote male-directed aggression regardless of the CNO concentration. n, Representative raster plots showing the behaviors towards male and female intruders after saline, 0.1 mg/kg CNO and 0.5 mg/kg CNO i.p. injection in PA\textsuperscript{Esr1+VMHvl} hM3Dq expressing animals. Scale bar: 60 s. o–z, 0.5 mg/kg CNO i.p. injection in PA\textsuperscript{Esr1+VMHvl} hM3Dq expressing animals caused a significant decrease in attack latency (s and y) and an increase in attack duration (t and z) towards both male and female intruders. Animals with 0.1 mg/kg CNO showed an increased trend of attack. Each test lasts 10 min. For animals that did not show the relevant behaviors within the testing period, the latency will be set at 600 s. n = 8 animals in b–m and o–z. All data in b–m and o–z are presented as mean ± s.e.m. Repeated measure One-way ANOVA with Geisser-Greenhouse’s correction followed by Tukey’s multiple comparison test in p; Friedman test followed by Dann’s multiple comparisons test in b–g, l–o and q–z. *p < 0.05; **p < 0.01. Brain atlas images in a are modified from ref. 50. For detailed statistics information, see Supplementary table 1.
Extended Data Fig. 9 | See next page for caption.
Optogenetic activation of PA^Esr1+VMHvl neurons promotes aggression while optogenetic activating PA^Esr1+MPN cells fails to cause behavioral change in naïve male mice, related to Fig. 6. a, Experimental schematics. Representative images showing the expressions of ChR2-EYFP (green) and DAPI (blue) in PA^Esr1+VMHvl (top) and PA^Esr1+MPN (bottom) cells. Scale bar: 200 μm. Dashed lines outline the PA. Solid white lines indicate the placement of optic fibers. c, Test schedule. d, e, Representative raster plots illustrating behaviors against various intruders during and between light stimulation. Scale bar: 60 s. Top showing light-on period in cyan. Bottom showing behaviors. f-j, Optogenetic activation of PA^Esr1+MPN cells did not cause any measurable change in sexual and aggressive behaviors towards any intruder. k-o, The percentage of trials that animals attacked (k), average latency to attack during each trial (l), and the average duration of attack per trial (m) towards male intruder, but not castrated male and female intruder, increased with light stimulation. n, o, Light stimulation did not change duration of sniffing (n) or mounting (o) towards any intruders. p-t, Animals expressing EYFP in PA^Esr1+VMHvl neurons showed no behavioral changes during light stimulation. n = 5 animals for each group. All data in f-t shown as mean ± s.e.m. Two-tailed paired t-test (i, k, l, m, n, s and t) and Wilcoxon matched-pairs signed rank test (g, h, j, l, o-t). *p < 0.05. For detailed statistics information, see Supplementary Table 1.
Extended Data Fig. 10 | A basic wiring diagram showing triple descending projections from the cerebral hemisphere to the behavior control column that drives social behaviors and general exploration, related to Figs. 1, 7. In parallel to the classical cortico-striato-nigral circuit that controls general exploration, PA/vSub (cortex equivalent), MeA (striatum equivalent) and BNST (pallidum equivalent) modulate the rostral part of the behavior control column (including MPN and VMHvl) through triple descending projections – a cortical excitatory projection, an striatal inhibitory projection and a pallidal disinhibitory projection. SNr: substantia nigra pars reticulate.
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Software and code

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Data collection

We listed all softwares used in the experiments and for analysis in the Methods section. We used a custom TDT program, OpenEx (Tucker-Davis Technologies) to collect the fiberphotometry signal and send a trigger signal to the pulse generator for optogenetics, and StreamPix5 (Norpix) for multiple camera recording. We used the customized Matlab code to track movement of mice. Epifluorescent images were captured with a virtual slide scanner (Olympus, VS120). Confocal images were captured with Zen program from LSM 510 or 700 (Zeiss). Differential interference contrast imaging to obtain the whole-cell recording (Siemenscope Pro 6000, Scientifica). Electrophysiological signals were recorded using an Axopatch 700B amplifier (Molecular Devices). The tissue samples for RNA-Seq were laser-dissected using a LMD06000 fluorescence microscope system (Leica microsystems). The cDNA libraries in RNA-Seq were sequenced with Illumina HiSeq 4000 using high output mode.

Data analysis

We listed all softwares used in the experiments and for analysis in the Methods section. We used customized code written in MATLAB 2013 to annotate behavior and to track the location of animals (https://github.com/pdollard/toolbox) and further analyze the time-locked photometry signal by a customized code. Attack events in a randomly selected set of videos were annotated using Deeplabcut and Simba (Mathis. et al., 2018 Nat. Neurosci., Nilson et al., 2020 bioRxiv). Epifluorescent and confocal microscopic imaging data were analyzed by ZEN2009 Light Edition and ImageJ.2P with custom settings. Whole-cell patch clamp data were analyzed with Igor Pro (Wavemetrics). Sequencing reads were aligned to the mouse genomes (build mm10/GRCm38) using the splice-aware STAR aligner and then the counts for each gene were generated using featureCounts. Differential gene expression analysis were performed using the limma R package with normalized gene reads through an open-source software DEP (http://ge-lab.org/dep/). All statistical analysis were performed using GraphPad Prism 7 or MATLAB 2018a. The custom codes will be available from corresponding authors upon reasonable request.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. Github). See the Nature Research guidelines for submitting code & software for further information.
No statistical methods were used to pre-determine sample sizes but our sample sizes in the data are similar with previous works in this field. The experiments were not done blindly in the study, since the experimental conditions (control vs experimental groups) were obvious to the experimenters and the analyses were carried out objectively by using a recording system and not subjective to human bias.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf.

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**

No statistical methods were used to pre-determine sample sizes but our sample sizes in the data are similar with previous works in this field (Hashikawa et al., 2017 Nat Neurosci., Fang et al., 2018 Neuron).

**Data exclusions**

For single retrograde tracing experiments, 3/4 and 3/6 animals that showed correct unilateral targeting in MPN and VMHvl, respectively, were included in the final analysis. For dual retrograde CTB tracing experiments, 3/5 animals with correct targeting in both MPN and VMHvl were included. For microdissection of distinct subregions in the PA, 3/5 animals with correct targeting in both MPN and VMHvl were included in the final analysis. For microdissection of distinct subregions in the PA with retrograde tracing, 2/2 and 2/4 animals that showed correct unilateral targeting in MPN and VMHvl, respectively, were included in the final analysis. For dual-retrograde tracing, 3/7 animals that showed correct unilateral targeting in both MPN and VMHvl were included in the final analysis. For investigating the outputs of PAEsr1+MPN and PAEsr1+VMHvl projectors, 3/5 animals that showed correct unilateral targeting in the MPN and correct virus expression in the PA and 3/5 animals that showed correct unilateral targeting in the VMHvl and correct unilateral virus expression in the PA were included in the final analysis. For investigating the inputs of PAEsr1+MPN and PAEsr1+VMHvl cells, 3/5 animals that showed correct unilateral targeting in the MPN and correct virus expression in the PA and 3/6 animals that showed correct unilateral targeting in the VMHvl and correct virus expression in the PA were included in the final analysis. For the slice recording experiment, 10/12 animals that showed correct unilateral targeting in MPN, VMHvl and PA were included in the final analysis. For fiber photometric recording of the PAEsr1+MPN (PAEsr1+VMHvl) population, 5/8 animals that showed correct unilateral targeting in the VMHvl and correct GCaMP6f expression and fiber placement in the PA were included in the final analysis. For pharmacogenetic inhibition of PAEsr1+ cells, 5/9 test animals that showed correct bilateral targeting of PA were included in the final analysis. For pharmacogenetic inhibition of PAEsr1+MPN or PAEsr1+VMHvl, 8/10 test animals that showed correct bilateral targeting in the MPN and correct bilateral virus expression in the PA were included in the final analysis. 8/10 test animals that showed correct bilateral targeting in the VMHvl and correct bilateral virus expression in the PA were included. For pharmacogenetic activation of PAEsr1+MPN or PAEsr1+VMHvl cells, 8/14 test animals that showed correct bilateral targeting in the VMHvl and correct bilateral virus expression in the PA were included in the final analysis. For pharmacogenetic activation of PAEsr1+MPN or PAEsr1+VMHvl cells, 8/14 test animals that showed correct bilateral targeting in the VMHvl and correct bilateral virus expression in the PA were included in the final analysis. For pharmacogenetic inhibition of PAEsr1+MPN or PAEsr1+VMHvl, 8/10 test animals that showed correct bilateral targeting in the VMHvl and correct bilateral virus expression in the PA were included in the final analysis. 5/16 test animals that showed correct bilateral targeting in the VMHvl, correct bilateral expression of ChR2-EYFP in the PA (> 100 positive cells in all sections) and bilateral fiber placements in the PA were included in the final analysis.

**Replication**

Experimental findings were reliably reproduced among all subjects in all experiments comprised of multiple cohorts. GCaMP6 recordings were conducted at least with 3 cohorts of animals. Pharmacogenetic experiments were conducted with 3 cohorts of animals. Optogenetic experiments were conducted with 2 cohorts of animals.

**Randomization**

For GCaMP6 recording and pharmacogenetic inactivation, animals were screened for both aggressive behaviors and sexual behaviors before the surgery and randomly assigned to experimental and control groups. For optogenetic and pharmacogenetic activation, experimental and control animals were randomly selected from naive (no prior sexual experience or tested for aggression) animals. Intruder animals and objects were subsequently introduced into a subject’s cage, after we confirmed the order of presentation didn’t affect neural responses.

Blinding

The experiments were not done blindly in the study, since the experimental conditions (control vs experimental groups) were obvious to experimenters and the analyses were carried out objectively by using a recording system and not subjective to human bias. During annotation and cell counting, the experimenter was blind to the GCaMP6 signal or behavioral responses.
Antibodies used

We listed all antibodies used with their catalog number in Methods section of "Immunohistochemistry". Primary antibodies: Rabbit anti-Esr1 (sc-542, Lot #F1715, Santa Cruz), Goat anti-cFos (sc-52-G, Santa Cruz), rabbit anti-GFP (A11122, Thermo Fisher) and Sheep anti-DIG (Sigma-Aldrich, 11207733910). Secondary antibodies: Donkey anti-rabbit Alexa 488/594 (R37118/R37119, Life Technologies) or Donkey anti-goat Alexa 488 (A11055, Life Technologies).

Validation

http://www.scbt.com/datasheet-52-c-fos-4-antibody.html
http://www.scbt.com/datasheet-542-eralpha-mc-20-antibody.html
https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&productsubtype=antibody_primary&productId=A-11122&version=65

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

We described the subjects in Methods section (Mice). Mice were housed under a 12 h light-dark cycle (10 p.m. to 10 a.m. light), with food and water available ad libitum. Animals used for CTB retrograde tracing and RNA-Seq experiments were 14-16 week old wild-type C57BL/6N male purchased from Charles River. Male Esr1-2A-Cre (14-38 weeks, Jackson Stock No. 017911), male Vgat-ires-Cre × Ai6 (12-16 weeks, Jackson stock No. 016962 and 007906), male Vglut1-ires-Cre × Ai6 (12-16 weeks, Jackson stock No. 023527 and 007906) were used for functional, recording and histological experiments. Stimulus animals were adult C57BL/6N female and male or BALB/c male mice (> 8 weeks) purchased from Charles River. Stimulus animals were group housed. After surgery, all test animals were singly housed. All the experiments were performed during the dark cycle of the animals.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All animal experiments were performed according to protocols approved by NYU IACUC protocols for the care and use of laboratory animals. We complied with all pertinent ethical regulations.

Note that full information on the approval of the study protocol must also be provided in the manuscript.