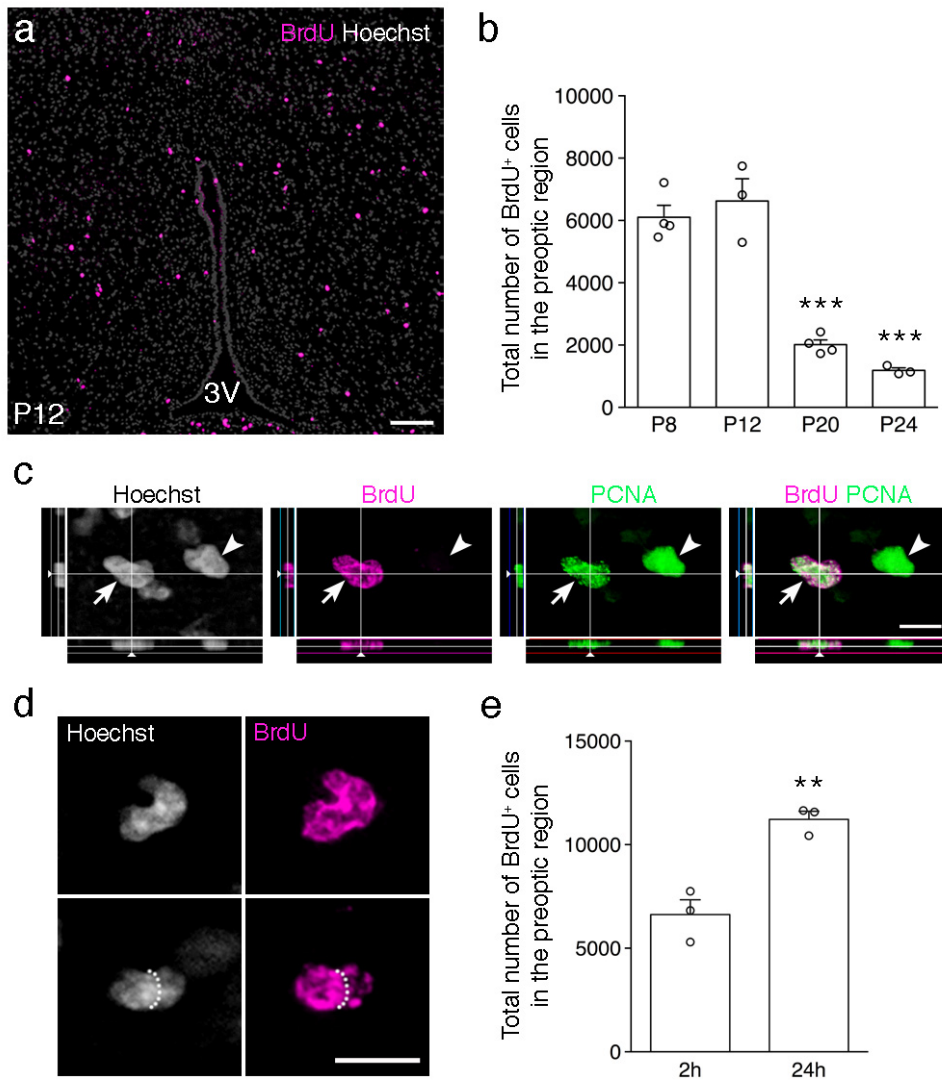


## SUPPLEMENTARY NOTE

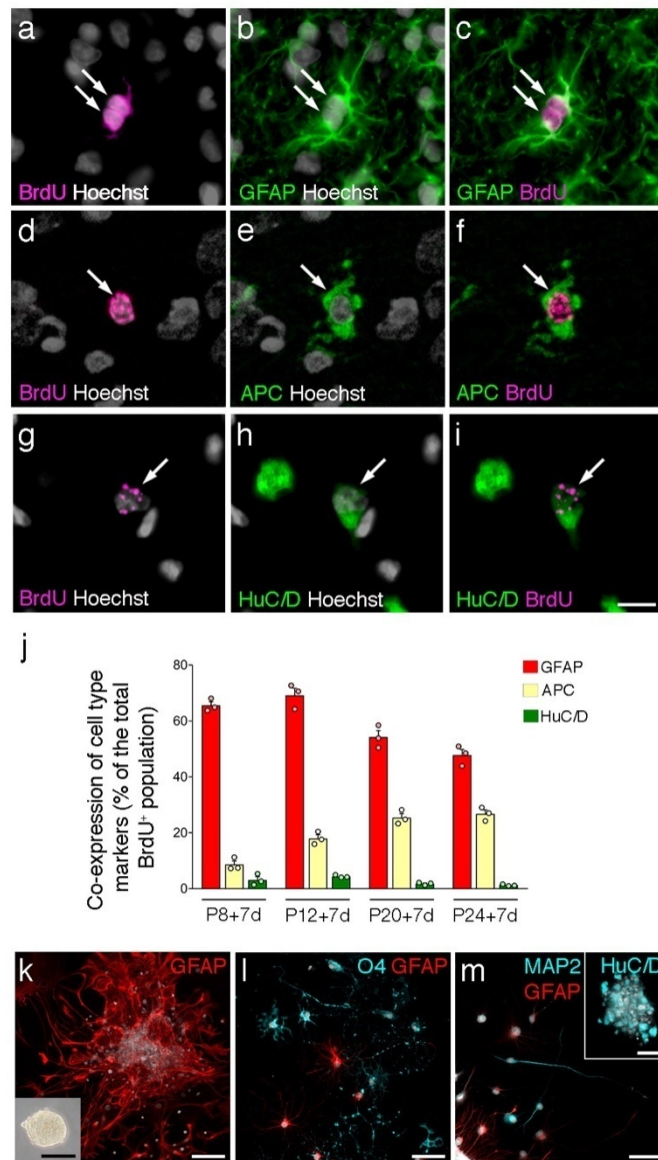
ErbB4 receptors are key regulators of both astrogenesis<sup>55</sup> and female sexual maturation<sup>37</sup>. Disruption of astrocytic ErbB4 signaling perturbs reproductive development and function due to decreased ability of astrocytes to stimulate GnRH neuronal activity<sup>28</sup>. We determined whether ErbB deficiency also alters the relationship between GnRH neurons and newborn astrocytes during postnatal development. In mice, as in rats, newborn cells were seen to be generated in close morphological association with GnRH neurons during the infantile period (**Supplementary Fig. 10a**) and to differentiate into astrocytes (**Supplementary Fig. 10b**), and this relationship persisted into adulthood (**Supplementary Fig. 10c**). The birth of new cells (**Supplementary Fig. 10d**) and their association with GnRH neurons (**Supplementary Fig. 10c**, compare white bars) was not affected by the disruption of ErbB4 signaling pathways in infancy. However, their association with GnRH neurons in adulthood was significantly reduced in ErbB4-deficient animals (**Supplementary Fig. 10c**), although the total number of BrdU<sup>+</sup> cells in the preoptic region remained unchanged between genotypes (**Supplementary Fig. 10e**), suggesting that astrocytic ErbB4 signaling is specifically required for the long-term maintenance of the association of infantile newborn cells with GnRH neurons in this species.

## SUPPLEMENTARY FIGURES



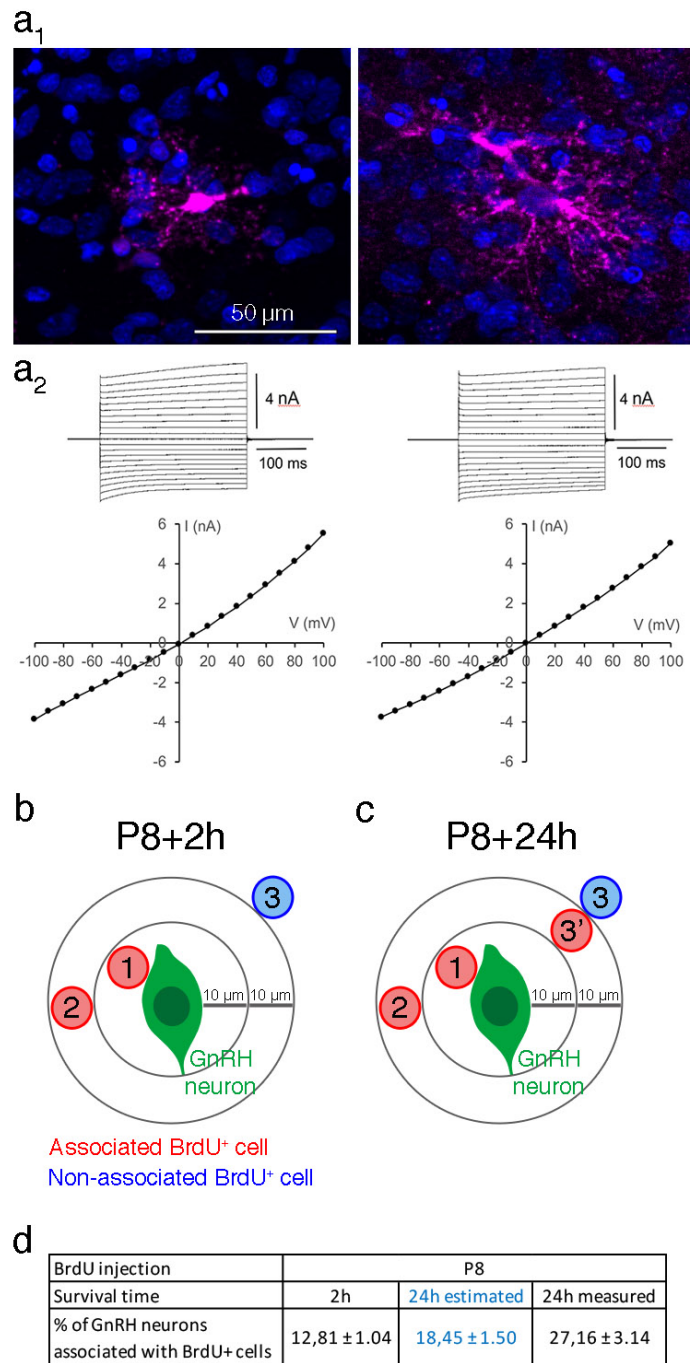
**Supplementary Figure 1. Proliferation in the preoptic region of infantile and juvenile female rats.** (a) Representative photomicrograph showing BrdU immunofluorescence (magenta) in the preoptic region of a rat injected with BrdU at P12 and sacrificed 2h later, illustrating quantification in **b**. 3V, third ventricle. Scale bar: 100  $\mu$ m. (b) Total number of BrdU<sup>+</sup> cells in the preoptic region of animals injected with BrdU at P8, P12, P20 or P24 and sacrificed 2h later (one-way ANOVA:  $F_{(3,10)} = 50.63$ ,  $P < 0.001$ ,  $n = 4, 3, 4$  and 3 rats per group; Tukey's multiple comparison test: P8 versus P20,  $q_{(10)} = 11.428$ ,  $P < 0.001$ ; P8 versus P24,  $q_{(10)} = 12.715$ ,  $P < 0.001$ ; P12 versus P20,  $q_{(10)} = 11.921$ ,  $P < 0.001$ ; P12 versus P24,  $q_{(10)} = 13.148$ ,  $P < 0.001$ ). (c) Two hours after injection, BrdU is seen in cells (magenta, arrow) expressing

PCNA, an endogenous cell proliferation marker (green). Note the presence of PCNA<sup>+</sup>/BrdU<sup>-</sup> cells (arrowhead) that correspond to proliferative cells that either have not reached or have already passed the S-phase during the 2h-exposure to BrdU. Scale bar: 10 μm. **(d)** Representative photomicrographs of BrdU immunolabeling (magenta) showing a mitotic figure and a pair of closely apposed cells (dotted line) twenty-four hours after BrdU injection. The experiment was performed in 17 animals. Scale bar: 10 μm. **(e)** Total number of BrdU<sup>+</sup> cells in the preoptic region of animals injected with BrdU at P12 and sacrificed 2h or 24h later (two-sided unpaired *t*-test,  $t_{(4)} = -5.638$ ,  $P = 0.005$ ;  $n = 3$  rats per group). **(a, c, d)** Sections were counterstained with the nuclear marker Hoechst (white). **(b, e)** Values shown are means ± s.e.m.; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .



**Supplementary Figure 2. The postnatal preoptic region contains multipotent progenitors.** (a-i) Representative double immunofluorescence labeling for BrdU (magenta) and GFAP (b, c, green), APC (e, f, green) or HuC/D (h, i, green) in the preoptic region of female rats injected with BrdU at P8 and sacrificed 7 days later, illustrating quantifications in j. Arrows point to co-labeled cells. Sections were counterstained with the nuclear marker Hoechst (white). Scale bar: 10  $\mu$ m. (j) Percentage of preoptic BrdU<sup>+</sup> cells that co-express GFAP, APC or HuC/D in animals injected with BrdU at P8, P12, P20 or P24 and sacrificed 7 days later ( $n = 3$  animals per group). Note that the proportion of BrdU<sup>+</sup> cells that co-express GFAP is higher during the infantile compared to the juvenile period (one-way ANOVA:  $F_{(3,8)} = 18.919$ ,  $P < 0.001$ ,  $n = 3$  rats per group; Tukey's multiple comparisons test: P8 versus P20,  $q_{(8)}$

= 5.046,  $P = 0.03$ ; P8 versus P24,  $q_{(8)} = 7.930$ ,  $P = 0.002$ ; P12 versus P20,  $q_{(8)} = 6.44$ ,  $P = 0.008$ ; P12 versus P24,  $q_{(8)} = 9.325$ ,  $P < 0.001$ , P8 versus P12,  $q_{(8)} = 1.395$ ,  $P = 0.761$ , P20 versus P24,  $q_{(8)} = 2.884$ ,  $P = 0.251$ ). Values shown are means  $\pm$  s.e.m. (**k-m**) *In vitro* isolation of multipotent progenitors from the postnatal preoptic region. Floating primary neurospheres form upon dissociation of the preoptic region and culture in the appropriate medium (inset in **k**, phase contrast photomicrograph). After 7 days in serum-containing medium, these cellular spheres adhere and differentiate into the three neural lineages, as attested by the expression of the astrocytic marker GFAP (**k-m**, red), the oligodendroglial marker O4 (**l**, turquoise) and the neuronal markers MAP2 (**m**, turquoise) and HuC/D (inset in **m**, turquoise) ( $n = 4$  cultures). Scale bars: 200  $\mu\text{m}$  (inset in **k**), 100  $\mu\text{m}$  (**k** main panel and inset in **m**), 50  $\mu\text{m}$  (**l** and **m** main panel). Cell nuclei were stained with Hoechst (white).

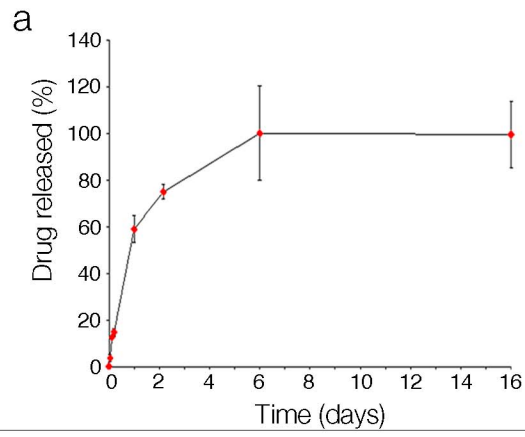


**Supplementary Figure 3. Relative contribution of BrdU<sup>+</sup> cell proliferation to the increased association between GnRH neurons and BrdU<sup>+</sup> cells 24h after BrdU injection.**

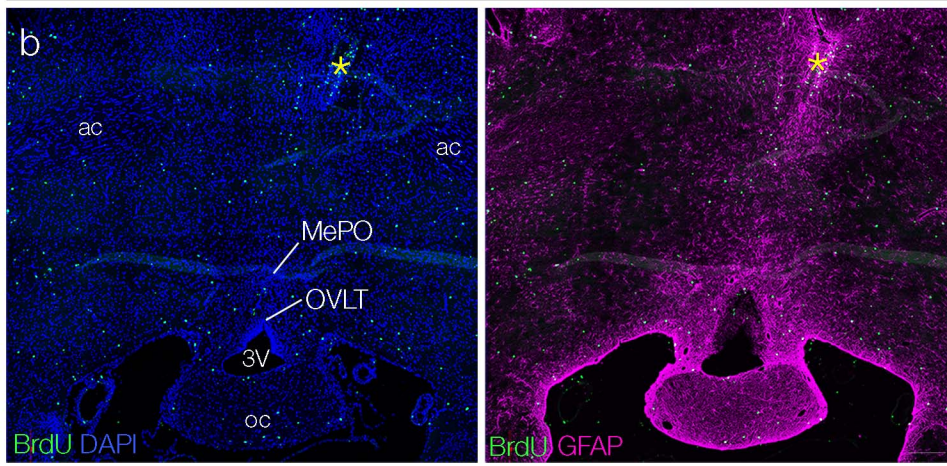
(a) Estimation of the radius of infantile astrocytes in the preoptic region of P11-P12 female rats using biocytin filling of patch-clamped astrocytes. (a<sub>1</sub>) Images showing two astrocytes filled with biocytin (magenta). Nuclei were stained with DAPI (blue). (a<sub>2</sub>) Whole-cell current (top) and

current-voltage relationship (bottom) of each recorded astrocyte. **(b)** Schematic representation of the association criterion used in the present study. Cells 1 and 2 are considered associated with the GnRH neuron since they are located at a distance  $\leq 10 \mu\text{m}$  from the GnRH neuronal cell body with no interposed cell nucleus in between. Cell 3 is not considered to be associated. **(c)** 24h after the BrdU injection, most BrdU<sup>+</sup> cells divide (**Supplementary Fig. 1e**) and give birth to a daughter cell. In this example, the daughter cell 3' born after the division of cell 3 is found in the association zone of the GnRH neuron. **(d)** By analyzing the environment of GnRH neurons 2h after a single injection of BrdU at P8 ( $n = 137$  GnRH neurons analyzed,  $N = 3$  rats), we determined the proportion of GnRH neurons that would be considered associated if a distant BrdU<sup>+</sup> cell (such as cell 3) gave birth to a closer daughter cell (such as cell 3'). Twenty-four hours after BrdU injection, the total number of BrdU<sup>+</sup> cells in the preoptic region increases 1.7-fold (**Fig. 6c and Supplementary Fig. 1e**), and numerous mitotic figures and pairs of closely apposed cells are observed (**Supplementary Fig. 1d**), indicating that the duration of the cell cycle is  $\sim 24\text{h}$ . Assuming that all BrdU<sup>+</sup> cells divide within 24h and that one daughter cell always forms closer to the GnRH neuron, the estimated proportion of GnRH neurons with associated BrdU<sup>+</sup> cells at P8+24h (24h estimated) is lower than the proportion actually measured (24h measured) (two-sided unpaired  $t$ -test,  $t_{(11)} = 2.817$ ,  $P = 0.017$ ;  $n = 8$  and 5 rats per group), indicating that proliferation alone cannot fully account for the increase in the association between GnRH neurons and BrdU<sup>+</sup> cells between P8+2h and P8+24h. Values shown are means  $\pm$  s.e.m.

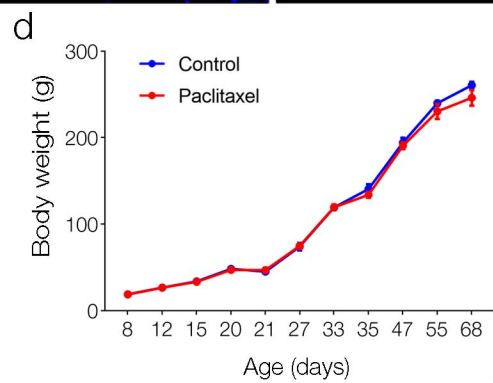
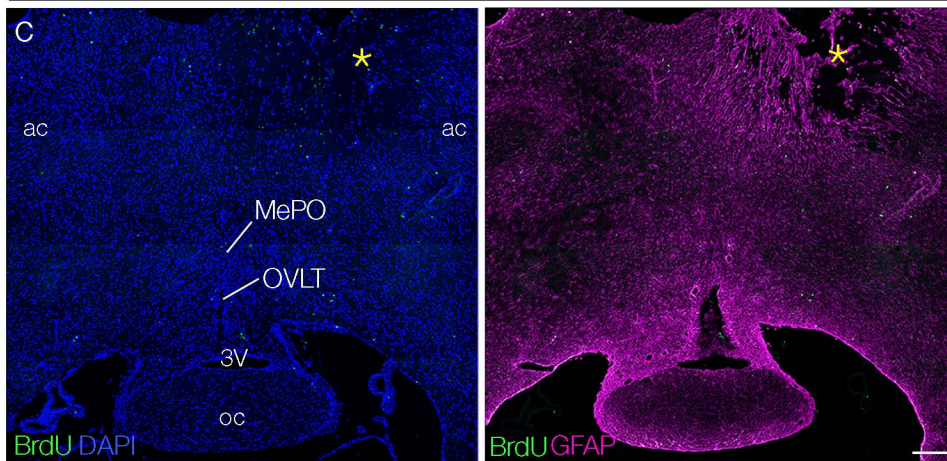




Control

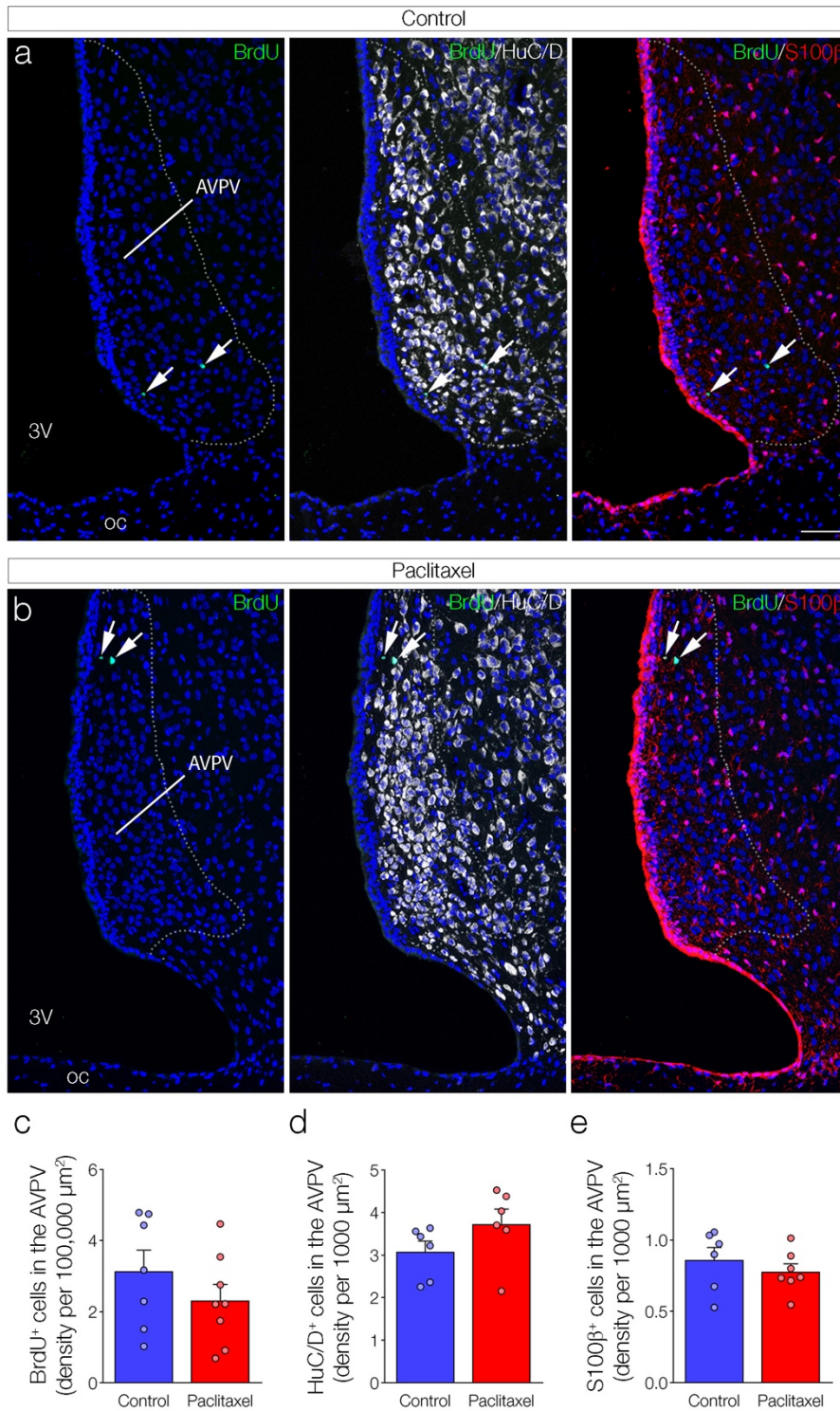


Paclitaxel



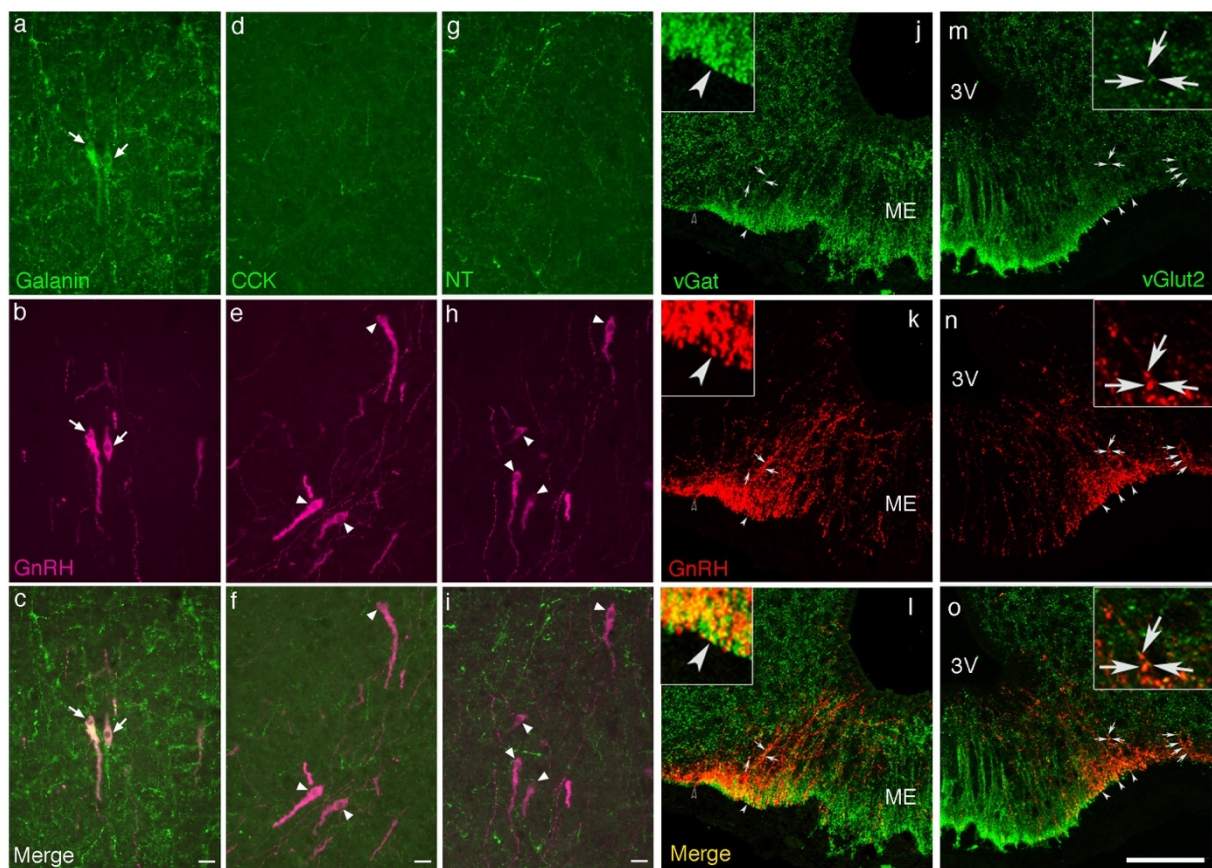


**Supplementary Figure 4. Stereotaxic injection of paclitaxel into the preoptic region of infantile female rats does not affect body weight.** (a) *In vitro* release of paclitaxel from PLGA-based microparticles (initial loading = 40% w/w). Paclitaxel release is complete by 6 days ( $n = 3$  independent experiments). (b,c) Representative coronal sections of the preoptic region of adult animals injected with blank- (b) or paclitaxel-loaded microparticles (c) immunolabeled for BrdU (green), and GFAP (magenta). Nuclei were stained with DAPI (blue). The yellow asterisk indicates the injection site. The staining was performed in 17 control and 18 paclitaxel-treated animals. 3V, third ventricle; ac, anterior commissure; MePO, median preoptic nucleus, oc, optic chiasm; OVLT, organum vasculosum laminae terminalis. Scale bar: 200  $\mu\text{m}$ . (d) Body weight of female rats injected with blank- (control) or paclitaxel-loaded microparticles (two-way repeated-measures ANOVA, treatment:  $F_{(1, 272)} = 3.86$ ,  $P = 0.0505$ ; age:  $F_{(10, 272)} = 1081$ ,  $P < 0.0001$ ; interaction:  $F_{(10, 272)} = 0.8569$ ,  $P = 0.5743$ ; subject matching:  $F_{(7,70)} = 1.070$ ,  $P = 0.3921$ ,  $n = 8$  to 17 rats per age). (a, d) Values shown are means  $\pm$  s.e.m.



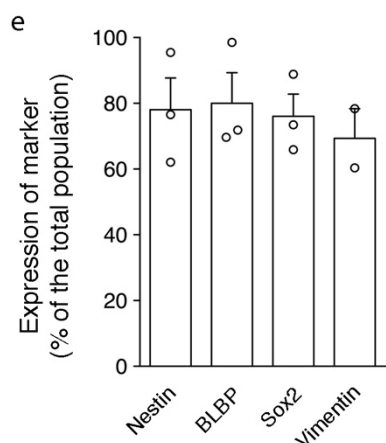
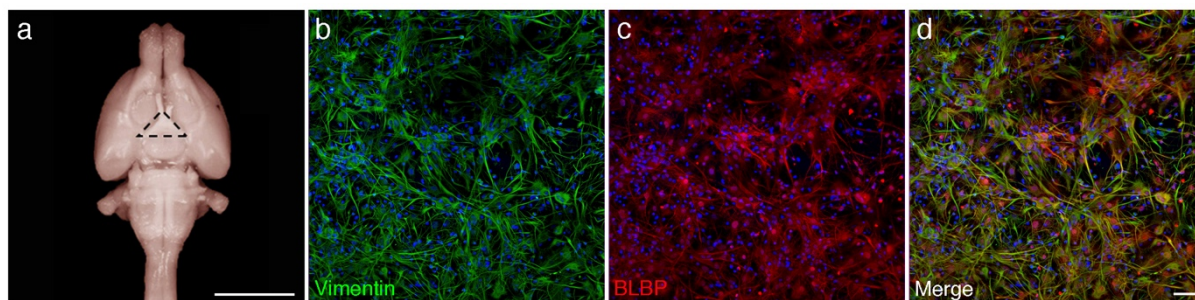
**Supplementary Figure 5. Stereotaxic injection of paclitaxel into the preoptic region of infantile female rats does not affect AVPV progenitors, neurons and astrocytes.**(a,b) Representative coronal sections of the preoptic region at the level of the anteroventral periventricular nucleus (AVPV) immunolabeled for BrdU (green), HuC/D (white) and

S100 $\beta$ (red), illustrating quantifications in **c-e**. The arrows point to BrdU<sup>+</sup> cells. Sections were counterstained with the nuclear marker Hoechst (blue). 3V, third ventricle; oc, optic chiasm. Scale bar: 100  $\mu$ m. (**c-e**) Density of BrdU<sup>+</sup> cells (**c**: two-sided unpaired *t*-test,  $t_{(13)} = 1.117$ ,  $P = 0.2841$ ;  $n = 7$  and 8 rats per group), HuC/D<sup>+</sup> neurons (**d**: two-sided unpaired *t*-test,  $t_{(10)} = 1.523$ ,  $P = 0.1587$ ;  $n = 6$  rats per group) and S100 $\beta$ <sup>+</sup> astrocytes (**e**: two-sided unpaired *t*-test,  $t_{(11)} = 0.8358$ ,  $P = 0.4210$ ;  $n = 6$  and 7 rats per group) in the AVPV of adult female rats after a single injection of BrdU at P8 (see **Figure 2a**). Values shown are means  $\pm$  s.e.m.

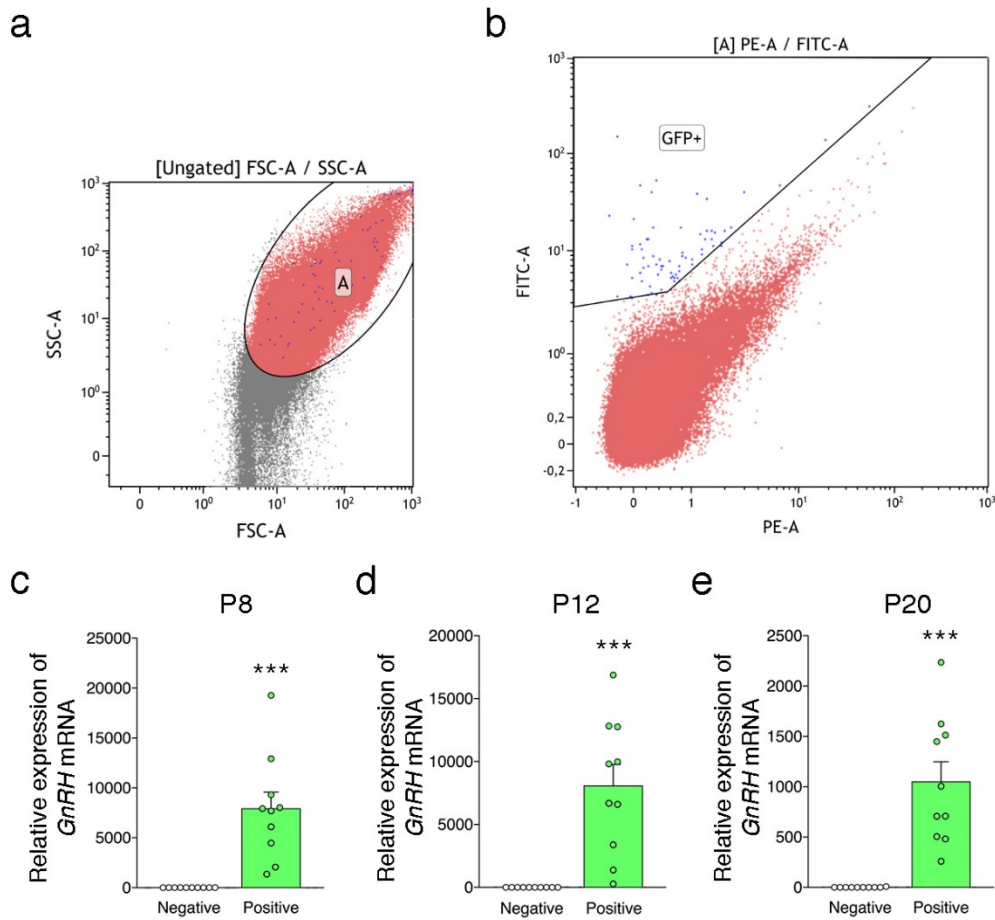


**Supplementary Figure 6. GnRH neurons express galanin, glutamate and GABA during the infantile period.** Double immunofluorescence labeling for GnRH (second and third horizontal rows, magenta in **b,e,h,c,f,i** and red ink,**l,n,o**) and galanin (**a,c**, green), cholecystokinin (CCK, **d,f**, green), neurotensin (NT, **g,i**, green), vGat (**j, l**, green) or vGluT2 (**m,o**, green) in the hypothalamus of infantile female rats. GnRH neuronal cell bodies express galanin (**a-c**, arrows) but not CCK (**d-f**, arrowheads) or NT (**g-i**, arrowheads). In the median eminence (ME), some GnRH neuronal axons (arrows) and terminals (arrowheads) express vGat (**j-l**) or vGluT2 (**m-o**), indicating their ability to produce GABA or glutamate, respectively. Experiments were performed in P8 (vGluT2 and vGat immunolabeling,  $n = 3$ ) or P12 female rats (galanin, CCK and NT immunolabeling,  $n = 2$ ). 3V, third ventricle. Scale bars: 20  $\mu\text{m}$  (**a-i**) and 50  $\mu\text{m}$  (**j-o**; 150  $\mu\text{m}$  in insets).

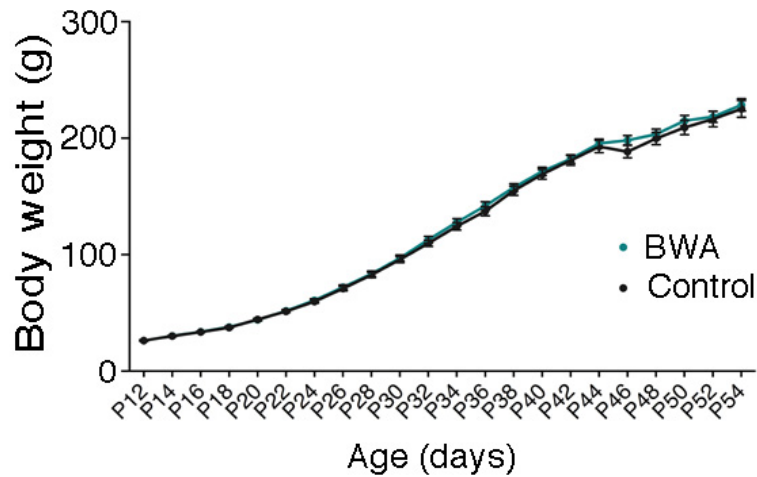




**Supplementary Figure 7. Characterization of primary cultures of progenitors from the rat preoptic region.** (a) Photomicrograph of the ventral part of a rat brain showing the microdissected region (dotted triangle). Scale bar: 1 cm. (b-d) Immunofluorescence labeling for vimentin (b) and BLBP (c) in one progenitor culture representative of three independent cultures. Cell nuclei were stained with Hoechst (blue). (d) merge of (b) and (c). Scale bar: 50  $\mu$ m. (e) Percentage of cells expressing nestin, BLBP, Sox2 and vimentin in progenitor cultures. Values shown are means  $\pm$  s.e.m.  $n = 3$  independent cultures.

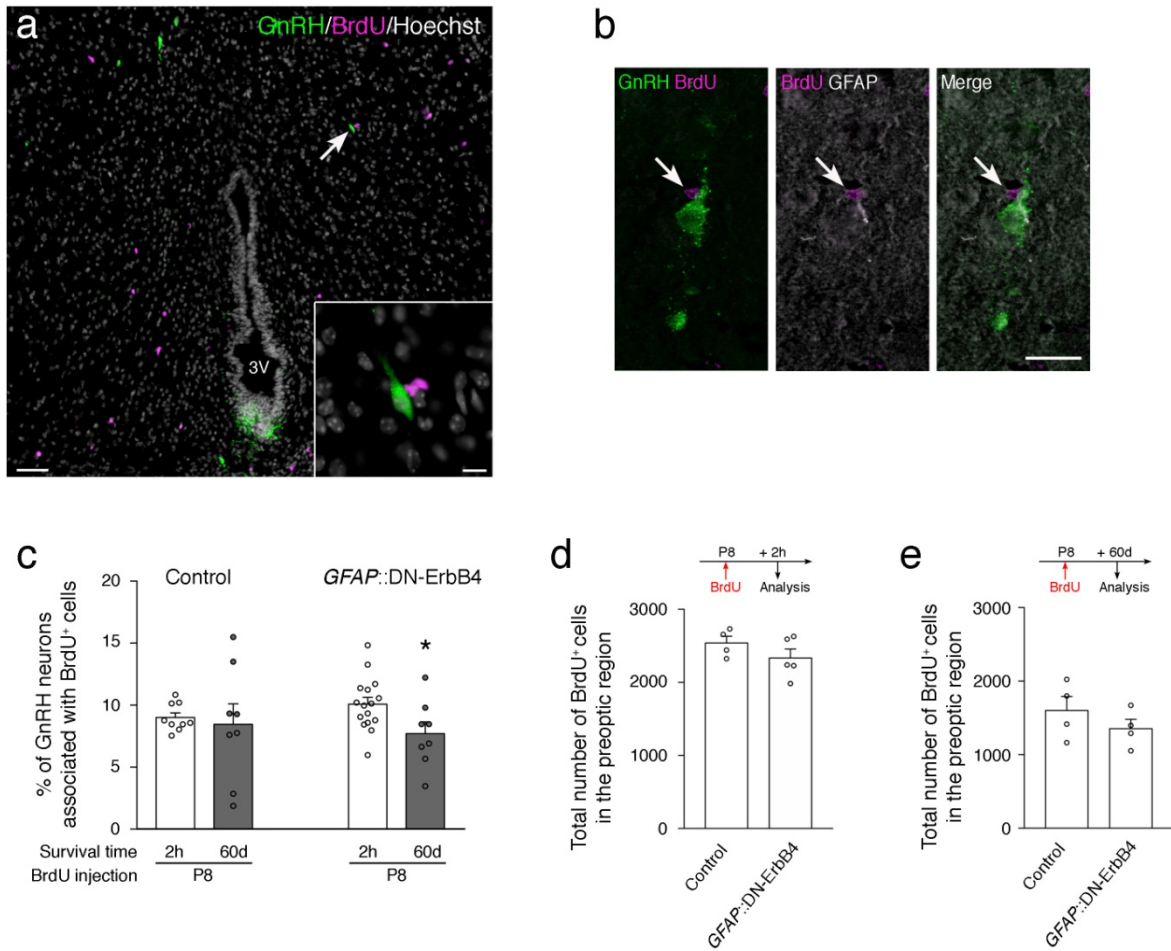


**Supplementary Figure 8. Isolation of hypothalamic GnRH neurons from GnRH-EGFP rats.** (a, b) Gating strategy for GnRH-EGFP neuron isolation by FACS used for experiments shown in **Fig. 5b-c** and **Fig. 7a,c**. (c-e) Real-time PCR analysis of the expression of *Gnrh* mRNA in EGFP-positive- or negative cells at different postnatal ages (P8, Mann-Whitney test:  $U = 100$ ,  $P < 0.001$ ,  $n = 10$  rats per group; P12, Mann-Whitney test:  $U = 100$ ,  $P < 0.001$ ,  $n = 10$  rats per group; P20, Mann-Whitney test:  $U = 100$ ,  $P < 0.001$ ,  $n = 10$  rats per group). Values are expressed relative to control values, set at 1. Values shown are means  $\pm$  s.e.m.; \*\*\*  $P < 0.001$ .



**Supplementary Figure 9. Inhibition of DP1 signaling in the preoptic region of infantile female rats does not affect body weight.** Body weight of animals treated at P8 with the DP1 antagonist BWA868C (BWA) via a stainless steel cannula stereotaxically implanted in the preoptic region (two-way repeated-measures ANOVA, treatment:  $F_{(1,17)} = 0.3324$ ,  $P = 0.5718$ ; time:  $F_{(21,357)} = 2236$ ,  $P < 0.0001$ ; interaction:  $F_{(21,357)} = 0.6202$ ,  $P = 0.9040$ ; subject matching:  $F_{(17,357)} = 38.12$ ,  $P < 0.0001$ ,  $n = 9$  and  $10$  rats per group). Values shown are means  $\pm$  s.e.m.





**Supplementary Figure 10. Mice deficient in astroglial ErbB4 signaling show reduced morphological association between GnRH neurons and astrocytes born during the infantile period.** (a) Representative coronal section of the preoptic region of a P8 wild-type mouse immunolabeled for GnRH (green) and BrdU (magenta), illustrating quantifications performed in c. Inset, higher magnification view of the GnRH neuron indicated by the arrow in the main panel, showing the physical proximity with a BrdU<sup>+</sup> cell. Sections were counterstained with the nuclear marker Hoechst (blue). 3V, third ventricle. Scale bars: 50  $\mu$ m in main panel, 10  $\mu$ m in inset. (b) Triple immunofluorescence labeling for GnRH (green), BrdU (magenta) and GFAP (white) in the preoptic region of a female wild-type mouse injected with BrdU at P8 and sacrificed 7 days later, showing a BrdU/GFAP double-labeled cell associated with a GnRH neuron (arrow). The experiment was performed in 4 animals. Scale bar: 10  $\mu$ m. (c) Proportion of GnRH neurons associated with BrdU<sup>+</sup> cells 2h or 60 d after a single injection of BrdU at P8 in wild-type (control) and transgenic mice expressing a dominant-negative form of ErbB4

driven by the GFAP promoter (*Gfap*::DN-ErbB4) (Control, Mann-Whitney test:  $U = 40$ ,  $P = 0.736$ ,  $n = 9$  and 8 mice per group; *GFAP*::DN-ErbB4, two-sided unpaired  $t$ -test:  $t_{(22)} = 2.372$ ,  $P = 0.027$ ;  $n = 16$  and 8 mice per group). (**d**, **e**) Total number of BrdU<sup>+</sup> cells in the preoptic region of wild-type (control) and *Gfap*::DN-ErbB4 transgenic mice injected with BrdU at P8 and analyzed 2h later (**d**: two-sided unpaired  $t$ -test:  $t_{(7)} = 1.266$ ,  $P = 0.246$ ;  $n = 4$  and 5 mice per group) or 60d later (**e**: two-sided unpaired  $t$ -test:  $t_{(6)} = 1.069$ ,  $P = 0.326$ ;  $n = 4$  mice per group). (**c-e**) Values shown are means  $\pm$  s.e.m.; \*  $P < 0.05$ .

## SUPPLEMENTARY TABLES

<b>Antigen</b>	<b>Host &amp; Clonality</b>	<b>Dilution</b>	<b>Clone, Source, Reference, RRID number</b>
APC	mouse monoclonal	1:100	clone CC-1, Millipore Cat# OP80, RRID:AB_2057371
BLBP	rabbit polyclonal	1:500	Millipore Cat# ABN14, RRID:AB_10000325
BrdU	rat monoclonal	1:500	clone BU1/75 (ICR1), Bio-Rad Cat# OBT0030, RRID:AB_609568
BrdU	rat monoclonal	1:500	clone BU1/75 (ICR1), Abcam Cat# ab6326, RRID:AB_305426
CCK	rabbit polyclonal	1:2000	antiserum coded IS-15/8, Philippe Ciofi
Galanin	rabbit polyclonal	1:3000	antiserum coded IS-42, Philippe Ciofi
GFAP	rabbit polyclonal	1:1000	Agilent Cat# Z0334, RRID:AB_10013382
GFAP	mouse monoclonal	1:500	clone G-A-5, Sigma-Aldrich Cat# G3893, RRID:AB_477010
GnRH	rabbit polyclonal	1:3000	antiserum coded IS-83L, Philippe Ciofi
GnRH	guinea pig polyclonal	1:2000	antiserum coded IS-8, Philippe Ciofi
HuC/D	mouse monoclonal	1:500	clone 16A11, Molecular Probes Cat# A-21271, RRID:AB_221448
Ki67	rabbit polyclonal	1:2000	Abcam Cat# ab15580, RRID:AB_443209
MAP2	mouse monoclonal	1:500	clone HM-2, Sigma-Aldrich Cat# M4403, RRID:AB_477193
nestin	mouse monoclonal	1:300	clone rat-401, Millipore Cat# MAB353, RRID:AB_94911
NT	rabbit polyclonal	1:3000	antiserum coded IS-2/7, Philippe Ciofi
O4	mouse monoclonal	1:300	clone # O4, R and D Systems Cat# MAB1326, RRID:AB_357617
Olig2	goat polyclonal	1:100	R and D Systems Cat# AF2418, RRID:AB_2157554
PCNA	mouse monoclonal	1:10,000	clone PC10, Santa Cruz Biotechnology Cat# sc-56, RRID:AB_628110
S100	rabbit polyclonal	ready-to-use	IR504, Dako
Sox2	goat polyclonal	1:500	Santa Cruz Biotechnology Cat# sc-17320, RRID:AB_2286684
vGaT	guinea pig polyclonal	1:750	Synaptic Systems Cat# 131 004, RRID:AB_887873
vGluT2	guinea pig polyclonal	1:750	Synaptic Systems Cat# 135 404, RRID:AB_887884
Vimentin	chicken polyclonal	1:2000	Millipore Cat# AB5733, RRID:AB_11212377

**Supplementary Table 1.** List of primary antibodies.

<b>Species</b>	<b>Conjugated</b>	<b>Dilution</b>	<b>Source, Reference, RRID number</b>
Donkey anti-chicken IgG	Alexa 488	1:500	Jackson ImmunoResearch Labs Cat# 703-545-155, RRID:AB_2340375
Donkey anti-guinea pig IgG	Alexa 488	1:500	Jackson ImmunoResearch Labs Cat# 706-545-148, RRID:AB_2340472
Donkey anti-goat IgG	Alexa 647	1:500	Molecular Probes Cat# A-21447, RRID:AB_141844
Donkey anti-mouse IgG	Alexa 488	1:500	Molecular Probes Cat# A-21202, RRID:AB_141607
Donkey anti-mouse IgG	Alexa 647	1:500	Molecular Probes Cat# A-31571, RRID:AB_162542
Goat anti-mouse IgM	Fluorescein	1:500	Vector Laboratories Cat# FI-2020, RRID:AB_2336185
Donkey anti-rabbit IgG	Alexa 488	1:500	Molecular Probes Cat# A-21206, RRID:AB_2535792
Donkey anti-rabbit IgG	Alexa 568	1:500	Thermo Fisher Scientific Cat# A10042, RRID:AB_2534017
Donkey anti-rat IgG	Alexa 488	1:500	Molecular Probes Cat# A-21208, RRID:AB_141709
Donkey anti-rat IgG	Alexa 568	1:500	Thermo Fisher Scientific Cat# A-21209, RRID:AB_2535795

**Supplementary Table 2.** List of secondary antibodies.

<b>Gene</b>	<b>Probe ID</b>
Actb	Rn00667869_m1
Cebpb	Rn00824635_s1
Dicer1	Rn01518055_m1
Gnrh1	Rn00562754_m1
Gpr44 (prostaglandin D2 receptor 2 or DP2)	Rn02349421_s1
Kiss1r (Kisspeptin 1 receptor or Gpr54)	Rn00576940_m1
Otx2	Rn01414598_m1
Pla2g4a	Rn00591916_m1
Ptgdr1 (prostaglandin D2 receptor or DP1)	Rn00824628_m1
Ptgds	Rn00564605_m1
Ptger1 (prostaglandin E2 receptor 1 or EP1)	Rn00565349_m1
Ptger2 (prostaglandin E receptor 2 or EP2)	Rn00579419_m1
Ptger3 (prostaglandin E2 receptor 3 or EP3)	Rn01636929_m1
Ptger4 (prostaglandin E receptor 4 or EP4)	Rn00583420_m1
Ptges (prostaglandin E synthase or Pges)	Rn00572047_m1
Ptgs-1 (prostaglandin-endoperoxide synthase 1 or Cox-1)	Rn00566881_m1
Ptgs-2 (prostaglandin-endoperoxide synthase 2 or Cox-2)	Rn01483828_m1
Rn45s	Rn03928990_g1
Zeb1	Rn01538408_m1

**Supplementary Table 3.** List of TaqMan primers.