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4 **GnRH neurons recruit astrocytes in infancy to facilitate network**
5 **integration and sexual maturation**

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50 **ABSTRACT**

51 Neurons producing gonadotropin-releasing hormone (GnRH), which control fertility, complete
52 their nose-to-brain migration by birth. However, their function depends on integration within a
53 complex neuroglial network during postnatal development. Here, we show that GnRH
54 neurons during the infantile period use a prostaglandin-D2/DP1-receptor signaling
55 mechanism to recruit newborn astrocytes that “escort” them into adulthood, and the
56 impairment of postnatal hypothalamic gliogenesis dramatically alters sexual maturation by
57 preventing this recruitment, a process mimicked by the endocrine disruptor bisphenol-A. DP1
58 signaling inhibition in the infantile preoptic region, where GnRH cell bodies reside, disrupts
59 the correct wiring and firing of GnRH neurons, alters minipuberty or the first activation of the
60 hypothalamic-pituitary-gonadal axis during infancy, and delays the timely acquisition of
61 reproductive capacity. These findings uncover a previously unknown neuron-to-neural-
62 progenitor communication pathway and demonstrate that postnatal astrogenesis is a basic
63 component of a complex set of mechanisms used by the neuroendocrine brain to control
64 sexual maturation.

65

66 INTRODUCTION

67 Neuroendocrine GnRH neurons, which ensure species survival by controlling the
68 ability of individuals to reproduce, are principally located in the preoptic region, a basal
69 forebrain region harboring diverse neuronal populations¹. Unlike other hypothalamic neurons
70 driving bodily functions, GnRH neurons are not born in the brain but originate in the olfactory
71 placode, and migrate from the nose to the brain during embryogenesis². Although this
72 migration is complete at birth, GnRH neurons recruit other neuronal populations, notably
73 those expressing kisspeptin and neuronal nitric oxide synthase (nNOS), during postnatal
74 development to establish a complex circuit that fine-tunes GnRH production and release by
75 providing precisely coordinated excitatory and inhibitory inputs^{3, 4}, thus orchestrating sexual
76 maturation, puberty and adult fertility⁵. However, the mechanisms by which GnRH neurons
77 sculpt their own microenvironment and establish the connections necessary to control
78 reproduction remain unexplored.

79 The GnRH network is not exclusively neuronal but includes at least two glial cell
80 types: tanycytes, which control the access of GnRH neurosecretory terminals to the
81 pericapillary space of pituitary portal vessels, into which the hormone is released, and
82 astrocytes, whose communication with GnRH neurons is essential for reproductive function⁶.
83 Glial cells, especially astrocytes, also help generate physiological responses by forming a
84 hub with neurons that integrates internal and external cues within the adult brain⁶.
85 Additionally, they act as cartographers of synaptogenesis and neural circuit formation in the
86 developing central nervous system^{7, 8}. Conversely, studies in the neocortex suggest that
87 astrocyte formation is regulated by extrinsic signals generated by neurons, **the development**
88 **of which** precedes gliogenesis⁹. We therefore asked whether GnRH neurons could
89 themselves be responsible for the circuit formation subtending reproductive function in
90 adulthood by establishing a glial entourage during postnatal development.

91 Here, we demonstrate that GnRH neurons in the infantile period attract newborn
92 progenitor cells that differentiate into astrocytes and remain closely associated with them,
93 essentially “escorting” these neurons into adulthood. Additionally, local inhibition of infantile

94 gliogenesis in the preoptic region, which harbors GnRH neuronal somata, delays puberty.
95 The interaction between GnRH neurons and newborn astrocytes underlying correct sexual
96 maturation depends on reciprocal prostaglandin-mediated signaling, and especially the
97 expression of the prostaglandin D2 receptor DP1 by these newborn cells. Our findings thus
98 identify a novel neuron-to-neural-progenitor communication mechanism that unfolds during
99 the infantile period and times the pubertal activation of the reproductive axis.

100

101 **RESULTS**

102 **GnRH neurons assemble a glial entourage during early infancy**

103 GnRH neuronal somata in rats are loosely distributed between the medial
104 septum/diagonal bands of Broca and the preoptic nuclei, being densest in the median
105 preoptic nucleus (MePO) at the level of the organum vasculosum laminae terminalis (OVLT).
106 To determine if the birth of new cells in the vicinity of GnRH neurons shapes their
107 microenvironment, we used the thymidine analog, bromodeoxyuridine (BrdU) to map cell
108 proliferation in the OVLT and its surroundings, referred to hereafter as the “preoptic region”,
109 in female rats at different stages of postnatal development [postnatal day (P)1 and P5:
110 neonatal period; P8 and P12: infantile period; P20 and P24: juvenile period, i.e. after
111 weaning] (**Fig. 1a**). In agreement with a previous report¹⁰, more cells incorporated BrdU in
112 the infantile versus the juvenile period (**Supplementary Fig. 1a,b**). BrdU colocalization with
113 proliferating-cell nuclear antigen (PCNA), an endogenous cell-cycle marker (**Supplementary**
114 **Fig. 1c**), the occurrence of pairs of closely apposed BrdU⁺ nuclei (**inset in Fig. 1b**,
115 **Supplementary Fig. 1d**), and doubling of the BrdU⁺ cell population between 2h and 24h
116 after BrdU injection (**Supplementary Fig. 1e**) strongly suggested that BrdU-labeled cells
117 were entering the cell cycle. Seven days after BrdU injection, most newborn cells expressed
118 either glial fibrillary acidic protein (GFAP) in conjunction with an astrocyte-like morphology,
119 denoting their differentiation into astrocytes, or adenomatosis polyposis coli (APC) – a
120 marker of oligodendrocytes, while a very small number expressed the neuronal marker,
121 HuC/D (**Supplementary Fig. 2a-j**). The presence of multipotent progenitors in the postnatal

122 preoptic region was confirmed *in vitro* by culturing clonally-derived neurospheres capable of
123 differentiating into all three neural cell types (**Supplementary Fig. 2k-m**).

124 Interestingly, co-immunolabeling revealed the close proximity of some GnRH
125 neuronal cell bodies to BrdU⁺ newborn cells (**Fig. 1b**). Biocytin filling of patch-clamped
126 astrocytes in living brain slices from infantile rats showed that preoptic astrocytes had a
127 radius of 20-40 μm (**Supplementary Fig. 3a**). We therefore decided to look for newborn
128 progenitors, which presumably have smaller arborizations, at a conservative distance of 10
129 μm from GnRH neurons. Two hours after BrdU injection, GnRH neurons accompanied by
130 BrdU⁺ progenitors were more frequent in neonatal and infantile rats than in juvenile animals
131 (**Fig. 1c**). GnRH neurons preferentially associated with proliferating cells, since 12.8% of
132 GnRH neurons were juxtaposed to BrdU⁺ cells 2h after BrdU injection at P8, versus only
133 3.6% of non-GnRH neurons (**Fig. 1c,d**). At this time-point, these proximal BrdU⁺ cells
134 expressed the progenitor cell marker, Sox2, but not GFAP (**Fig. 1e-g**). Twenty-four hours
135 later, 27.2% of GnRH neurons were associated with BrdU⁺ cells labelled by a single injection
136 at P8 (**Fig. 1c**). This increased association persisted even after seven days, when 22.3% of
137 GnRH neurons had a companion BrdU⁺ cell (**Fig. 1c,d**). Notably, this proportion reached
138 $80.3 \pm 2.7\%$ ($n = 4$ rats) when the BrdU regimen was increased to three injections per day
139 from P6 to P8 (**Fig. 1h**). Because the number of GnRH neurons in the preoptic region
140 remained unchanged during postnatal development (367.2 ± 43.5 neurons at P8 versus 342.9
141 ± 22.3 neurons at P15, two-sided unpaired t -test, $t_{(14)} = 0.498$, $P = 0.626$; $n = 8$ rats per
142 group), the increased proportion of GnRH neurons associated with BrdU⁺ cells could either
143 stem from a global increase in the number of BrdU⁺ cells in the preoptic region after
144 proliferation (**Supplementary Fig. 1e**) or the migration of BrdU⁺ cells towards GnRH
145 neurons. We analyzed the neighborhood of GnRH neurons 2h after a single BrdU injection at
146 P8, and identified all neurons that would be considered associated with a BrdU⁺ cell 24h later
147 if the proliferation of existing BrdU⁺ cells were to give rise to a daughter cell lying within 10μm
148 of the GnRH neuronal soma (**Supplementary Fig. 3b,c**). However, even under the very

149 broad assumption that every proliferative event would give rise to a newborn cell within the
150 perimeter described, the resulting theoretical percentage of GnRH neurons with associated
151 BrdU⁺ cells was still significantly lower than the actual percentage measured
152 (**Supplementary Fig. 3d**), indicating that cell division in the neighborhood of GnRH neurons
153 could not fully account for the increased proportion of GnRH neurons with a BrdU⁺
154 companion cell. GnRH neurons are thus likely attract newborn cells to their immediate
155 vicinity during the early infantile period.

156 The association between GnRH neurons and cells born at P8 was maintained until
157 adulthood (**Fig. 1c**). Notably, the increase in GnRH neurons with BrdU⁺ companions between
158 2h and 7 days after BrdU injection was seen only in the group injected at P8 (**Fig. 1c**),
159 indicating that the beginning of the infantile period is a critical time-window for GnRH neurons
160 to enrich their immediate cellular environment. Triple immunolabeling for GnRH, BrdU and
161 GFAP, APC or HuC/D showed that P8-born cells accompanying GnRH neurons mainly
162 differentiated into GFAP-expressing astrocytes (**Fig. 1g,i-k**). Physical proximity to GnRH
163 neurons did not alter the fate of P8-born cells (phenotype of BrdU⁺ cells in the vicinity of
164 GnRH neurons (n = 6-7 rats) vs. all newborn cells in the preoptic region (n = 3 rats): GFAP,
165 Mann-Whitney test: $U = 9$, $P = 0.833$; APC, Mann-Whitney test: $U = 6$, $P = 0.500$; HuC/D,
166 Mann-Whitney test: $U = 3$, $P = 0.167$) (compare **Supplementary Fig. 2j** and **Fig. 1g**).

167

168 **Blocking infantile preoptic cell proliferation delays puberty**

169 To determine if the birth of new cells in the infantile preoptic region is involved in
170 sexual maturation, we locally inhibited cell proliferation in P6 or P7 female rats by
171 stereotaxically injecting biodegradable poly(D,L-lactic-co-glycolic acid) (PLGA)-based
172 microparticles (**Fig. 2a,c**), designed to release the antimitotic compound, paclitaxel, in a time-
173 controlled manner¹¹, just above the MePO. An initial assay revealed that this release was
174 complete by 6 days, i.e. before the end of the infantile period when proliferation in the
175 preoptic area peaks (**Supplementary Fig. 4a**). Control animals were injected with blank-

176 loaded microparticles (**Fig. 2a,b**), and a single BrdU injection carried out at P8 as before in
177 both groups. We then monitored puberty onset and adult estrous cyclicity. At adulthood,
178 paclitaxel-treated animals showed decreased cell density (**Fig. 2c,d**, **Supplementary Fig.**
179 **4b,c**), fewer BrdU⁺ cells at the injection site (**Fig. 2e**, **Supplementary Fig. 4b,c**) and a lower
180 proportion of GnRH neurons associated with BrdU⁺ cells in the MePO (**Fig. 2f**), although the
181 total number of GnRH neurons in the preoptic region remained unaffected (**Fig. 2g**). In the
182 anteroventral periventricular nucleus (AVPV), a more ventral preoptic nucleus that extends
183 well beyond the OVLT caudally and relays the estrogen positive feedback¹², chronic
184 intracerebroventricular administration of another antimetabolic, AraC, during the peripubertal
185 period has been shown to block proliferation¹³. However, paclitaxel treatment did not affect
186 the number of BrdU⁺ cells, neurons or astrocytes in the AVPV (**Supplementary Fig. 5**),
187 indicating that our administration method had extremely localized antimetabolic effects.
188 Paclitaxel-treated animals exhibited a marked delay in puberty onset compared to control
189 animals, as indicated by delayed vaginal opening (**Fig. 2h,i**) and first estrus (**Fig. 2j,k**). In
190 addition, while the two milestones coincided in control animals, they were uncoupled in
191 paclitaxel-treated animals, which experienced their first estrus ~3.5 days after vaginal
192 opening (**Fig. 2l,m**), a phenomenon rarely observed in rats⁵. Notably, paclitaxel-treated
193 animals had similar body weights to control animals (**Supplementary Fig. 4d**), indicating that
194 the delay in sexual maturation was not due to a growth deficit. Paclitaxel-treated animals also
195 exhibited fewer complete 4-day estrous cycles (**Fig. 2l,n**). Taken together, these results
196 suggest that the presence of newborn cells in the environment of preoptic GnRH neurons
197 during the infantile period, rather than GnRH neurons *per se*, is necessary for the timely
198 onset of puberty and contributes to fertility in adulthood.

199

200 **Endocrine disruptors alter GnRH neuron-progenitor interaction**

201 A common adverse condition for the development and function of the gonadotropic
202 axis is exposure to endocrine-disrupting chemicals (EDCs) during perinatal life. While the
203 gonads have long been considered the main targets of EDCs, a growing number of studies

204 highlight their neuroendocrine effects, such as GnRH neuronal network alterations¹⁴.
205 Intriguingly, rat pups exposed for the first 15 days after birth to a very low but environmentally
206 relevant dose¹⁵ of the plasticizer bisphenol A (BPA; 25 ng/kg per day) tend towards delayed
207 vaginal opening¹⁶, phenocopying our paclitaxel-treated rat pups. Additionally, these BPA-
208 exposed rats also demonstrate an altered frequency of GnRH secretion at P20¹⁶, suggesting
209 a perturbation of the first postnatal activation of the hypothalamic-pituitary-gonadal (HPG)
210 axis, known as minipuberty. **This phenomenon**, which occurs at P12 in rats and 1 month of
211 age in humans, helps establish an adult pattern of pulsatile GnRH secretion **that, in** baby
212 girls and female rodents alike, **triggers** an FSH surge that is essential for subsequent
213 gonadal maturation and hence, fertility^{5, 17}. To verify whether the adverse effects of EDCs on
214 gonadotropic axis maturation could stem from their interference with the ability of GnRH
215 neurons to recruit neighboring cells, we treated female rat pups with 25 ng/kg per day of BPA
216 from P1¹⁶, then injected them with BrdU at P8 and assessed GnRH neuron association with
217 BrdU⁺ cells 2h and 7d later (**Fig. 3a**). Contrary to paclitaxel, BPA exposure did not affect cell
218 birth near GnRH neurons at P8 (**Fig. 3b**). Additionally, the total number of GnRH neurons in
219 the preoptic region at P8 also remained unchanged (255.7 ± 34.95 in control P8 rats versus
220 218.9 ± 30.38 in BPA-treated P8 rats, Mann-Whitney test: $U = 22$, $P = 0.3282$; $n = 8$ rats per
221 group). However, unlike untreated rats, BPA-exposed animals showed reduced association
222 between GnRH neurons and P8-born BrdU⁺ cells 1 week later (**Fig. 3b**) even though there
223 was no depletion of BrdU⁺ cells in the preoptic region (**Fig. 3c**), further supporting the view
224 that rather than proliferation, it is the recruitment of new glial progenitors by GnRH neurons
225 that influences later reproductive function. From a broader viewpoint, these results also open
226 up the possibility that the effects of EDCs on fertility and brain maturation are at least partly
227 due to their interference with the ability of select neuronal populations to shape their glial
228 environment during postnatal development.

229

230 **GnRH neurons use PGD2 to recruit preoptic progenitor cells**

231 Considering that the increased proportion of GnRH neurons associated with BrdU⁺
232 cells between P8 + 2h and P8 + 24h is likely due to BrdU⁺ cell recruitment rather than
233 proliferation (**Supplementary Fig. 3**), we wondered whether GnRH neurons themselves
234 released chemotropic factors to attract glial progenitors that would eventually differentiate
235 into astrocytes in their vicinity and remain associated with them into adulthood (**Fig. 1i**). We
236 first probed a set of molecules expressed in female rodent GnRH neurons: GnRH,
237 neurotensin (NT), cholecystokinin (CCK), galanin, glutamate and GABA^{18, 19}. **Double**
238 immunolabeling **showed** that infantile GnRH neuronal perikarya contained galanin but lacked
239 CCK and NT (**Supplementary Fig. 6a-i**). However, the vesicular transporters for glutamate
240 and γ -aminobutyric acid (GABA), v-Glut and v-GAT, respectively, colocalized at the GnRH
241 termination field in the median eminence (**Supplementary Fig. 6j-o**), suggesting that
242 postnatal GnRH neurons could also influence proliferating cells through the local glutamate
243 or GABA release.

244 To assess the putative chemotactic effects of GnRH, galanin, glutamate and GABA
245 produced by GnRH neurons, we next developed adherent primary cultures of progenitor cells
246 from the rat preoptic region at P1-P2 (**Supplementary Fig. 7a**). Cultured cells exhibited an
247 antigenic profile typical of neural progenitors, predominantly expressing nestin, BLBP, Sox2
248 and vimentin (**Fig. 4a, Supplementary Fig. 7b-e**). However, in transwell assays, their
249 migration was not significantly affected by GnRH, galanin, glutamate or GABA (**Fig. 4b**),
250 suggesting that other factors might be responsible for their chemoattraction by GnRH
251 neurons.

252 Next, since postnatal progenitor recruitment by GnRH neurons occurs in the global
253 context of astrogenesis⁸, and GnRH neurons are tightly surrounded by GFAP-expressing
254 processes at P8 (**Fig. 1f**), we searched for candidate genes in GnRH neurons **that could be**
255 regulated by astroglial factors. We conducted a transcriptomic analysis of GnV3 cells, an *in*
256 *vitro* model of postnatal rat GnRH neurons²⁰, exposed to medium conditioned with or without
257 hypothalamic astrocytes for 4 days (**Fig. 4c**). In all, 512 genes were differentially expressed
258 (212 upregulated and 300 downregulated) when GnV3 cells were treated with astrocyte-

259 conditioned medium (See Source Data Files in **Supplementary Data 1**). The most
260 upregulated gene was *Ptgds* encoding brain-type prostaglandin D2 (PGD2) synthase, a
261 molecule with potent chemoattractant properties²¹, which displayed a 133-fold increase in
262 response to astrocyte-conditioned medium (**Supplementary Data 1**). RT-PCR analysis
263 confirmed this upregulation (**Fig. 4d**). Moreover, PGD2 stimulated the passage of preoptic
264 neural progenitors from the upper to the lower compartment in transwell migration assays
265 (**Fig. 4b**), but did not affect their proliferation (**Fig. 4e**). As gliogenesis continues during
266 postnatal development, these data suggest that GnRH neurons respond to incoming glial
267 signals by synthesizing PGD2 and thus attracting newborn cells in their vicinity.

268 PGD2 functions *via* the G protein-coupled receptors, DP1 (*Ptgdr1*) and DP2 (*Gpr44*)
269 ^{21, 22}, both expressed in primary cultures of preoptic neural progenitors (**Fig. 4f**). Since DP1
270 signaling is already known to be involved in regulating hypothalamic function²², we used
271 BWA868C, a specific antagonist of DP1^{22, 23}, to determine whether the effect of PGD2 on
272 hypothalamic progenitor cell migration was mediated by DP1. BWA868C treatment of
273 preoptic neural progenitors not only drastically inhibited the stimulatory effect of PGD2 on cell
274 migration (**Fig. 4g,h**), it also reduced the PGD2-induced phosphorylation of the mitogen-
275 activated protein kinase (MAPK) ERK (**Fig. 4i**), confirming that PGD2 signaling was active in
276 these progenitors.

277

278 **PGD2/DP1 signaling mediates preoptic precursor recruitment**

279 Next, to investigate whether GnRH neurons produce PGD2 during postnatal
280 development *in vivo*, we analyzed *Ptgds* expression in cells isolated by fluorescence-
281 activated cell sorting (FACS) from transgenic rats expressing enhanced green fluorescent
282 protein (EGFP) under the control of the GnRH promoter²⁴ using real-time PCR (**Fig. 5a**,
283 **Supplementary Fig. 8a,b**). *Gnrh* mRNA was only detected in the EGFP-positive cell fraction,
284 demonstrating the accuracy of the sorting procedure (**Supplementary Fig. 8c-e**). Using this
285 approach, we found that *Ptgds* transcripts were indeed enriched in rat GnRH neurons and

286 that their expression significantly increased between P8 and P20 in these neurons (**Fig. 5b**)
287 but remained unchanged in preoptic cells lacking EGFP (**Fig. 5c**).

288 To gain further insight into the involvement of the PGD2/DP1 signaling pathway in the
289 communication between GnRH neurons and neural progenitors *in vivo*, we evaluated *Ptgds*
290 and *Ptgdl* expression in the postnatal preoptic region. Multiplex *in situ* hybridization in
291 female rats at P8 confirmed the expression of *Ptgds* transcripts in GnRH neurons (**Fig. 5d**,
292 upper row), while neighboring Sox2-expressing neural progenitors expressed *Ptgdl* mRNA
293 (**Fig. 5d**, lower row). Moreover, the fraction of GnRH neurons expressing *Ptgds* increased
294 between P8 and P12, an increase still seen at P20 (**Fig. 5e**), explaining the increased *Ptgds*
295 expression observed in FACS-sorted cells above (**Fig. 5b**); however, the abundance of
296 *Ptgds* transcripts per GnRH neuron did not significantly increase across the infantile period
297 (**Fig. 5f**). In contrast, *Ptgdl* expression in Sox2⁺ progenitors showed no significant variation
298 between P8 and P20 (**Fig. 5g,h**). Altogether, these data show that the increased *Ptgds*
299 expression in GnRH neurons during infantile development corresponds to an increase in the
300 proportion of PGD2-synthesizing GnRH neurons during the period of enhanced astrogenesis
301 in the preoptic area (**Supplementary Fig. 1, Supplementary Fig. 2**).

302 To confirm that the PGD2/DP1 signaling pathway is indeed involved in progenitor
303 cells recruitment by GnRH neurons *in vivo*, we acutely injected BWA868C stereotaxically into
304 the preoptic region of P7 female rats, 2h after a single BrdU injection, and quantified the
305 proportion of GnRH neurons associated with BrdU⁺ cells after 24h (**Fig. 6a**). This proportion
306 increased in control animals but not in BWA868C-treated rats (**Fig. 6b**). Since BWA868C
307 treatment did not affect the total number of BrdU⁺ cells in the preoptic region (**Fig. 6c**), the
308 inhibitory effect of BWA868C on the physical proximity between GnRH neurons and BrdU⁺
309 cells was not due to neural progenitors depletion **but to the blockade of the trophic influence**
310 **of GnRH neurons on nearby glial progenitors**. Together **with our *in vitro* findings**, these
311 results indicate that functional PGD2/DP1 signaling is necessary for GnRH neurons to recruit
312 **or attract** a glial entourage during the infantile period in rats.

313

314 **Inhibiting DP1 disrupts GnRH network maturation and function**

315 To assess the functional involvement of DP1 signaling in postnatal sexual maturation,
316 we implanted BWA868C pellets (10 nM in cocoa butter) in the preoptic region of P8 female
317 rats²⁵ and monitored the occurrence of the first postnatal FSH surge indicative of minipuberty
318 (**Fig. 6d**). Similar to acute injection, BWA868C released chronically from a pellet also
319 significantly hampered the association of GnRH neurons with BrdU⁺ cells one week later
320 (**Fig. 6e**), although the total number of BrdU⁺ cells in the preoptic region remained unaffected
321 (**Fig. 6f**). While the treatment had no effect on somatic growth (**Supplementary Fig. 9**), the
322 FSH surge at minipuberty was blunted in BWA868C-treated pups in comparison to vehicle-
323 treated littermates (**Fig. 6g**), suggesting that the activity of infantile GnRH neurons was
324 impaired by their inability to attract newborn astrocytic precursors.

325 Next, since minipuberty has previously been shown to be a critical period for the
326 switch in the transcriptional control of GnRH expression⁴, we assessed whether BWA868C
327 treatment at P7 influenced this process. RT-qPCR analyses in FACS-isolated rat GnRH
328 neurons at P12-P13 indicated that BWA868C implantation had no apparent impact on *Gnrh*
329 expression (**Fig. 7a**). However, it decreased the expression of *Cebpb*, a repressor of the
330 *Gnrh* promoter, but left unchanged the expression of another repressor, *Zeb1*, as well as
331 *Kiss1r* and *Otx2*, two stimulators of *GnRH* expression, and *Dicer*, an RNase-III
332 endonuclease essential for microRNA biogenesis and the microRNA-mediated infantile *Gnrh*
333 transcriptional switch⁴ (**Fig. 7a**). Surprisingly, BWA868C treatment also impacted several
334 other genes involved in prostaglandin synthesis and signaling in infantile GnRH neurons
335 (**Fig. 7b**). In addition to a significant decrease in *Ptgds* expression (**Fig. 7c**), in line with our
336 transcriptomic analysis showing *Ptgds* regulation by astrocyte-derived factors (**Fig. 4d**,
337 **Supplementary Data 1**), BWA868C treatment downregulated transcripts for *Ptger1* and
338 *Ptger3*, encoding the PGE2 receptors EP1 and EP3, respectively, in infantile rat GnRH
339 neurons (**Fig. 7c**). These results are in keeping with previous *in vitro* studies showing that
340 astrocyte-conditioned medium modulates the sensitivity of GnRH neurons to astrocytic

341 PGE2, a stimulator of GnRH release, by upregulating EP1 and EP3 expression²⁶, and
342 suggest that the BWA868C-mediated decrease in astrocyte recruitment by GnRH neurons
343 interferes with astrocytic PGE2-stimulated GnRH release rather than GnRH production.

344 We next investigated whether, in addition to altering astrocyte-to-GnRH-neuron
345 communication processes, BWA868C treatment could also influence neuronal connectivity,
346 in particular the apposition of glutamatergic or GABAergic terminals onto GnRH neuronal cell
347 bodies, and consequently GnRH neuronal activity during the infantile period. The BWA868C-
348 mediated inhibition of DP1 signaling in the preoptic region significantly decreased the
349 number of appositions of vesicular glutamate transporter 2 (vGluT2)-immunoreactive punctae
350 but not of vesicular GABA transporter (vGAT)-immunoreactive punctae on GnRH somata at
351 P15 (**Fig. 7d**).

352 Given this robust decrease in putative excitatory synaptic inputs to GnRH neurons,
353 we next performed *ex vivo* whole-cell patch-clamp recordings of GnRH neurons in living
354 brain slices containing the OVLT from P10-P15 infantile *Gnrh::Egfp* rats treated either with
355 vehicle or with BWA868C *in vivo* at P7-P8 (**Fig. 7e,f**). We found no significant difference
356 between the two conditions in the resting membrane potential (V_m) (**Fig. 7g**), membrane
357 resistance (R_m) (**Fig. 7h**), voltage threshold for action potential (AP) generation (V_{TH}) (**Fig.**
358 **7i**) or time constant (control time constant = 0.47 ± 0.07 ms, $n = 15$ cells from 8 rats;
359 BWA868C time constant = 0.43 ± 0.05 ms, $n = 22$ cells from 7 rats; Mann-Whitney test: $U =$
360 149.5 , $P = 0.641$). These findings demonstrate that the integrative properties and intrinsic
361 excitability of GnRH neurons were not modified by BWA868C treatment. However, we
362 observed a significant attenuation of the spontaneous firing rate in GnRH neurons partly
363 deprived of glutamatergic synapses (**Fig. 7j** and **f**, left), reminiscent of the increased
364 interpulse interval of GnRH neurosecretion in BPA-treated rat pups¹⁶. This was not
365 accompanied by modifications in spike properties such as full amplitude (control spike
366 amplitude = 57.73 ± 2.75 mV, $n = 10$ cells from 8 rats; BWA868C spike amplitude = $61.13 \pm$
367 1.88 mV, $n = 10$ cells from 8 rats; two-sided unpaired t -test, $t(18) = 1.02$, $P = 0.321$), half-

368 width duration (control half-width duration = 1.85 ± 0.17 ms, $n = 10$ cells from 8 rats;
369 BWA868C half-width duration = 1.74 ± 0.07 ms, $n = 10$ cells from 8 rats; Mann-Whitney test:
370 $U = 49$, $P = 0.970$) or after-hyperpolarization potential (AHP) amplitude (control AHP
371 amplitude = 7.52 ± 0.55 mV, $n = 10$ cells from 8 rats; BWA868C AHP amplitude = $7.18 \pm$
372 0.30 mV, $n = 10$ cells from 8 rats; two-sided unpaired t -test, $t(18) = 0.535$, $P = 0.599$) or
373 duration (control AHP duration = 470.9 ± 69.52 ms, $n = 10$ cells from 8 rats; BWA868C AHP
374 duration = 465.7 ± 65.53 ms, $n = 10$ cells from 8 rats; two-sided unpaired t -test, $t(18) = 0.055$,
375 $P = 0.957$) as measured from isolated APs. We further analyzed spike generation in neurons
376 in which clear baseline synaptic activity was detected on the trace ($n = 10$ neurons from 8
377 rats per condition). In the control group, in 5 neurons, firing was triggered by a slowly
378 developing temporal summation of fast depolarizing events (**Fig. 7f**, top right), reminiscent of
379 depolarizing postsynaptic potentials. This dramatically differed from most BWA868C-treated
380 neurons, in which AP firing was apparently elicited by intrinsic mechanisms ($n = 4$ neurons,
381 **Fig. 7f**, bottom right, AP #1 and #2) or the cooperative effect of intrinsic and synaptic
382 mechanisms ($n = 4$ neurons, **Fig. 7f**, bottom right, AP #3). Only two BWA868C-treated
383 neurons analyzed displayed APs elicited by an excitatory synaptic barrage. Altogether, these
384 findings indicate that BWA868C treatment does not alter the resting membrane or integrative
385 properties of GnRH neurons but significantly reduces their functional output, likely due to
386 decreased glutamatergic synaptic connections.

387 Finally, we analyzed the effects of infantile BWA868C treatment on the acquisition of
388 mature reproductive function. Even though BWA868C implantation in the preoptic region
389 during the infantile period did not alter the age at vaginal opening (**Fig. 8a,b**), which
390 coincided with first estrus (**Fig. 8a**), it significantly delayed the onset of regular estrous
391 cyclicity (**Fig. 8a,c**). During the first two weeks after vaginal opening, BWA868C-treated
392 females spent more time in estrus at the expense of proestrus, indicating a dysregulation of
393 pulsatile GnRH release, in contrast to control (vehicle-treated) littermates, which quickly
394 acquired a regular 4-day estrous cycle.

395 Altogether, these results show that the integrity of GnRH neuron-glia communication
396 in the preoptic region during the infantile period is critical for the recruitment of newborn glial
397 precursors that accompany these neurons into adulthood as well as the establishment of
398 functional synaptic inputs to GnRH neurons. Furthermore, this communication, mediated by
399 PGD2/DP1 signaling, is essential for the correct initiation of sexual maturation, reciprocal
400 signaling by astrocytes of the GnRH neural network, and **adult pattern of estrous cyclicity**.

401

402 **DISCUSSION**

403 The ability to reproduce is acquired through a long and complex process that relies on
404 the fine regulation of GnRH neuronal network activity during the postnatal period. This
405 process involves intrinsic and extrinsic modifications to functional gene networks^{4, 27} and
406 neuronal circuits converging onto GnRH neurons^{28, 29}, as well as the increased stimulatory
407 influence of glial cells⁶. Our data provide the first neuroanatomical and physiological
408 evidence that GnRH neurons build their own glial environment during a circumscribed period
409 after birth via a specific cell-cell communication pathway with neural progenitors born during
410 a wave of astrogenesis, and that this reciprocal communication is essential for the maturation
411 of the hypothalamic neuroendocrine system controlling the survival of the species.

412 Astrocytes have long been known to play a role in the control of GnRH neuronal
413 function, particularly during postnatal sexual maturation²⁵, being sophisticated sensors⁸ and
414 regulators of neuronal activity via both the timely trafficking of energy metabolites³⁰ and
415 gliotransmission³¹. They communicate with GnRH neurons by releasing bioactive molecules
416 such as PGE2^{32, 33}, known to be released at active synapses in response to glutamate^{34, 35}.
417 Glutamate-stimulated PGE2 release within the GnRH neural network may involve the
418 activation of the ErbB signaling pathway³⁶, also important for puberty onset^{25, 37}, in
419 hypothalamic astrocytes. Here, we pinpoint the production of another prostaglandin, PGD2,
420 by GnRH neurons themselves as a potent communication signal used by these neurons to
421 attract infantile astroglial progenitors to their vicinity. Our findings demonstrate that inhibiting

422 cell proliferation or impairing PGD2/DP1 signaling in the preoptic region during this period
423 significantly alters sexual maturation, indicating that astrogenesis in the vicinity of GnRH
424 neurons during postnatal development is an important component of the homeostatic
425 mechanism required for GnRH neural network maturation. While recent advances in glial cell
426 biology have provided substantial insights into how astrocytes influence neuronal function
427 (see ³⁸ for review), the concept that neurons could stably recruit astrocytic progenitors as
428 companions or “escorts”, i.e. allow newborn neighboring astrocytes to become
429 structurally/functionally associated with them during development and maintain this
430 relationship into adulthood, has rarely been evoked.

431 Interestingly, the birth of new cells in the infantile preoptic region could also be
432 modulated by neuronal factors released by other components of the GnRH neuroglial
433 network. For example, some incoming fibers from the developing arcuate nucleus of the
434 hypothalamus release β -endorphin^{39, 40}, which has recently been shown to activate quiescent
435 neural stem cells in the adult brain⁴¹, and could thus be a candidate to regulate this infantile
436 proliferation. While we cannot rule out an indirect influence of the proliferation or signaling of
437 neural progenitors on reproductive function through similar mechanisms in other components
438 of the GnRH network, e.g. by altering astrocyte-kisspeptin-neuron interactions in the adjacent
439 AVPV, the fact that paclitaxel did not modify BrdU+ cells in this nucleus suggests that the
440 physiological changes we observed were selectively due to perturbed astrocyte-GnRH
441 neuron association, rather than alterations in the neuroglial interactions of afferent neurons.
442 Nevertheless, paclitaxel affected the reproductive phenotype more severely than BWA868C,
443 suggesting that the progenitor depletion might have been harder to compensate for than
444 simply inadequate GnRH neuron connectivity due to impaired association with glial
445 progenitors.

446 Interestingly, deficient DP1 signaling in the preoptic region also blunts the FSH surge
447 at P12, i.e. during minipuberty, the first centrally driven and gonad-independent activation of
448 the HPG axis⁵. This phenotype is highly reminiscent of that of mice in which astrocytic
449 function is selectively altered by expressing a dominant-negative ErbB4 receptor (DN-ErbB4)

450 under the control of the GFAP promoter³⁷. On the other hand, the disrupted estrous cycle
451 triggered by blocking preoptic DP1 signaling in rats phenocopies transgenic mice expressing
452 a dominant negative mutation of the adhesion molecule SynCAM1 under the GFAP
453 promoter⁴². SynCAM1 expression, required to maintain the physical contact between
454 astrocytes and GnRH neuronal cell bodies⁴³, is activated by astroglial ErbB4 signaling⁴².
455 Altogether, these findings evoke a scenario in which the birth of new astrocytes during
456 postnatal development promotes a selective increase in the expression of the PGD2-
457 producing enzyme, PGDS, in infantile GnRH neurons via an as yet unidentified signaling
458 pathway involving soluble factors. The choice of candidate signaling factors is large, with
459 astrocytes releasing not only gliotransmitters but numerous neuroactive substances,
460 including thrombospondin, TNF α and TGF β 1 (see for review ⁴⁴⁻⁴⁶). Regardless of its identity,
461 PGD2 released by GnRH neurons attracts DP1-expressing neural progenitors that eventually
462 differentiate into astrocytes and remain associated with **these** neurons until adulthood, **an**
463 **“escort” function that could facilitate** their integration into functional networks and sexual
464 maturation (**Extended Data Fig. 1**), and that could require other neuron-to-glia
465 communication processes similar to those involving infantile ErbB4 signaling^{42, 43}. While such
466 experiments in rats are beyond the technical scope of this study, preliminary experiments in
467 *Gfap::DN-ErbB4* mice indicate that newborn cells that are morphologically associated with
468 infantile GnRH neurons fail to accompany them into adulthood, in contrast to wild-type
469 littermates, despite the unaltered proliferation and long-term survival of these cells
470 (**Supplementary Note; Supplementary Fig. 10**).

471 Astrocytes also play key roles in the control of synapse formation and functional efficacy⁴⁷,
472 ⁴⁸, shaping synaptic properties to fit ongoing developmental and functional needs, thus
473 providing contextual guidance to the synapses^{49, 50}. During postnatal development, synaptic
474 punctae in the brain appear concurrently with astrogenesis⁴⁷. Remarkably, our results show
475 that preventing GnRH neurons from attracting glial progenitors in their immediate
476 neighborhood by blocking DP1 signaling markedly decreases the number of vGluT2
477 appositions on GnRH neurons and significantly attenuates GnRH neuron firing, primarily by

478 decreasing the rate of depolarizing postsynaptic potentials. Since the FSH surge at
479 minipuberty is also blunted in these pups, we can postulate that the birth of new astrocytes
480 near infantile GnRH neurons is required for the establishment of excitatory glutamatergic
481 inputs onto GnRH neurons, confirming the long-suspected notion that such afferents
482 contribute to the gonad-independent maturation of the HPG axis in infantile rats^{51, 52}. These
483 excitatory synaptic inputs are thought to develop after GABAergic inputs^{53, 54}, which,
484 contrarily, do not appear to require infantile gliogenesis for their establishment or
485 maintenance (**Fig. 7d**). Intriguingly, in the interconnected GnRH neuroglial network⁶,
486 glutamate spillover at the synaptic cleft by the co-activation of metabotropic and AMPA
487 glutamatergic receptors in hypothalamic astrocytes³⁶ or their progenitors may initiate a
488 signaling cascade that allows ErbB4 receptor recruitment³⁶, promotes astrogenesis⁵⁵ and
489 thus leads to the stabilization and maturation of glutamatergic synapses. This causes
490 increased PGE2 synthesis and release by astrocytes³⁶, which can then signal back to
491 GnRH-secreting neurons⁶. An infantile GnRH neuron-dependent modulation of astrogenesis
492 may therefore contribute to building a signaling network capable of delivering coordinated
493 trans-synaptic and astroglial inputs to GnRH neurons themselves for the initiation of puberty
494 and the timely acquisition of adult reproductive capacity (**Extended Data Fig. 1**)⁴.

495 Recent human and animal studies increasingly demonstrate that the GnRH neural
496 network is particularly sensitive to environmental pollutants, in particular endocrine
497 disruptors, during early postnatal development¹⁴. Exposure to EDCs such as the plasticizer
498 BPA during the critical perinatal period is indeed reported to be associated with early or late
499 puberty onset and subsequent reproductive impairments in both boys and girls (see for
500 review¹⁴), and to affect the central control of puberty, including glutamatergic and
501 GABAergic inputs to GnRH neurons, in preclinical animal models^{16, 56, 57}. While the underlying
502 mechanisms remain elusive, glial cells, which play a central role in GnRH neuronal network
503 development and function⁶, would be the obvious suspects to mediate this EDC-induced
504 pathophysiological process¹⁴. Our results intriguingly show that treating infantile rats with
505 very low but environmentally relevant doses of BPA¹⁵ known to cause alterations in sexual

506 maturation, phenocopying paclitaxel-treated rats¹⁶, alters the recruitment of newborn
507 neighboring cells by GnRH neurons at minipuberty, without appearing to affect overall cell
508 proliferation in the infantile preoptic region. This raises the possibility that early-life exposure
509 to chemicals in contact with food⁵⁸, such as BPA, may perturb puberty onset and durably
510 impact reproductive function at least partially by interfering with the capacity of GnRH
511 neurons to build a proper glial environment through altered PGDS expression⁵⁹ or ErbB
512 signaling⁶⁰, resulting in their miswiring during postnatal development.

513 To summarize, our findings shed light on a hitherto unsuspected neuron-to-neural-
514 progenitor communication process that shapes the glial environment of GnRH neurons
515 during postnatal development and is required for the proper wiring and function of these
516 neurons, which control the survival of the species in mammals.

517

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535

536 **AUTHOR CONTRIBUTIONS**

537 A.S., A.-S. P. and V.P. designed the experiments. G.P., M.M., C.A., T.L., S.G., D.F., V.M., M.
538 M.-L., A C.-R., V.D., D.M., A.L., M.T.S., P.C., A.S. and V.P. performed the experiments. A.S.
539 and V.P. analyzed the data. J.S., G.C. and F.P. contributed material. All authors discussed
540 the results and made edits to the manuscript. A.S., S.R., S.R.O. and V.P. wrote the
541 manuscript.

542

543 **COMPETING FINANCIAL INTERESTS**

544 The authors declare no competing financial interests.

545

546 **FIGURE LEGENDS**

547 **Figure 1. Postnatally-born astrocytes preferentially associate with GnRH neuronal cell**
548 **bodies. (a)** Experimental protocol. **(b)** Representative coronal section of the preoptic region
549 immunolabeled for GnRH (green) and BrdU (magenta), nuclei counterstained with Hoechst
550 (white), illustrating quantifications in **c**. Arrow, arrowheads: GnRH neuronal cell bodies. Inset:
551 higher magnification of the GnRH neuron indicated, showing physical proximity to a BrdU⁺
552 cell (yellow arrows). Scale bars: 100µm (main panel), 10 µm (inset). **(c)** Percentage of GnRH
553 neurons associated with BrdU⁺ cells 2h, 24h, 7d or 60d after a single BrdU injection at P1,
554 P5, P8, P12, P20 or P24 (*n*=9,6,7,6,8,5,10,5,5,6,9,3,3,6,9,3,4,9,5 rats/group) (two-way
555 ANOVA: age at BrdU injection, $F_{(5,73)}=44.92$, $P<0.0001$; survival time (2h, 7d), $F_{(1,73)}=3.116$,
556 $P=0.0817$; interaction, $F_{(5,73)}= 8.392$, $P< 0.0001$; Tukey's multiple comparison test, P8+2h
557 versus P8+7d: $q_{(73)} = 8.583$, $P<0.0001$; P8, Kruskal-Wallis one-way ANOVA on ranks:
558 $H_{(4)}=20.98$, $P=0.0001$; Dunn's multiple comparison: 2h versus 24h, $P=0.0274$; 2h versus 60d,
559 $P>0.9999$). **(d)** Percentage of GnRH- or HuC/D-expressing neurons associated with BrdU⁺
560 cells 2h or 7d after a single BrdU injection at P8 (% of GnRH neurons versus % of HuC/D⁺

561 neurons associated with BrdU⁺ cells: Mann-Whitney test, 2h: $U=0$, $P=0.004$; $n=8,4$
562 rats/group, respectively; 7d: $U=0$, $P=0.002$; $n=10,4$ rats/group).**(e,f,i-k)** Representative triple
563 immunofluorescence labeling for GnRH (green), BrdU (magenta) and Sox2 (**e**, white), GFAP
564 (**f,i**, white), APC (**j**, white) or HuC/D (**k**, white) in the female rat preoptic region injected with
565 BrdU at P8 and sacrificed 2h (**e,f**) or 7d later (**i-k**), illustrating quantifications in **g**. Yellow
566 arrows: BrdU⁺ cells associated with GnRH neurons co-expressing the indicated markers.
567 Scale bars: 20 μ m. **(g)** Percentage of BrdU⁺ cells associated with GnRH neurons co-
568 expressing Sox2, GFAP, APC or HuC/D in animals injected with BrdU at P8 and sacrificed
569 2h or 7d later (GFAP, Mann-Whitney test: $U=0$, $P=0.0012$, $n=6,7$ rats/group). **(h)** Co-
570 immunolabeling for GnRH (green) and BrdU (magenta), nuclei counterstained with Hoechst
571 (white), in female rats injected with BrdU thrice daily from P6 to P8 and sacrificed at P15,
572 showing two GnRH neurons with companion BrdU⁺ cells (yellow arrows). $n=4$ animals. Scale
573 bar: 20 μ m. **(c,d,g)** Values: means \pm s.e.m.; * $P<0.05$; ** $P<0.01$; i.p., intraperitoneal; neo,
574 neonatal period; OVLT, organum vasculosum laminae terminalis.

575

576 **Figure 2. Inhibiting GnRH neuron association with newborn glia delays the onset of**
577 **puberty and impairs mature estrous cyclicity.** **(a)** Diagram of the experimental protocol,
578 with a coronal view of the rat brain showing the anatomical localization of the microparticle
579 implantation site (red line) in the preoptic region (dashed area). **(b, c)** Coronal sections from
580 adult animals injected with blank- (b) or paclitaxel-loaded microparticles (c) at P6,
581 immunolabeled for GnRH (green) and counterstained with the nuclear marker Hoechst
582 (white). The region delimited by the dashed white line in **c** shows the hypocellular region
583 around the injection site (asterisk). ac, anterior commissure. Scale bars: 200 μ m.**(d-g)** Cell
584 nuclear density at the injection site (**d**: two-tailed unpaired t -test, $t_{(10)}=4.366$, $P=0.0014$, $n=6$
585 rats/group), number of BrdU⁺ cells at the injection site (**e**: two-tailed unpaired t -test, $t_{(11)}=4.2$,
586 $P=0.0015$; $n=7,6$ rats/group), percentage of GnRH neurons associated with BrdU⁺ cells (**f**:
587 two-tailed unpaired t -test with Welch's correction, $t_{(6,9)}=4.24$, $P=0.0040$; $n=7$ rats/group) and
588 total number of GnRH neurons in the preoptic region (**g**: two-tailed unpaired t -test,

589 $t_{(15)}=2.075$, $P=0.0556$; $n=7,10$ rats/group) of control and paclitaxel-treated rats. (h-i)
590 Cumulative percentage (h) and age of vaginal opening (i: Mann-Whitney test: $U=69.5$,
591 $P=0.0428$, $n=17,14$ rats/group) in control and paclitaxel-treated rats. (j-k) Cumulative
592 percentage (j) and age of first estrus (k: Mann-Whitney test: $U=32$, $P=0.0002$, $n=17,14$
593 rats/group) in control and paclitaxel-treated rats. (l) Representative estrous cycle profiles of
594 control and paclitaxel-implanted animals showing the day of vaginal opening (vo) and first
595 estrus (red dot). The first paclitaxel-treated animal has a normal estrous cycle while the next
596 three are examples of altered estrous cyclicity. D, diestrus; E, estrus; P, proestrus. (m)
597 Difference between age at vaginal opening and at first estrus in control and paclitaxel-treated
598 animals (Mann-Whitney test: $U=28.5$, $P<0.0001$, $n=17,14$ rats/group). (n) Percentage of
599 complete estrous cycles in control and paclitaxel-treated animals (Mann-Whitney test:
600 $U=80.5$, $P=0.0156$, $n=17,18$ rats/group). (d-g,i,k,m-n) Values: means \pm s.e.m.* $P<0.05$;
601 ** $P<0.01$; *** $P<0.001$.

602

603 **Figure 3. Early exposure to bisphenol A inhibits infantile GnRH neuron-progenitor**
604 **association.** (a) Schematic of the protocol used to evaluate the effect of bisphenol A (BPA)
605 on the association between GnRH neurons and progenitors. (b) Percentage of GnRH
606 neurons associated with BrdU⁺ cells in control and BPA-treated animals (one-way ANOVA:
607 $F_{(3,26)}=16.6$, $P<0.0001$, $n=11,4, 8,7$ rats/group; Tukey's multiple comparisons test: 2h control
608 versus 7d control, $q_{(26)}=7.739$, $P<0.0001$; 2h BPA versus 7d BPA, $q_{(26)}=4.46$, $P=0.0198$; 2h
609 control versus 2h BPA, $q_{(26)}=1.412$, $P=0.7517$). (c) Total number of BrdU⁺ cells 2h and 7d
610 after a single injection of BrdU at P8 in animals treated with BPA from P1 to P15 (two-tailed
611 unpaired t -test, $t_{(11)}=0.4553$, $P=0.6578$; $n=6,7$ rats/group). (b,c) Values: means \pm
612 s.e.m.* $P<0.05$; *** $P<0.001$.

613

614 **Figure 4. PGD2 is a chemoattractant for preoptic progenitor cells *in vitro*.** (a)
615 Immunofluorescence labeling for Sox2, nestin and BLBP in one primary progenitor culture
616 representative of three independent cultures. Cell nuclei were stained with Hoechst (blue).

617 Insets: higher magnification views of cells co-expressing the three markers (arrows). Scale
618 bars: 50 μ m. **(b)** Quantification of transwell assays showing the effect of different factors on
619 the migration of preoptic progenitor cells across a porous membrane. Epidermal growth
620 factor (EGF) was used as a positive control⁶¹ (Kruskal-Wallis one-way ANOVA on ranks:
621 $H_{(6)}=25.731$, $P<0.001$; Dunn's multiple comparison method: control versus treatment: PGD2,
622 $Q_{(6)}=4.255$, $P<0.001$; EGF, $Q_{(6)}=3.925$, $P=0.0018$; $n=18,19,12,17,10,10,12$ wells/condition;
623 $N=5$ independent experiments). **(c)** Strategy used to identify astrocyte-regulated gene
624 expression in GnRH neurons (See **Supplementary Data 1**). **(d)** Real-time PCR analysis of
625 *Ptgds* mRNA levels in GnV3 cells treated with hypothalamic astrocyte-conditioned medium
626 (ACM) or non-conditioned medium (control) (Mann-Whitney test: $U=195$, $P<0.001$; $n=13,15$
627 replicates/condition; $N=3$ independent experiments). ACM values are expressed relative to
628 control values, arbitrarily set at 1. **(e)** Percentage of preoptic progenitor cells expressing the
629 proliferation marker Ki67 after *in vitro* treatment with the same factors as in **b**. Fibroblast
630 growth factor 2 (FGF2) was used as a positive control⁶² (Kruskal-Wallis one-way ANOVA on
631 ranks: $H_{(6)}=38.576$, $P<0.001$; Dunn's multiple comparison method: control versus FGF2,
632 $Q_{(6)}=4.798$, $P<0.001$; $n=23,22,16,17,15,16,16$ wells/condition; $N = 3$ independent
633 experiments). **(f)** Real-time PCR analysis of *Ptgdl* and *Gpr44* mRNA levels in primary
634 preoptic progenitor cell cultures ($n=4$ cultures). Values expressed relative to mRNA levels in
635 the total brain, arbitrarily set at 1 (blue line). **(g)** Representative photomicrographs of the
636 Hoechst-stained lower side of transwell assay membranes, illustrating quantifications in **h**.
637 Scale bar: 100 μ m. **(h)** Quantification of transwell assays (Kruskal-Wallis one-way ANOVA on
638 ranks: $H_{(5)}=25.995$, $P<0.001$; Dunn's multiple comparison method: control versus PGD2,
639 $Q_{(5)}=3.277$, $P=0.0157$; PGD2 versus PGD2 + BWA 10nM, $Q_{(5)}=3.321$, $P=0.0135$; PGD2
640 versus PGD2 + BWA 1 μ M, $Q_{(5)}=4.548$, $P<0.001$; $n=11,11,12,12,11,10$ wells/condition; $N=3$
641 independent experiments). **(i)** ERK1/2 phosphorylation (pERK) in progenitors treated with
642 PGD2 (1 μ M) and/or the DP1 receptor antagonist BWA868C (BWA). Representative
643 immunoblot of three independent experiments. tERK, total ERK. **(b,d-f,h)** Values: means \pm
644 s.e.m.* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

645

646 **Figure 5. *In vivo* expression of *Ptgds* and *Ptgdl* in the preoptic region of infantile**
647 **female rats. (a)** Procedure used to isolate GnRH neurons from the preoptic region (POA) of
648 GnRH-EGFP transgenic rats. FACS, fluorescence-activated cell sorting; Pos, EGFP-positive
649 fraction; Neg, EGFP-negative fraction. **(b,c)** Real-time PCR analysis of the expression of
650 *Ptgds* mRNA in EGFP-positive- (b) or negative cells (c) at different postnatal ages (**b**:
651 Kruskal-Wallis one-way ANOVA on ranks: $H_{(2)}=7.476$, $P=0.024$, $n=10$ rats/group; Dunn's
652 multiple comparisons method: P8 versus P20, $Q_{(2)}=3.844$, $P=0.0191$; **c**: Kruskal-Wallis one-
653 way ANOVA on ranks: $H_{(2)}=5.280$, $P=0.071$, $n=10$ rats/group). Values expressed relative to
654 P8 values, arbitrarily set at 1. n.s., non-significant. **(d)** Representative confocal images of
655 fluorescent *in situ* hybridization for *Gnrh*, *Sox2*, *Ptgds* and *Ptgdl* mRNAs in the preoptic
656 region of P8 female rats, illustrating quantifications in **e-h**. (Upper row) Arrow: a GnRH
657 neuron containing *Ptgds* mRNA (magenta dots) and surrounded by *Sox2*-expressing cells
658 (arrowheads, white dots). (Lower row) Arrow: a GnRH neuron surrounded by *Sox2*-
659 expressing cells (white arrowheads). Purple arrowheads: two *Sox2*-expressing cells next to
660 the GnRH neuron, co-expressing *Ptgdl* mRNA (magenta dots). No such dots could be seen
661 in any section of the negative controls (see methods). Sections were counterstained with the
662 nuclear marker DAPI (blue). Scale bar: 5 μ m. **(e,f)** Percentage of GnRH neurons that contain
663 *Ptgds* mRNA (**e**: one-way ANOVA: $F_{(2,16)}=10.96$, $P=0.001$, $n=7,6,6$ rats/group; Tukey's
664 multiple comparison test: P8 versus P12, $q_{(16)}=5.185$, $P=0.0056$; P8 versus P20, $q_{(16)}=6.046$,
665 $P=0.0016$) and of the average number of *Ptgds* dots per *Ptgds*-expressing GnRH neuron (**f**:
666 Kruskal-Wallis one-way ANOVA on ranks: $H_{(3)}=5.484$, $P=0.0585$, $n=7,6,6$ rats/group)
667 determined from fluorescent *in situ* hybridization experiments. **(g,h)** Percentage of *Sox2*-
668 expressing cells that contain *Ptgdl* mRNAs (**g**: one-way ANOVA: $F_{(2,15)}=1.528$, $P=0.2489$,
669 $n=6$ rats/group) and average number of *Ptgdl* dots per *Sox2/Ptgdl*-co-expressing cell (**h**:
670 Kruskal-Wallis one-way ANOVA on ranks: $H_{(3)}=3.029$, $P=0.2306$, $n=6$ rats/group) determined
671 from fluorescent *in situ* hybridization experiments. **(b-c,e-h)** Values: means \pm s.e.m.* $P<0.05$;
672 ** $P<0.01$.

673

674 **Figure 6. Activation of DP1 signaling in the infantile preoptic region controls GnRH**
675 **neuron-progenitor association, and is involved in sexual maturation.** (a) Protocol used
676 to evaluate the involvement of DP1 signaling in the recruitment of progenitors by GnRH
677 neurons. Drawing: coronal view of the rat brain showing the anatomical localization of the
678 injection site, i.e. the preoptic region (dashed area). i.c., intracerebral; i.p., intraperitoneal. (b)
679 Percentage of GnRH neurons associated with BrdU⁺ cells 24h after a single BrdU injection at
680 P7 in the absence (control) or presence of the DP1 antagonist BWA868C (BWA) (one-way
681 ANOVA: $F_{(2,11)}=12.933$, $P=0.001$, $n=4,5,5$ rats/group; Tukey's multiple comparison test: 2h
682 versus 24h groups: 24h-control, $q_{(11)}=6.778$, $P=0.002$; 24h-BWA, $q_{(11)}=1.763$, $P=0.452$; 24h-
683 control versus 24h-BWA, $q_{(11)}=5.319$, $P=0.008$). (c) Total number of BrdU⁺ cells in the
684 preoptic region of control or BWA-treated animals (one-way ANOVA: $F_{(2,11)}=20.887$, $P<0.001$,
685 $n=4,5,5$ rats/group; Tukey's multiple comparison test: 2h versus 24h-control, $q_{(11)}=8.019$,
686 $P<0.001$; 2h versus 24h-BWA, $q_{(11)}=8.102$, $P<0.001$; 24h-control versus 24h-BWA,
687 $q_{(11)}=0.0883$, $P=0.998$). (d) Protocol used to evaluate the involvement of DP1 signaling in
688 sexual maturation. (e) Percentage of GnRH neurons associated with BrdU⁺ cells 7d after a
689 single BrdU injection at P8 in the absence or presence of BWA868C (two-tailed unpaired t -
690 test, $t_{(6)}=2.532$, $P=0.045$; $n=3,5$ rats/group). (f) Total number of BrdU⁺ cells in the preoptic
691 region of control or BWA-treated animals (two-tailed unpaired t -test, $t_{(6)}=0.771$, $P=0.470$;
692 $n=3,5$ rats/group). (g) Circulating FSH levels at P12 in BWA868C (BWA)-injected- and
693 control animals (two-tailed unpaired t -test, $t_{(20)}=3.075$, $P=0.006$; $n=8,14$ rats/group). Values:
694 means \pm s.e.m.; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

695

696 **Figure 7. Blocking DP1 signaling in the infantile period impairs GnRH neuron**
697 **maturation and functional activation.** (a,c) RT-PCR in control versus BWA868C-treated
698 FACS-sorted GnRH-EGFP neurons for (a) *Gnrh* and upstream regulators (Mann-Whitney
699 tests: *Gnrh*, $U=27$, $P=0.7925$; *Cebpb*, $U=8$, $P=0.0426$; *Kiss1r*, $U=27$, $P>0.9999$; two-tailed
700 unpaired t -tests: *Dicer*, $t_{(13)}=0.6232$, $P=0.5439$; *Otx2*, $t_{(11)}=0.7906$, $P=0.4459$; *Zeb1*,

701 $t_{(14)}=0.2605$, $P=0.7983$; $n=6,10,6,8,6,9,6,9,5,8,6,10$ rats/group) and (c) prostaglandin
702 pathway genes (Mann-Whitney tests: *Ptgs-2*, $U=18$, $P=0.3277$; *Ptges*, $U=19$, $P=0.3884$;
703 *Ptger1*, $U=4$, $P=0.0140$; *Ptger2*, $U=16$, $P=0.2238$; *Ptger3*, $U=4$, $P=0.0048$; two-tailed
704 unpaired *t*-tests: *Pla2g4a*, $t_{(14)}=0.1837$, $P=0.8569$; *Ptgs-1*, $t_{(13)}=0.1135$, $P=0.9113$; *Ptgds*,
705 $t_{(11)}=2.671$, $P=0.0218$; *Ptger4*, $t_{(14)}=1.656$, $P=0.12$; $n=6,10,6,9,6,9,6,7,6,9,6,7,6,9,6,9,6,10$
706 rats/group). Values expressed relative to control values, set at 1. (b) Prostaglandin
707 biosynthesis. (d) Representative GnRH neuronal soma (red) with apposed vGluT2- (left,
708 green) and vGAT- (right, green) immunoreactive (ir) punctae (arrowheads) in control (top)
709 and BWA868C-treated animals (bottom). Scale bar: 10 μ m. Bar graphs: vGluT2- (two-tailed
710 unpaired *t*-test, $t_{(12)}=3.057$, $P=0.0099$; $n=7$ rats/group) and vGAT-immunoreactive punctae
711 (two-tailed unpaired *t*-test, $t_{(10)}=1.61$, $P=0.1384$; $n=6$ rats/group) per GnRH soma. (e)
712 Representative fluorescent (top) and infrared-differential-interference-contrast (IR-DIC)
713 (bottom) photomicrographs of acute brain slice from a *Gnrh::Egfp* infantile female rat,
714 showing a patched preoptic GnRH neuron (green). Scale bar: 25 μ m. (f) Left. Whole-cell
715 recording of spontaneous activity in representative GnRH neurons from control (top) and
716 BWA868C-treated (bottom) animals. Arrowhead bottom left: resting membrane potential.
717 Right. Expansion of action potentials (APs) indicated at left. Note: Control cell APs (top) were
718 driven by temporal summation of small-amplitude depolarizing events (oblique lines in
719 expansion), while APs from BWA868C-treated animals (bottom) were preceded by a smooth,
720 relatively fast depolarization without synaptic events, except in recording #3. Threshold for
721 AP generation: dashed line. (g-j) Electrophysiological cell properties: mean resting
722 membrane potential (V_m) (g: Mann-Whitney test: $U=602$, $P=0.760$; $n=37$ cells, 21 control
723 rats; $n=34$ cells, 14 BWA868C-treated rats), membrane resistance (R_m) (h: Mann-Whitney
724 test: $U=157$, $P=0.589$; $n=16$ cells, 8 control rats; $n=22$ cells, 7 BWA868C-treated rats),
725 voltage threshold for AP generation (VTH) (i: two-tailed unpaired *t*-test, $t_{(18)}=0.963$, $P=0.348$;
726 $n=10$ cells, 8 control rats; $n=10$ cells, 8 BWA868C-treated rats), spontaneous firing rate (j:
727 Mann-Whitney test: $U=285.5$, $P<0.0001$; $n=37$ cells, 21 control rats; $n=34$ cells, 14
728 BWA868C-treated rats). Values: means \pm s.e.m.; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

729

730 **Figure 8. Inhibition of DP1 signaling in the infantile period leads to perturbed estrous**

731 **cyclicity. (a)** Examples of estrous cycles in two control (*upper panels*) and two BWA-treated

732 animals (*lower panels*). Red dots: first estrus. D, diestrus; E, estrus; P, proestrus; vo, vaginal

733 opening. **(b)** Age at vaginal opening in BWA868C (BWA)-treated and control animals (two-

734 tailed unpaired *t*-test, $t_{(17)}=0.837$, $P=0.414$; $n=9,10$ rats/group). **(c)** Quantification of time

735 spent in the different phases of the estrous cycle during the first 15 days after vaginal

736 opening in control and BWA-treated animals (Diestrus, two-tailed unpaired *t*-test, $t_{(17)}=-1.160$,

737 $P=0.262$; Proestrus, Mann-Whitney test: $U=12.5$, $P=0.006$; Estrus, Mann-Whitney test:

738 $U=71.5$, $P=0.023$; $n=9,10$ rats/group). **(b-c)** Values: means \pm s.e.m. * $P<0.05$; ** $P<0.01$.

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- 897
- 898

899 METHODS

900

901 **Animals.** Sprague-Dawley rats were obtained from Janvier Laboratories (Saint Berthevin,
902 France). GnRH-EGFP transgenic rats, which express the enhanced green fluorescent
903 protein (EGFP) under the control of the rat GnRH promoter (NBRP Rat No: 0469, The

904 National BioResource Project for the Rat, Japan)^{24, 63} was a kind gift from Dr. Masakatsu
905 Kato (University of Tokyo Health Sciences, Tokyo, Japan). Wild-type and transgenic mice
906 expressing a dominant-negative form of the ErbB4 receptor in astrocytes (*GFAP::DN-ErbB4*)
907 were generated as described previously³⁷. The animals were maintained at 23-25°C on a
908 12-h light-dark cycle, with food and water available *ad libitum*. Animal studies were approved
909 by the Institutional Ethics Committee for the Care and Use of Experimental Animals of the
910 University of Lille (APAFIS#2617-2015110517317420 v5). The bisphenol A treatment
911 experiment was performed on Wistar rats purchased from the University of Liège and carried
912 out with the approval of the Belgian Ministry of Agriculture and the Ethical Committee at the
913 University of Liège. Animals were raised in polypropylene cages (Ref 1291H006, Tecnilab,
914 323 Netherlands), fed with low phytoestrogen chow (V135 R/Z low phytoestrogen pellets,
915 SSNIFF 324 Diet, Netherlands). Water was supplied in glass bottles to reduce EDC
916 contamination. All experiments were performed in accordance with the guidelines for animal
917 use specified by the European Union Council Directive of September 22, 2010 (2010/63/EU).
918 All experiments were performed in female animals, except the preparation of primary cultures
919 of preoptic progenitors, for which both sexes were used.

920

921 **BrdU injections.** Unless otherwise stated, animals received a single intraperitoneal (i.p.)
922 injection of BrdU (Sigma) at a dose of 300 mg/kg body weight at different postnatal ages and
923 were allowed to survive for 2h, 24h, 7 days or \geq 60 days. A group of rats received 3 i.p.
924 injections of BrdU (150 mg/kg) per day for 3 consecutive days (P6-P8) and were sacrificed at
925 P15.

926

927 **Stereotactic brain injection of paclitaxel-loaded microparticles.** The preparation,
928 physicochemical characterization and drug release kinetics of paclitaxel-loaded, poly(D,L-
929 lactic-co-glycolic acid) (PLGA)-based microparticles have been described¹¹. Briefly,
930 Paclitaxel (Zyo Pharma Trade, Hamburg, Germany) was encapsulated into PLGA-based

931 microparticles at a drug loading of 40% (w/w) (Carlina Technologies, Angers, France). Drug-
932 free microparticles were prepared accordingly without adding paclitaxel. Female rats at P6 or
933 P7 ($n = 35$) were anesthetized with an i.p. dose of a mixture of ketamine hydrochloride
934 (50 mg/ml; 60 mg/kg) and xylazine hydrochloride (2%; 10 mg/kg), secured in a stereotaxic
935 apparatus, and injected medially with 1 μ l of a suspension of 50 μ g paclitaxel-loaded or drug-
936 free microparticles in sterile water using a microsyringe with a 21 gauge needle (Hamilton,
937 Reno, NV) into the preoptic region of the hypothalamus, dorsal to the MePO (bregma:
938 0.0 mm, 6 mm deep from the skull)⁶⁴. All animals received an i.p. injection of BrdU at P8 and
939 were sacrificed at adult age. Brains were processed for immunofluorescent labeling of BrdU,
940 GnRH and GFAP, and cell nuclei counter-staining using the nuclear marker Hoechst or DAPI
941 to determine the implantation site of microparticles. Animals with an implantation site located
942 outside the preoptic region were excluded from the analysis.

943

944 **Bisphenol-A treatment.** Exposure to bisphenol A (BPA) was performed as described
945 previously¹⁶. Briefly, female pups were subcutaneously injected (0.05 mL) with either vehicle
946 (corn oil) or BPA (Ref 23,9658; Sigma-Aldrich) at a dose of 25 ng/kg.d. Injections were given
947 every 24 hours from P1 to P15. All animals received an i.p. injection of BrdU at P8 and were
948 sacrificed 2 hours or 7 days later. Brains were extracted and processed for
949 immunofluorescent staining of BrdU and GnRH.

950

951 **Stereotaxic brain infusions of BWA868C.**

952 *Acute injections.* Female rats received an i.p. injection of BrdU at P7. A group of animals ($n =$
953 4) was sacrificed 2h after the injection. The other animals were placed in a stereotaxic frame
954 (Kopf® Instruments, California) under anesthesia (isoflurane), and 2 burr holes were drilled
955 0.5 mm apart from the bregma, according to a rat brain atlas⁶⁴. A 10 μ l Hamilton syringe was
956 slowly inserted into the preoptic region (5.2 mm deep relative to the dura), and 1 μ l of a
957 solution containing 10 nM BWA868C (Cayman Chemical, Ann Arbor, Michigan, USA) ($n = 5$)
958 or vehicle (saline solution 0.9%, $n = 5$) was injected bilaterally using an infusion pump (KD

959 Scientific, Holliston, MA) over 5 min. Animals were killed 24h later and their brains processed
960 for immunofluorescent labeling of BrdU and GnRH.

961 *BWA868C implants.* Female rats were placed in a stereotaxic frame under anesthesia, and
962 one burr hole was drilled at the level of the bregma as detailed above. A stainless steel 5 mm
963 cannula filled at its tip with BWA868C (10 nM) or vehicle mixed with melted cocoa butter was
964 stereotaxically implanted in the preoptic region at the level of the bregma, as previously
965 described⁶⁵. After implantation, the cannulas were fixed to the skull with superglue, and the
966 skin wound sutured. Blood was collected via submandibular bleeding from animals at P12 for
967 plasma FSH measurements using a procedure described previously by others⁶⁶.

968

969 **Physiological measurements.**

970 *Puberty onset and estrous cyclicity.* Animals were monitored daily for imperforation of the
971 vaginal membrane (i.e. vaginal opening). After vaginal opening, vaginal smears were
972 performed daily and analyzed under an inverted microscope to identify the specific day of the
973 estrous cycle. The percentage of complete estrus cycles was calculated as the number of
974 occurrences of the sequence “Diestrus-Diestrus-Proestrus-Estrus”, defined as a complete
975 estrus cycle, divided by the theoretical maximum possible number of complete estrus cycles
976 over the recorded period of time for each animal (> 2 weeks).

977 *Follicle-stimulating hormone level measurements.* Serum FSH levels were measured
978 using radioimmunoassay as previously described⁴. The accuracy of hormone measurements
979 was confirmed by the assessment of rodent serum samples of known concentration (external
980 controls).

981

982

983 **Electrophysiology.**

984 *Brain slices preparation.* Electrophysiological recordings were performed on living brain
985 slices containing the preoptic region from 10- to 15-days-old GnRH-EGFP transgenic rats.
986 Rats were anesthetized with isoflurane and decapitated. The brain was removed and rapidly
987 placed in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 120 NaCl, 3.2 KCl,

988 1 NaH₂PO₄, 26 NaHCO₃, 1 MgCl₂, 2 CaCl₂, 10 glucose (300 mOsm, pH 7.4) and bubbled
989 with 95% O₂-5% CO₂. 200- μ m coronal slices were cut using a vibratome (VT1200; Leica).
990 Slices were incubated at 34°C in oxygenated aCSF for a recovery period of 1 hour, and then
991 at room temperature until recording.

992 *Patch-clamp recording.* For patch-clamp recording, individual brain slices were placed in a
993 submerged recording chamber (Warner Instruments), immobilized by a nylon grid and
994 continuously perfused at 3 ml/min with oxygenated aCSF maintained at 32.8°C by a heater
995 controller (TC-344C; Warner Instruments). GnRH neurons were visualized and identified
996 under 10x and 40x magnification using an upright fluorescent microscope with infrared
997 differential interference contrast (Leica DM-LFSA) and an ORCA-Flash 4.0 digital CMOS
998 camera (Hamamatsu). Recording pipettes were pulled from borosilicate glass capillaries (1.5
999 mm outer diameter; 1.12 mm inner diameter; World Precision Instruments) using P1000
1000 Flaming Brown puller (Sutter Instruments Co) and had resistance from 7 to 9 M Ω when filled
1001 with an internal solution containing (in mM): 140 K-gluconate, 10 KCl, 1 EGTA, 2 Na₂-ATP
1002 and 10 HEPES, pH 7.3 with KOH. Pipettes were placed in contact with GnRH neurons using
1003 a PCS-5400 micromanipulator (Thorlabs). Whole-cell patch-clamp recordings were
1004 performed in current-clamp mode using a Multiclamp 700B Amplifier, digitized with the
1005 Digidata 1322A interface and acquired with pClamp 10.2 software (Molecular Devices). Data
1006 were filtered at 1 kHz and sampled at 5 kHz. Recordings were analyzed with Clampfit 10.2
1007 from pClamp software (Molecular Devices). Resting membrane potential, spontaneous firing
1008 rate, membrane resistance, voltage threshold for action potential generation, full amplitude
1009 and half width duration of action potentials, after-hyperpolarizing potential amplitude and
1010 duration, and time constant were determined as previously described^{32, 67}. Only cells which
1011 showed less than 20% change in access resistance throughout the recording were included
1012 in this study. Junction potential was corrected in the data analysis.

1013 *Biocytin labeling of preoptic astrocytes.* Slices were incubated during 30 minutes in 100 nM
1014 sulforhodamine 101 (SR101, Sigma), a fluorescent dye that is selectively taken up by
1015 astrocytes, and were pretreated for 20 minutes with 50 μ M carbenoxolone (Sigma) to inhibit

1016 gap junctions. Biocytin (2 mg/ml, Sigma) was added to the internal solution and astrocytes
1017 were patched in whole-cell configuration for 20 minutes. **Electrical membrane properties of**
1018 **astrocytes were measured in voltage-clamp mode by applying a series of voltage pulses**
1019 **from -100 mV to +100 mV (300 ms, 10 mV increments) from a holding potential of -80 mV as**
1020 **previously described³⁰. Astrocytes showed typical glial cell membrane properties including a**
1021 **highly polarized membrane potential and a linear current-voltage (I-V) relationship³⁰.** Slices
1022 were fixed with 4% paraformaldehyde overnight at 4°C, incubated with Streptavidin Alexa
1023 Fluor 647 (1:250, Invitrogen) in PBS containing 0.5% Triton X-100 overnight at 4°C,
1024 counterstained with DAPI (1:1000) and mounted on glass slides with Mowiol.

1025

1026 **Preoptic neurosphere cultures.** The preoptic region of female rats aged 10 or 19 days was
1027 dissected and minced on 80 µm nylon mesh (Buisine, Clermont de l'Oise, France). The
1028 dissected region corresponded to a 1-mm-thick triangular tissue located behind the diagonal
1029 band of Broca just before the OVLT, i.e., the beginning of the third ventricle, and the optic
1030 chiasm. We checked that our dissection excluded the subventricular zone of the lateral
1031 ventricle. The cell suspension was transferred to a culture flask (BD Biosciences, San Jose,
1032 CA) and cultured in neurosphere medium composed of Dulbecco's Modified Eagle's Medium
1033 (DMEM)/F-12 with L-glutamine, 15 mM HEPES (#31330-038) supplemented with 2% (v/v) B-
1034 27 (#17504-044), 20 ng/ml EGF (human recombinant; #PHG0311), 20 ng/ml FGF2 (human
1035 recombinant; #13256-029), 2 mM L-glutamine (25030-024), and 100 units of penicillin and
1036 100 µg of streptomycin/ml (#15140-122) (all from Gibco, Carlsbad, CA) under a humid
1037 atmosphere of 5% CO₂-95% air at 37°C. Cells were grown in hypoxic conditions by covering
1038 flask caps with parafilm. Neurospheres were formed after ~1 week. Thereafter, the
1039 neurospheres were collected twice a week through centrifugation, dissociated by mechanical
1040 trituration and passaged in half-renewed neurosphere medium to form further generations of
1041 neurospheres. Neurospheres were kept for up to 31 passages, the longest time examined.

1042 *Clonogenicity.* To evaluate the generation of secondary neurospheres from single cells,
1043 primary neurospheres were dissociated into single cells by either mechanical dissociation

1044 through 20 µm nylon mesh (Buisine) or enzymatic digestion using StemProAccutase Cell
1045 Dissociation Reagent (Gibco) for 10 min at room temperature. Cells were plated in 96-well
1046 culture plates at a density of 50, 75 or 100 cells per well in 50 µl of neurosphere medium.
1047 Wells containing cell aggregates 24h after plating were excluded from the analysis. Cultures
1048 were fed with 100 % (v/v) fresh neurosphere medium every 3 days until day 10, when wells
1049 were examined for the presence of neurospheres.

1050 *Differentiation experiments.* To evaluate their differentiation potential, neurospheres were
1051 transferred to 24-well plates on poly-L-lysine (0.01%; #P4832, Sigma)-coated glass
1052 coverslips and grown in DMEM/F12 with L-glutamine, 15 mM HEPES (#31330-038)
1053 supplemented with 10% (v/v) fetal bovine serum (#10270106), 2 mM L-glutamine, and 100
1054 units of penicillin and 100 µg of streptomycin/ml (all from Gibco) for 7 days.

1055

1056 **Adherent cultures of preoptic progenitors.** The preoptic region of rats aged 1 or 2 days
1057 was dissected as described above and minced on 20 µm nylon mesh (Buisine). The cell
1058 suspension was transferred to a culture flask (BD Biosciences) coated with poly-D-lysine (10
1059 µg/ml, P7405, Sigma) and laminin (10 µg/ml, L6274, Sigma) and cultured in neurosphere
1060 medium under a humid atmosphere of 5% CO₂-95% air at 37°C. The medium was changed
1061 every 3 to 4 days. Cells were passaged once, when 70-80% confluence was reached, and
1062 were then used for migration assays, snap frozen in dry ice for gene expression analysis or
1063 seeded on 24-well plates glass coverslips for proliferation assays or immunocytochemical
1064 characterization.

1065

1066 **GnV3 cell culture and treatment.** Cells were grown in Neurobasal-A medium (10888-022)
1067 supplemented with 2% (v/v) B-27, 100 units of penicillin and 100 µg of streptomycin/ml, 25
1068 mM GlutaMAX (35050-038), 1% fetal bovine serum, 50 µg/ml FGF2 (all from Gibco) and 1
1069 µg/µl doxycycline (D-9891, Sigma) as described⁶⁸. All experiments were performed on GnV3
1070 cells at their 13th or 14th passage.

1071 *Preparation of hypothalamic astrocyte-conditioned medium.* Primary astrocyte cultures
1072 were prepared from the hypothalamus of 1-2-day-old rat pups and grown in DMEM/F12
1073 medium (#31330-038) supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine,
1074 and 100 units of penicillin and 100 µg of streptomycin/ml (all from Gibco) as previously
1075 described³⁶. After reaching confluence (i.e., after ~7-10 days), contaminants were removed
1076 by overnight shaking. The growth medium was replaced by astrocyte-defined medium (ADM)
1077 composed of DMEM (41965-039) supplemented with 2 mM L-glutamine, 5 µg/ml insulin (I-
1078 5500, Sigma) and 100 µM putrescine (P-5780, Sigma). After two days, the culture medium
1079 (now ACM, astrocyte-conditioned medium) was collected, centrifuged at 1,000 rpm and the
1080 supernatant was kept at -80°C until use.

1081 *Treatment of GnV3 cells with hypothalamic astrocyte-conditioned medium.* One day
1082 before treatment, GnV3 cells were plated at a density of 2×10^5 cells/cm² in their growth
1083 medium. Cells were treated with ADM (control condition) or ACM for a total of 4 days, with a
1084 culture medium renewal after every 2 days. At the end of the 4-day treatment, GnV3 cells
1085 were collected in Trizol (Invitrogen) for microarray analysis or lysis buffer (Qiagen) for RT-
1086 qPCR analysis, and stored at -80°C until use. Treatment of GnV3 cells was performed in
1087 three experiments using ACM from three different primary astrocyte cultures.

1088

1089 ***In vitro migration assay.*** Boyden microchemotaxis chambers were used according to the
1090 manufacturer's instructions (Neuro Probe, Gaithersburg MD, USA). In brief, primary preoptic
1091 progenitors were harvested, re-suspended in control medium (Neurobasal-A medium
1092 supplemented with 2% (v/v) B-27, 2 mM L-glutamine, and 100 units of penicillin and 100 µg
1093 of streptomycin/ml (all from Gibco)) and plated in 24-well plate chambers at a density of
1094 150,000 cells per well on the open-bottom wells of the upper compartment, previously coated
1095 with poly-D-lysine and laminin. The two compartments of each well were separated by a
1096 polyvinylpyrrolidone-free polycarbonate porous membrane (8 µm pore size). The lower
1097 chamber was filled with control medium containing GnRH (100 ng/ml, GeneCust, Ellange,
1098 Luxembourg), PGD2 (1 µM, Sigma), galanin (2 µM, Tocris Bioscience, Bristol, UK),

1099 glutamate (10 μ M, Sigma), GABA (2 μ M, Sigma) or EGF (100 ng/ml, Gibco). BWA868C (10
1100 nM or 1 μ M) was added to the cell suspension in the upper compartment. After 18h of
1101 incubation in 5% CO₂, 95% air at 37°C, cells attached to the upper side of the filter were
1102 mechanically removed with a cotton swab. Cells that had migrated to the lower side were
1103 fixed with 4% paraformaldehyde (30 minutes at room temperature) and stained with Hoechst
1104 (1:10,000). Cells were photographed with a 20x objective and counted using ImageJ 1.52p
1105 software (NIH, Bethesda).

1106

1107 ***In vitro* proliferation assay.** Primary preoptic progenitors were transferred to 24-well plates
1108 on poly-D-lysine/laminin-coated glass coverslips and grown in neurosphere medium. Once
1109 cells were 70-80% confluent, they were transferred to neurosphere medium devoid of FGF2
1110 (starvation medium) for 24h prior to treatment with GnRH, PGD2, galanin, glutamate, GABA
1111 (same concentrations as in migration assays) or FGF2 (100 ng/ml) in starvation medium for
1112 24h. Coverslips were fixed and processed for Ki67 immunolabeling.

1113

1114 **Immunofluorescence labeling**

1115 *Tissue preparation.* Animals were anesthetized by i.p. injection of pentobarbital (70
1116 mg/kg). They were perfused transcardially with a rinse of saline solution (0.9% NaCl),
1117 followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer(PB) (pH 7.4). The
1118 brains were removed and immersed in the same fixative for 2-3h at 4°C. They were then
1119 transferred to PB containing 20% sucrose until they had sunk, embedded in Tissue Tek®
1120 (Sakura Finetek, Villeneuve d'Ascq, France), and frozen in liquid nitrogen. Frozen 14- μ m
1121 coronal sections containing the preoptic region (0.20 mm to -0.30 mm relative to bregma)⁶⁴
1122 were prepared using a cryostat (CM3050S, Leica, Nussloch, Germany) and mounted on
1123 chrome-alum-gelatin coated slides, air-dried and subjected to immunolabeling.

1124 *Immunohistofluorescence.* The sections were subjected to microwave pretreatment in a
1125 solution of sodium citrate 0.01 M (pH 6) for 4 minutes at 800 W followed by 2 cycles 5
1126 minutes each at 400 W for antigen retrieval. After cooling at room temperature and washing

1127 in PB, sections were incubated with primary antibodies (**Supplementary Table 1**) diluted in
1128 PB containing 0.9% sodium chloride, 0.3% Triton X-100 and 2% normal donkey serum
1129 (PBSTS) overnight at 4°C. Sections were then incubated with appropriate secondary
1130 antibodies at room temperature for 1h (**Supplementary Table 2**). After nuclei staining with
1131 Hoechst or DAPI (1/1,000), sections were coverslipped in Mowiol mounting medium (20%
1132 Mowiol, 2.5% DABCO in Tris 0.2 M pH 8.5). Control sections were incubated in the absence
1133 of primary antibody. Omission of the primary antibody resulted in no staining.

1134 *Immunocytofluorescence.* Coverslips were fixed in 4% paraformaldehyde (15 min at room
1135 temperature), incubated with PBS 0.1 M containing 0.1% Triton X-100 and 0.3% normal
1136 donkey serum for 30 minutes at room temperature, and subjected to immunofluorescent
1137 labeling following the same procedure as for tissue sections. For O4 immunodetection, the
1138 anti-O4 antibody (**Supplementary Table 1**) was applied to living cells for 2h at 37°C before
1139 fixation.

1140

1141 **Fluorescent *in situ* hybridization.** FISH was performed on frozen brain sections of the
1142 preoptic region of female rats at P8, P12 and P20 with the RNAscope® Multiplex Fluorescent
1143 Kit v2 according to the manufacturer's protocol (Advanced Cell Diagnostics, Inc., Newark,
1144 CA, USA). Specific probes were used to detect *Ptgds* (486051, NM_013015.2, target region
1145 2 - 702), *Ptgdl* (486041, NM_022241.1, target region 224 - 1157), *Gnrh1* (502431-C2,
1146 NM_012767.2, target region 2 - 454) and *Sox2* (502441-C3, NM_001109181.1, target region
1147 51 - 2079) mRNAs. Hybridization with a probe against the *Bacillus subtilis* dihydrodipicolinate
1148 reductase (*dapB*) gene (320871) was used as negative control.

1149

1150 **Microscopy**

1151 Analysis of sections and acquisition of images were done using an Axio Imager Z2 Apo-
1152 Tome microscope equipped with a motorized stage (Zeiss, Germany) and an ORCA-Flash
1153 4.0 V2 camera (Hamamatsu, Japan) driven by the Zen 2.3 (blue edition) imaging software
1154 (Zeiss). For FISH experiments and immunofluorescence labeling for vGluT2 and vGAT,

1155 acquisition of images was performed using an inverted confocal microscope (LSM 710,
1156 Zeiss, Jena, Germany). High magnification photomicrographs were acquired with a 63x
1157 objective (NA 1.4) using the Airyscan detector (Zeiss). Images to be used for figures were
1158 pseudocolored, adjusted for brightness and contrast and merged using Adobe Photoshop
1159 22.1.0 (Adobe Systems, San Jose, CA).

1160

1161 **Microscopic analyses.** All analyses were done in the preoptic region, as defined in the
1162 Swanson stereotaxic rat brain atlas, between plate 17 (i.e., beginning of the OVLT) and plate
1163 20 (i.e., crossing of the anterior commissure)⁶⁹, with a particular attention to the MePO,
1164 where most GnRH neurons that stimulate the LH surge are located⁷⁰. Analyses were made
1165 on one out of every 5 sections. Estimation of total cell populations in the preoptic region was
1166 obtained by multiplying the number of cells per section by the total number of sections per
1167 preoptic region, and then correcting for split nuclei⁷¹.

1168 *Morphological association between GnRH neurons and BrdU⁺ cells.* Quantification was
1169 made under the fluorescent microscope at the 40x objective. A BrdU⁺ cell was considered
1170 morphologically associated with a GnRH neuron when it was located at a distance \leq the size
1171 of the BrdU⁺ nucleus (i.e., $\leq 10 \mu\text{m}$) from the GnRH neuron cell body with no interposed cell
1172 nucleus in between (**Supplementary Fig. 3**).

1173 *Number of cell nuclei at the injection site of microparticle-injected rats.* The total number of
1174 Hoechst-stained nuclei was automatically quantified using the ImageJ software on
1175 microphotographs of the injection site taken at the 20x objective. Quantification represents
1176 the mean number of Hoechst-stained nuclei in a 20x microscopic field, corresponding to a
1177 surface area of 0.5 mm^2 , counted on 3 sections per animal ($n = 12$).

1178 *Number of BrdU⁺ cells at the injection site of microparticle-injected rats.* Analysis was
1179 performed on photomontages of the preoptic region taken at the 20x objective. Quantification
1180 represents the mean number of BrdU⁺ cells in a 1 mm square centered on the injection site,
1181 counted on 3 to 4 sections per animal ($n = 13$).

1182 *Quantification of vGluT2 and vGaT appositions to GnRH neuron cell bodies.* Analysis was
1183 performed on high magnification images of GnRH neuron somata located at the level of the
1184 OVLT using the IMARIS 9.3 software (Bitplane) as described before ⁷². Briefly, the number of
1185 vGluT2- or vGaT-immunoreactive punctae closely apposed with the GnRH neuron soma was
1186 quantified on 3D reconstructions from raw confocal image z-stacks using a channel of
1187 colocalization. A punctum was considered apposed when no black pixels were visible
1188 between the GnRH and the vGluT2 or vGaT fluorescence. The value was normalized to the
1189 number of optical sections through the z-depth of the neuron and the surface area of the
1190 neuronal soma. The resulting value was multiplied by the average number of optical sections
1191 and the average surface area of all neurons analyzed to provide a number of punctae per
1192 GnRH soma.

1193

1194 **Western blot analyses.** Preoptic primary progenitors were starved for 24h in DMEM/F-12
1195 with L-glutamine, 15 mM HEPES supplemented with 2 mM L-glutamine, 100 units of
1196 penicillin and 100 µg of streptomycin/ml, 5 µg/ml insulin (Sigma) and 16 µg/ml putrescine
1197 (Sigma). The cells were treated with PGD2 1 µM and/or BWA868C (10 nM or 1 µM) in fresh
1198 medium for 7 minutes and snap-frozen on dry ice. Proteins were extracted and subjected to
1199 size-fractionation as previously described ⁴. The proteins were then transferred onto 0.2 µm
1200 pore-size nitrocellulose membranes (Invitrogen) using the NuPAGE system (Invitrogen).
1201 Membranes were blocked for 1 h in blocking buffer (Tris buffer saline (0.05 M Tris, pH 7.4,
1202 0.15 M NaCl) with 0.05% Tween 20 (TBST) and 5% nonfat milk) at room temperature and
1203 incubated overnight at 4°C with the appropriate primary antibody diluted in blocking buffer
1204 (rabbit polyclonal anti-phospho-p44/42 MAPK (Thr202/Tyr204) (pErk) #9101, 1:1,000; rabbit
1205 polyclonal anti-p44/42 MAPK (Erk) #9102, 1:1,000, Cell Signaling Technology, Beverly, MA,
1206 USA). After washing with TBST, membranes were exposed to horseradish peroxidase-
1207 conjugated secondary antibodies (goat anti-rabbit IgG, PI1000, 1:2000, Vector) diluted in
1208 blocking buffer for 1 h at room temperature. Immunoreactions were visualized using the ECL

1209 detection kit (NEL101; PerkinElmer, Boston, MA). Immunoblots were scanned using a
1210 desktop scanner (Epson Expression 1680 PRO) and Adobe Photoshop.

1211

1212 **Isolation of hypothalamic GnRH neurons using fluorescence-activated cell sorting.**

1213 The preoptic regions of *Gnrh::Egfp* transgenic rats were microdissected and enzymatically
1214 dissociated using a Papain Dissociation System (Worthington, Lakewood, NJ) to obtain
1215 single-cell suspensions. FACS was performed using an ARIA SORP cell sorter cytometer
1216 and FACSDiva 8.0 software (BD Biosciences). Data were analyzed using the Kaluza 2.0
1217 software (Beckman Coulter). The sort decision was based on measurements of EGFP
1218 fluorescence (excitation: 488 nm, 50 mW; detection: GFP bandpass 530/30 nm,
1219 autofluorescence bandpass 695/40 nm) by comparing cell suspensions from the preoptic
1220 region and from the cerebral cortex of *Gnrh::Egfp* animals. A total of 174 ± 20 (range, 44 –
1221 700) EGFP-positive cells were sorted per animal and directly lysed into 10 μ l of extraction
1222 buffer containing 0.1% Triton[®] X-100 (Sigma) and 0.4 unit/ μ l RNaseOUT™ (Life
1223 technologies).

1224

1225 **Quantitative RT-PCR analyses.** Gene expression analyses were performed on FACS-
1226 sorted GnRH neurons, primary preoptic progenitor cultures and GnV3 cells. For primary
1227 preoptic progenitors, RNAs were extracted as follows: 100 μ l of Trizol (Life Scientific,
1228 Carlsbad CA, USA) were used to lyse cells before adding 100 μ l of chloroform (Merck,
1229 Darmstadt, Germany) and centrifuging at 12,000 g for 15 minutes at 4°C. The aqueous
1230 phase was carefully collected and added to isopropanol at 1:1 before centrifuging at 12,000 g
1231 for 10 minutes at 4°C. The aqueous phase was then discarded and the pellet washed in 70%
1232 ethanol. After centrifugation at 12,000 g for 5 minutes at 4°C, the pellet was left to air-dry and
1233 then diluted in 10 μ l DEPC water. The purity and quantity of RNA were determined by UV
1234 spectroscopy (Nanodrop 1000, Thermo Scientific, Waltham MA, USA). Messenger RNAs
1235 obtained from FACS-sorted GnRH neurons or primary progenitors were reverse transcribed
1236 using SuperScript[®] III Reverse Transcriptase (Life Technologies). For sorted cells, a linear

1237 preamplification step was performed using the TaqMan®PreAmp Master Mix Kit protocol
1238 (Applied Biosystems). Real-time PCR was carried out on Applied Biosystems 7900HT Fast
1239 Real-Time PCR System using exon-boundary-specific TaqMan® Gene Expression Assays
1240 (Applied Biosystems) (**Supplementary Table 3**). Gene expression data were analyzed using
1241 SDS 2.4.1 and Data Assist 3.0.1 software (Applied Biosystems) with R45S and actin as
1242 control housekeeping mRNAs following a standardized procedure ⁷³. For GnV3 cells, RNAs
1243 were purified using the RNeasy Mini Kit (Qiagen, Basel, Switzerland) according to the
1244 manufacturer's instructions. The solution was treated with DNase (RNase-Free DNase Set,
1245 Qiagen, Basel, Switzerland) and eluted with 30 µl of water. RNA concentrations were
1246 determined with a Nanodrop® (ND-1000 Spectrophotometer, Witec, Luzern, Switzerland).
1247 Two hundred ng of RNA was reverse transcribed using the RT High Capacity RNA-to-cDNA
1248 Kit (Applied Biosystems, Rotkreuz, Switzerland). Real-Time PCR analysis was performed on
1249 2 µl of cDNA using Power SYBR Green Taq polymerase master mix (Applied Biosystems,
1250 Rotkreuz, Switzerland). The primer sequences used were: Ptgds forward: 5'-
1251 TGGGTCTCTTGGGATTCCA-3', Ptgds reverse: 5'-CCCCAGGAACTTGTCTTGTTG-3';
1252 polymerase 2a (Polr2a, as endogenous control) forward: 5'-
1253 GTAAGGGCCACTATCTTCATCATCA-3', Polr2a reverse: 5'-
1254 CCCTCATCATACTGGTCACATC-3'.

1255

1256 **Microarray hybridization and data analysis.** RNA purification and microarray hybridization
1257 were performed as previously described ²⁰. Total RNA was extracted from the cells using
1258 Trizol (Invitrogen), according to the manufacturer's instructions. Only RNA samples with RNA
1259 integrity numbers greater than 9.0 were accepted for microarray analysis. Microarray
1260 hybridization was performed with Affymetrix GeneChip Rat Gene 1.0 ST arrays according to
1261 the manufacturer's instructions and scanned with the Gene Array scanner (Affymetrix). The
1262 fluorescence images obtained were processed using the BioConductoraffy package (DAFL,
1263 Lausanne). Expression values were measured using the Robust Multi-array Average (RMA)
1264 algorithm generating expression values on a log₂ scale. RMA includes background

1265 correction, quantile normalization and probe set summary by robust regression. GnV3
1266 signature of 22,336 transcripts was generated and annotated with Gene Symbol. Genes
1267 without an Entrez annotation were filtered out. The fold change was calculated individually
1268 for each gene. Differential hybridized features were identified using Bioconductor package
1269 “limma”⁷⁴. Additionally, the false discovery rate, i.e. the expected proportion of rejected null
1270 hypotheses that are wrongly rejected, was estimated by a resampling procedure⁷⁵. Genes
1271 that showed substantial up- or down-regulation by log fold changes (FC) > 1 with a FDR <
1272 0.05 were considered as differentially expressed. For each condition (ADM or ACM),
1273 microarray analyses were performed using RNA samples derived from three wells per
1274 condition. Only the list of genes showing significant up and down-regulation is provided in the
1275 **Supplementary Data 1**.

1276

1277 **Statistics.** No statistical methods were used to pre-determine sample sizes but our sample
1278 sizes are similar to those reported in previous publications^{4, 25, 26, 37, 76}. Studies were not
1279 formally randomized and investigators were not blind to treatment group. All analyses were
1280 performed using the SigmaStat 3.5 (Systat) or GraphPad Prism 7.05 (GraphPad) software.
1281 Outliers were identified using the ROUT (Q=1%) method. Statistical differences were
1282 evaluated using unpaired two-sided Student’s *t*-tests for comparison of two groups and one-
1283 or two-way analysis of variance (ANOVA) with Tukey’s *post hoc* tests for comparison of more
1284 than two groups. When the criterion for normality or equal variances was not met, the Mann-
1285 Whitney Rank Sum Test or the Kruskal-Wallis One Way ANOVA on Ranks Test was used to
1286 compare two or more groups, respectively. $P < 0.05$ was considered significant.

1287

1288 **Data availability.** Uncropped Western blots and Source Data Files for transcriptomic
1289 analysis of GnV3 cells are provided as Extended data. Additional data that support the
1290 findings of this study are available from the corresponding author upon request.

1291

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4 **GnRH neurons recruit astrocytes in infancy to facilitate network**
5 **integration and sexual maturation**

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51 **ABSTRACT**

52 Neurons producing gonadotropin-releasing hormone (GnRH), which control fertility, complete
53 their nose-to-brain migration by birth. However, their function depends on integration within a
54 complex neuroglial network during postnatal development. Here, we show that GnRH
55 neurons during the infantile period use a prostaglandin-D2/DP1-receptor signaling
56 mechanism to recruit newborn astrocytes that “escort” them into adulthood, and the
57 impairment of postnatal hypothalamic gliogenesis dramatically alters sexual maturation by
58 preventing this recruitment, a process mimicked by the endocrine disruptor bisphenol-A. DP1
59 signaling inhibition in the infantile preoptic region, where GnRH cell bodies reside, disrupts
60 the correct wiring and firing of GnRH neurons, alters minipuberty or the first activation of the
61 hypothalamic-pituitary-gonadal axis during infancy, and delays the timely acquisition of
62 reproductive capacity. These findings uncover a previously unknown neuron-to-neural-
63 progenitor communication pathway and demonstrate that postnatal astrogenesis is a basic
64 component of a complex set of mechanisms used by the neuroendocrine brain to control
65 sexual maturation.

66

67 **INTRODUCTION**

68 Neuroendocrine GnRH neurons, which ensure species survival by controlling the
69 ability of individuals to reproduce, are principally located in the preoptic region, a basal
70 forebrain region harboring diverse neuronal populations¹. Unlike other hypothalamic neurons
71 driving bodily functions, GnRH neurons are not born in the brain but originate in the olfactory
72 placode, and migrate from the nose to the brain during embryogenesis². Although this
73 migration is complete at birth, GnRH neurons recruit other neuronal populations, notably
74 those expressing kisspeptin and neuronal nitric oxide synthase (nNOS), during postnatal
75 development to establish a complex circuit that fine-tunes GnRH production and release by
76 providing precisely coordinated excitatory and inhibitory inputs^{3, 4}, thus orchestrating sexual
77 maturation, puberty and adult fertility⁵. However, the mechanisms by which GnRH neurons
78 sculpt their own microenvironment and establish the connections necessary to control
79 reproduction remain unexplored.

80 The GnRH network is not exclusively neuronal but includes at least two glial cell
81 types: tanycytes, which control the access of GnRH neurosecretory terminals to the
82 pericapillary space of pituitary portal vessels, into which the hormone is released, and
83 astrocytes, whose communication with GnRH neurons is essential for reproductive function⁶.
84 Glial cells, especially astrocytes, also help generate physiological responses by forming a
85 hub with neurons that integrates internal and external cues within the adult brain⁶.
86 Additionally, they act as cartographers of synaptogenesis and neural circuit formation in the
87 developing central nervous system^{7, 8}. Conversely, studies in the neocortex suggest that
88 astrocyte formation is regulated by extrinsic signals generated by neurons, the development
89 of which precedes gliogenesis⁹. We therefore asked whether GnRH neurons could
90 themselves be responsible for the circuit formation subtending reproductive function in
91 adulthood by establishing a glial entourage during postnatal development.

92 Here, we demonstrate that GnRH neurons in the infantile period attract newborn
93 progenitor cells that differentiate into astrocytes and remain closely associated with them,
94 essentially “escorting” these neurons into adulthood. Additionally, local inhibition of infantile

95 gliogenesis in the preoptic region, which harbors GnRH neuronal somata, delays puberty.
96 The interaction between GnRH neurons and newborn astrocytes underlying correct sexual
97 maturation depends on reciprocal prostaglandin-mediated signaling, and especially the
98 expression of the prostaglandin D2 receptor DP1 by these newborn cells. Our findings thus
99 identify a novel neuron-to-neural-progenitor communication mechanism that unfolds during
100 the infantile period and times the pubertal activation of the reproductive axis.

101

102 **RESULTS**

103 **GnRH neurons assemble a glial entourage during early infancy**

104 GnRH neuronal somata in rats are loosely distributed between the medial
105 septum/diagonal bands of Broca and the preoptic nuclei, being densest in the median
106 preoptic nucleus (MePO) at the level of the organum vasculosum laminae terminalis (OVLT).
107 To determine if the birth of new cells in the vicinity of GnRH neurons shapes their
108 microenvironment, we used the thymidine analog, bromodeoxyuridine (BrdU) to map cell
109 proliferation in the OVLT and its surroundings, referred to hereafter as the “preoptic region”,
110 in female rats at different stages of postnatal development [postnatal day (P)1 and P5:
111 neonatal period; P8 and P12: infantile period; P20 and P24: juvenile period, i.e. after
112 weaning] (**Fig. 1a**). In agreement with a previous report¹⁰, more cells incorporated BrdU in
113 the infantile versus the juvenile period (**Supplementary Fig. 1a,b**). BrdU colocalization with
114 proliferating-cell nuclear antigen (PCNA), an endogenous cell-cycle marker (**Supplementary**
115 **Fig. 1c**), the occurrence of pairs of closely apposed BrdU⁺ nuclei (**inset in Fig. 1b**,
116 **Supplementary Fig. 1d**), and doubling of the BrdU⁺ cell population between 2h and 24h
117 after BrdU injection (**Supplementary Fig. 1e**) strongly suggested that BrdU-labeled cells
118 were entering the cell cycle. Seven days after BrdU injection, most newborn cells expressed
119 either glial fibrillary acidic protein (GFAP) in conjunction with an astrocyte-like morphology,
120 denoting their differentiation into astrocytes, or adenomatosis polyposis coli (APC) – a
121 marker of oligodendrocytes, while a very small number expressed the neuronal marker,
122 HuC/D (**Supplementary Fig. 2a-j**). The presence of multipotent progenitors in the postnatal

123 preoptic region was confirmed *in vitro* by culturing clonally-derived neurospheres capable of
124 differentiating into all three neural cell types (**Supplementary Fig. 2k-m**).

125 Interestingly, co-immunolabeling revealed the close proximity of some GnRH
126 neuronal cell bodies to BrdU⁺ newborn cells (**Fig. 1b**). Biocytin filling of patch-clamped
127 astrocytes in living brain slices from infantile rats showed that preoptic astrocytes had a
128 radius of 20-40 μm (**Supplementary Fig. 3a**). We therefore decided to look for newborn
129 progenitors, which presumably have smaller arborizations, at a conservative distance of 10
130 μm from GnRH neurons. Two hours after BrdU injection, GnRH neurons accompanied by
131 BrdU⁺ progenitors were more frequent in neonatal and infantile rats than in juvenile animals
132 (**Fig. 1c**). GnRH neurons preferentially associated with proliferating cells, since 12.8% of
133 GnRH neurons were juxtaposed to BrdU⁺ cells 2h after BrdU injection at P8, versus only
134 3.6% of non-GnRH neurons (**Fig. 1c,d**). At this time-point, these proximal BrdU⁺ cells
135 expressed the progenitor cell marker, Sox2, but not GFAP (**Fig. 1e-g**). Twenty-four hours
136 later, 27.2% of GnRH neurons were associated with BrdU⁺ cells labelled by a single injection
137 at P8 (**Fig. 1c**). This increased association persisted even after seven days, when 22.3% of
138 GnRH neurons had a companion BrdU⁺ cell (**Fig. 1c,d**). Notably, this proportion reached
139 $80.3 \pm 2.7\%$ ($n = 4$ rats) when the BrdU regimen was increased to three injections per day
140 from P6 to P8 (**Fig. 1h**). Because the number of GnRH neurons in the preoptic region
141 remained unchanged during postnatal development (367.2 ± 43.5 neurons at P8 versus 342.9
142 ± 22.3 neurons at P15, two-sided unpaired t -test, $t_{(14)} = 0.498$, $P = 0.626$; $n = 8$ rats per
143 group), the increased proportion of GnRH neurons associated with BrdU⁺ cells could either
144 stem from a global increase in the number of BrdU⁺ cells in the preoptic region after
145 proliferation (**Supplementary Fig. 1e**) or the migration of BrdU⁺ cells towards GnRH
146 neurons. We analyzed the neighborhood of GnRH neurons 2h after a single BrdU injection at
147 P8, and identified all neurons that would be considered associated with a BrdU⁺ cell 24h later
148 if the proliferation of existing BrdU⁺ cells were to give rise to a daughter cell lying within $10\mu\text{m}$
149 of the GnRH neuronal soma (**Supplementary Fig. 3b,c**). However, even under the very

150 broad assumption that every proliferative event would give rise to a newborn cell within the
151 perimeter described, the resulting theoretical percentage of GnRH neurons with associated
152 BrdU⁺ cells was still significantly lower than the actual percentage measured
153 (**Supplementary Fig. 3d**), indicating that cell division in the neighborhood of GnRH neurons
154 could not fully account for the increased proportion of GnRH neurons with a BrdU⁺
155 companion cell. GnRH neurons are thus likely attract newborn cells to their immediate
156 vicinity during the early infantile period.

157 The association between GnRH neurons and cells born at P8 was maintained until
158 adulthood (**Fig. 1c**). Notably, the increase in GnRH neurons with BrdU⁺ companions between
159 2h and 7 days after BrdU injection was seen only in the group injected at P8 (**Fig. 1c**),
160 indicating that the beginning of the infantile period is a critical time-window for GnRH neurons
161 to enrich their immediate cellular environment. Triple immunolabeling for GnRH, BrdU and
162 GFAP, APC or HuC/D showed that P8-born cells accompanying GnRH neurons mainly
163 differentiated into GFAP-expressing astrocytes (**Fig. 1g,i-k**). Physical proximity to GnRH
164 neurons did not alter the fate of P8-born cells (phenotype of BrdU⁺ cells in the vicinity of
165 GnRH neurons (n = 6-7 rats) vs. all newborn cells in the preoptic region (n = 3 rats): GFAP,
166 Mann-Whitney test: $U = 9$, $P = 0.833$; APC, Mann-Whitney test: $U = 6$, $P = 0.500$; HuC/D,
167 Mann-Whitney test: $U = 3$, $P = 0.167$) (compare **Supplementary Fig. 2j** and **Fig. 1g**).

168

169 **Blocking infantile preoptic cell proliferation delays puberty**

170 To determine if the birth of new cells in the infantile preoptic region is involved in
171 sexual maturation, we locally inhibited cell proliferation in P6 or P7 female rats by
172 stereotaxically injecting biodegradable poly(D,L-lactic-co-glycolic acid) (PLGA)-based
173 microparticles (**Fig. 2a,c**), designed to release the antimitotic compound, paclitaxel, in a time-
174 controlled manner¹¹, just above the MePO. An initial assay revealed that this release was
175 complete by 6 days, i.e. before the end of the infantile period when proliferation in the
176 preoptic area peaks (**Supplementary Fig. 4a**). Control animals were injected with blank-

177 loaded microparticles (**Fig. 2a,b**), and a single BrdU injection carried out at P8 as before in
178 both groups. We then monitored puberty onset and adult estrous cyclicity. At adulthood,
179 paclitaxel-treated animals showed decreased cell density (**Fig. 2c,d**, **Supplementary Fig.**
180 **4b,c**), fewer BrdU⁺ cells at the injection site (**Fig. 2e**, **Supplementary Fig. 4b,c**) and a lower
181 proportion of GnRH neurons associated with BrdU⁺ cells in the MePO (**Fig. 2f**), although the
182 total number of GnRH neurons in the preoptic region remained unaffected (**Fig. 2g**). In the
183 anteroventral periventricular nucleus (AVPV), a more ventral preoptic nucleus that extends
184 well beyond the OVLT caudally and relays the estrogen positive feedback¹², chronic
185 intracerebroventricular administration of another antimetabolic, AraC, during the peripubertal
186 period has been shown to block proliferation¹³. However, paclitaxel treatment did not affect
187 the number of BrdU⁺ cells, neurons or astrocytes in the AVPV (**Supplementary Fig. 5**),
188 indicating that our administration method had extremely localized antimetabolic effects.
189 Paclitaxel-treated animals exhibited a marked delay in puberty onset compared to control
190 animals, as indicated by delayed vaginal opening (**Fig. 2h,i**) and first estrus (**Fig. 2j,k**). In
191 addition, while the two milestones coincided in control animals, they were uncoupled in
192 paclitaxel-treated animals, which experienced their first estrus ~3.5 days after vaginal
193 opening (**Fig. 2l,m**), a phenomenon rarely observed in rats⁵. Notably, paclitaxel-treated
194 animals had similar body weights to control animals (**Supplementary Fig. 4d**), indicating that
195 the delay in sexual maturation was not due to a growth deficit. Paclitaxel-treated animals also
196 exhibited fewer complete 4-day estrous cycles (**Fig. 2l,n**). Taken together, these results
197 suggest that the presence of newborn cells in the environment of preoptic GnRH neurons
198 during the infantile period, rather than GnRH neurons *per se*, is necessary for the timely
199 onset of puberty and contributes to fertility in adulthood.

200

201 **Endocrine disruptors alter GnRH neuron-progenitor interaction**

202 A common adverse condition for the development and function of the gonadotropic
203 axis is exposure to endocrine-disrupting chemicals (EDCs) during perinatal life. While the
204 gonads have long been considered the main targets of EDCs, a growing number of studies

205 highlight their neuroendocrine effects, such as GnRH neuronal network alterations¹⁴.
206 Intriguingly, rat pups exposed for the first 15 days after birth to a very low but environmentally
207 relevant dose¹⁵ of the plasticizer bisphenol A (BPA; 25 ng/kg per day) tend towards delayed
208 vaginal opening¹⁶, phenocopying our paclitaxel-treated rat pups. Additionally, these BPA-
209 exposed rats also demonstrate an altered frequency of GnRH secretion at P20¹⁶, suggesting
210 a perturbation of the first postnatal activation of the hypothalamic-pituitary-gonadal (HPG)
211 axis, known as minipuberty. This phenomenon, which occurs at P12 in rats and 1 month of
212 age in humans, helps establish an adult pattern of pulsatile GnRH secretion that, in baby
213 girls and female rodents alike, triggers an FSH surge that is essential for subsequent
214 gonadal maturation and hence, fertility^{5, 17}. To verify whether the adverse effects of EDCs on
215 gonadotropic axis maturation could stem from their interference with the ability of GnRH
216 neurons to recruit neighboring cells, we treated female rat pups with 25 ng/kg per day of BPA
217 from P1¹⁶, then injected them with BrdU at P8 and assessed GnRH neuron association with
218 BrdU⁺ cells 2h and 7d later (**Fig. 3a**). Contrary to paclitaxel, BPA exposure did not affect cell
219 birth near GnRH neurons at P8 (**Fig. 3b**). Additionally, the total number of GnRH neurons in
220 the preoptic region at P8 also remained unchanged (255.7 ± 34.95 in control P8 rats versus
221 218.9 ± 30.38 in BPA-treated P8 rats, Mann-Whitney test: $U = 22$, $P = 0.3282$; $n = 8$ rats per
222 group). However, unlike untreated rats, BPA-exposed animals showed reduced association
223 between GnRH neurons and P8-born BrdU⁺ cells 1 week later (**Fig. 3b**) even though there
224 was no depletion of BrdU⁺ cells in the preoptic region (**Fig. 3c**), further supporting the view
225 that rather than proliferation, it is the recruitment of new glial progenitors by GnRH neurons
226 that influences later reproductive function. From a broader viewpoint, these results also open
227 up the possibility that the effects of EDCs on fertility and brain maturation are at least partly
228 due to their interference with the ability of select neuronal populations to shape their glial
229 environment during postnatal development.

230

231 **GnRH neurons use PGD2 to recruit preoptic progenitor cells**

232 Considering that the increased proportion of GnRH neurons associated with BrdU⁺
233 cells between P8 + 2h and P8 + 24h is likely due to BrdU⁺ cell recruitment rather than
234 proliferation (**Supplementary Fig. 3**), we wondered whether GnRH neurons themselves
235 released chemotropic factors to attract glial progenitors that would eventually differentiate
236 into astrocytes in their vicinity and remain associated with them into adulthood (**Fig. 1i**). We
237 first probed a set of molecules expressed in female rodent GnRH neurons: GnRH,
238 neurotensin (NT), cholecystokinin (CCK), galanin, glutamate and GABA^{18, 19}. Double
239 immunolabeling showed that infantile GnRH neuronal perikarya contained galanin but lacked
240 CCK and NT (**Supplementary Fig. 6a-i**). However, the vesicular transporters for glutamate
241 and γ -aminobutyric acid (GABA), v-Glut and v-GAT, respectively, colocalized at the GnRH
242 termination field in the median eminence (**Supplementary Fig. 6j-o**), suggesting that
243 postnatal GnRH neurons could also influence proliferating cells through the local glutamate
244 or GABA release.

245 To assess the putative chemotactic effects of GnRH, galanin, glutamate and GABA
246 produced by GnRH neurons, we next developed adherent primary cultures of progenitor cells
247 from the rat preoptic region at P1-P2 (**Supplementary Fig. 7a**). Cultured cells exhibited an
248 antigenic profile typical of neural progenitors, predominantly expressing nestin, BLBP, Sox2
249 and vimentin (**Fig. 4a, Supplementary Fig. 7b-e**). However, in transwell assays, their
250 migration was not significantly affected by GnRH, galanin, glutamate or GABA (**Fig. 4b**),
251 suggesting that other factors might be responsible for their chemoattraction by GnRH
252 neurons.

253 Next, since postnatal progenitor recruitment by GnRH neurons occurs in the global
254 context of astrogenesis⁸, and GnRH neurons are tightly surrounded by GFAP-expressing
255 processes at P8 (**Fig. 1f**), we searched for candidate genes in GnRH neurons that could be
256 regulated by astroglial factors. We conducted a transcriptomic analysis of GnV3 cells, an *in*
257 *vitro* model of postnatal rat GnRH neurons²⁰, exposed to medium conditioned with or without
258 hypothalamic astrocytes for 4 days (**Fig. 4c**). In all, 512 genes were differentially expressed
259 (212 upregulated and 300 downregulated) when GnV3 cells were treated with astrocyte-

260 conditioned medium (See Source Data Files in **Supplementary Data 1**). The most
261 upregulated gene was *Ptgds* encoding brain-type prostaglandin D2 (PGD2) synthase, a
262 molecule with potent chemoattractant properties²¹, which displayed a 133-fold increase in
263 response to astrocyte-conditioned medium (**Supplementary Data 1**). RT-PCR analysis
264 confirmed this upregulation (**Fig. 4d**). Moreover, PGD2 stimulated the passage of preoptic
265 neural progenitors from the upper to the lower compartment in transwell migration assays
266 (**Fig. 4b**), but did not affect their proliferation (**Fig. 4e**). As gliogenesis continues during
267 postnatal development, these data suggest that GnRH neurons respond to incoming glial
268 signals by synthesizing PGD2 and thus attracting newborn cells in their vicinity.

269 PGD2 functions *via* the G protein-coupled receptors, DP1 (*Ptgdr1*) and DP2 (*Gpr44*)
270 ^{21, 22}, both expressed in primary cultures of preoptic neural progenitors (**Fig. 4f**). Since DP1
271 signaling is already known to be involved in regulating hypothalamic function²², we used
272 BWA868C, a specific antagonist of DP1^{22, 23}, to determine whether the effect of PGD2 on
273 hypothalamic progenitor cell migration was mediated by DP1. BWA868C treatment of
274 preoptic neural progenitors not only drastically inhibited the stimulatory effect of PGD2 on cell
275 migration (**Fig. 4g,h**), it also reduced the PGD2-induced phosphorylation of the mitogen-
276 activated protein kinase (MAPK) ERK (**Fig. 4i**), confirming that PGD2 signaling was active in
277 these progenitors.

278

279 **PGD2/DP1 signaling mediates preoptic precursor recruitment**

280 Next, to investigate whether GnRH neurons produce PGD2 during postnatal
281 development *in vivo*, we analyzed *Ptgds* expression in cells isolated by fluorescence-
282 activated cell sorting (FACS) from transgenic rats expressing enhanced green fluorescent
283 protein (EGFP) under the control of the GnRH promoter²⁴ using real-time PCR (**Fig. 5a**,
284 **Supplementary Fig. 8a,b**). *Gnrh* mRNA was only detected in the EGFP-positive cell fraction,
285 demonstrating the accuracy of the sorting procedure (**Supplementary Fig. 8c-e**). Using this
286 approach, we found that *Ptgds* transcripts were indeed enriched in rat GnRH neurons and

287 that their expression significantly increased between P8 and P20 in these neurons (**Fig. 5b**)
288 but remained unchanged in preoptic cells lacking EGFP (**Fig. 5c**).

289 To gain further insight into the involvement of the PGD2/DP1 signaling pathway in the
290 communication between GnRH neurons and neural progenitors *in vivo*, we evaluated *Ptgds*
291 and *Ptgdl* expression in the postnatal preoptic region. Multiplex *in situ* hybridization in
292 female rats at P8 confirmed the expression of *Ptgds* transcripts in GnRH neurons (**Fig. 5d**,
293 upper row), while neighboring Sox2-expressing neural progenitors expressed *Ptgdl* mRNA
294 (**Fig. 5d**, lower row). Moreover, the fraction of GnRH neurons expressing *Ptgds* increased
295 between P8 and P12, an increase still seen at P20 (**Fig. 5e**), explaining the increased *Ptgds*
296 expression observed in FACS-sorted cells above (**Fig. 5b**); however, the abundance of
297 *Ptgds* transcripts per GnRH neuron did not significantly increase across the infantile period
298 (**Fig. 5f**). In contrast, *Ptgdl* expression in Sox2⁺ progenitors showed no significant variation
299 between P8 and P20 (**Fig. 5g,h**). Altogether, these data show that the increased *Ptgds*
300 expression in GnRH neurons during infantile development corresponds to an increase in the
301 proportion of PGD2-synthesizing GnRH neurons during the period of enhanced astrogenesis
302 in the preoptic area (**Supplementary Fig. 1, Supplementary Fig. 2**).

303 To confirm that the PGD2/DP1 signaling pathway is indeed involved in progenitor
304 cells recruitment by GnRH neurons *in vivo*, we acutely injected BWA868C stereotaxically into
305 the preoptic region of P7 female rats, 2h after a single BrdU injection, and quantified the
306 proportion of GnRH neurons associated with BrdU⁺ cells after 24h (**Fig. 6a**). This proportion
307 increased in control animals but not in BWA868C-treated rats (**Fig. 6b**). Since BWA868C
308 treatment did not affect the total number of BrdU⁺ cells in the preoptic region (**Fig. 6c**), the
309 inhibitory effect of BWA868C on the physical proximity between GnRH neurons and BrdU⁺
310 cells was not due to neural progenitors depletion but to the blockade of the trophic influence
311 of GnRH neurons on nearby glial progenitors. Together with our *in vitro* findings, these
312 results indicate that functional PGD2/DP1 signaling is necessary for GnRH neurons to recruit
313 or attract a glial entourage during the infantile period in rats.

314

315 **Inhibiting DP1 disrupts GnRH network maturation and function**

316 To assess the functional involvement of DP1 signaling in postnatal sexual maturation,
317 we implanted BWA868C pellets (10 nM in cocoa butter) in the preoptic region of P8 female
318 rats²⁵ and monitored the occurrence of the first postnatal FSH surge indicative of minipuberty
319 (**Fig. 6d**). Similar to acute injection, BWA868C released chronically from a pellet also
320 significantly hampered the association of GnRH neurons with BrdU⁺ cells one week later
321 (**Fig. 6e**), although the total number of BrdU⁺ cells in the preoptic region remained unaffected
322 (**Fig. 6f**). While the treatment had no effect on somatic growth (**Supplementary Fig. 9**), the
323 FSH surge at minipuberty was blunted in BWA868C-treated pups in comparison to vehicle-
324 treated littermates (**Fig. 6g**), suggesting that the activity of infantile GnRH neurons was
325 impaired by their inability to attract newborn astrocytic precursors.

326 Next, since minipuberty has previously been shown to be a critical period for the
327 switch in the transcriptional control of GnRH expression⁴, we assessed whether BWA868C
328 treatment at P7 influenced this process. RT-qPCR analyses in FACS-isolated rat GnRH
329 neurons at P12-P13 indicated that BWA868C implantation had no apparent impact on *Gnrh*
330 expression (**Fig. 7a**). However, it decreased the expression of *Cebpb*, a repressor of the
331 *Gnrh* promoter, but left unchanged the expression of another repressor, *Zeb1*, as well as
332 *Kiss1r* and *Otx2*, two stimulators of *GnRH* expression, and *Dicer*, an RNase-III
333 endonuclease essential for microRNA biogenesis and the microRNA-mediated infantile *Gnrh*
334 transcriptional switch⁴ (**Fig. 7a**). Surprisingly, BWA868C treatment also impacted several
335 other genes involved in prostaglandin synthesis and signaling in infantile GnRH neurons
336 (**Fig. 7b**). In addition to a significant decrease in *Ptgds* expression (**Fig. 7c**), in line with our
337 transcriptomic analysis showing *Ptgds* regulation by astrocyte-derived factors (**Fig. 4d**,
338 **Supplementary Data 1**), BWA868C treatment downregulated transcripts for *Ptger1* and
339 *Ptger3*, encoding the PGE2 receptors EP1 and EP3, respectively, in infantile rat GnRH
340 neurons (**Fig. 7c**). These results are in keeping with previous *in vitro* studies showing that
341 astrocyte-conditioned medium modulates the sensitivity of GnRH neurons to astrocytic

342 PGE2, a stimulator of GnRH release, by upregulating EP1 and EP3 expression²⁶, and
343 suggest that the BWA868C-mediated decrease in astrocyte recruitment by GnRH neurons
344 interferes with astrocytic PGE2-stimulated GnRH release rather than GnRH production.

345 We next investigated whether, in addition to altering astrocyte-to-GnRH-neuron
346 communication processes, BWA868C treatment could also influence neuronal connectivity,
347 in particular the apposition of glutamatergic or GABAergic terminals onto GnRH neuronal cell
348 bodies, and consequently GnRH neuronal activity during the infantile period. The BWA868C-
349 mediated inhibition of DP1 signaling in the preoptic region significantly decreased the
350 number of appositions of vesicular glutamate transporter 2 (vGluT2)-immunoreactive punctae
351 but not of vesicular GABA transporter (vGAT)-immunoreactive punctae on GnRH somata at
352 P15 (**Fig. 7d**).

353 Given this robust decrease in putative excitatory synaptic inputs to GnRH neurons,
354 we next performed *ex vivo* whole-cell patch-clamp recordings of GnRH neurons in living
355 brain slices containing the OVLT from P10-P15 infantile *Gnrh::Egfp* rats treated either with
356 vehicle or with BWA868C *in vivo* at P7-P8 (**Fig. 7e,f**). We found no significant difference
357 between the two conditions in the resting membrane potential (V_m) (**Fig. 7g**), membrane
358 resistance (R_m) (**Fig. 7h**), voltage threshold for action potential (AP) generation (V_{TH}) (**Fig.**
359 **7i**) or time constant (control time constant = 0.47 ± 0.07 ms, $n = 15$ cells from 8 rats;
360 BWA868C time constant = 0.43 ± 0.05 ms, $n = 22$ cells from 7 rats; Mann-Whitney test: $U =$
361 149.5 , $P = 0.641$). These findings demonstrate that the integrative properties and intrinsic
362 excitability of GnRH neurons were not modified by BWA868C treatment. However, we
363 observed a significant attenuation of the spontaneous firing rate in GnRH neurons partly
364 deprived of glutamatergic synapses (**Fig. 7j** and **f**, left), reminiscent of the increased
365 interpulse interval of GnRH neurosecretion in BPA-treated rat pups¹⁶. This was not
366 accompanied by modifications in spike properties such as full amplitude (control spike
367 amplitude = 57.73 ± 2.75 mV, $n = 10$ cells from 8 rats; BWA868C spike amplitude = $61.13 \pm$
368 1.88 mV, $n = 10$ cells from 8 rats; two-sided unpaired *t*-test, $t(18) = 1.02$, $P = 0.321$), half-

369 width duration (control half-width duration = 1.85 ± 0.17 ms, $n = 10$ cells from 8 rats;
370 BWA868C half-width duration = 1.74 ± 0.07 ms, $n = 10$ cells from 8 rats; Mann-Whitney test:
371 $U = 49$, $P = 0.970$) or after-hyperpolarization potential (AHP) amplitude (control AHP
372 amplitude = 7.52 ± 0.55 mV, $n = 10$ cells from 8 rats; BWA868C AHP amplitude = $7.18 \pm$
373 0.30 mV, $n = 10$ cells from 8 rats; two-sided unpaired t -test, $t(18) = 0.535$, $P = 0.599$) or
374 duration (control AHP duration = 470.9 ± 69.52 ms, $n = 10$ cells from 8 rats; BWA868C AHP
375 duration = 465.7 ± 65.53 ms, $n = 10$ cells from 8 rats; two-sided unpaired t -test, $t(18) = 0.055$,
376 $P = 0.957$) as measured from isolated APs. We further analyzed spike generation in neurons
377 in which clear baseline synaptic activity was detected on the trace ($n = 10$ neurons from 8
378 rats per condition). In the control group, in 5 neurons, firing was triggered by a slowly
379 developing temporal summation of fast depolarizing events (**Fig. 7f**, top right), reminiscent of
380 depolarizing postsynaptic potentials. This dramatically differed from most BWA868C-treated
381 neurons, in which AP firing was apparently elicited by intrinsic mechanisms ($n = 4$ neurons,
382 **Fig. 7f**, bottom right, AP #1 and #2) or the cooperative effect of intrinsic and synaptic
383 mechanisms ($n = 4$ neurons, **Fig. 7f**, bottom right, AP #3). Only two BWA868C-treated
384 neurons analyzed displayed APs elicited by an excitatory synaptic barrage. Altogether, these
385 findings indicate that BWA868C treatment does not alter the resting membrane or integrative
386 properties of GnRH neurons but significantly reduces their functional output, likely due to
387 decreased glutamatergic synaptic connections.

388 Finally, we analyzed the effects of infantile BWA868C treatment on the acquisition of
389 mature reproductive function. Even though BWA868C implantation in the preoptic region
390 during the infantile period did not alter the age at vaginal opening (**Fig. 8a,b**), which
391 coincided with first estrus (**Fig. 8a**), it significantly delayed the onset of regular estrous
392 cyclicity (**Fig. 8a,c**). During the first two weeks after vaginal opening, BWA868C-treated
393 females spent more time in estrus at the expense of proestrus, indicating a dysregulation of
394 pulsatile GnRH release, in contrast to control (vehicle-treated) littermates, which quickly
395 acquired a regular 4-day estrous cycle.

396 Altogether, these results show that the integrity of GnRH neuron-glia communication
397 in the preoptic region during the infantile period is critical for the recruitment of newborn glial
398 precursors that accompany these neurons into adulthood as well as the establishment of
399 functional synaptic inputs to GnRH neurons. Furthermore, this communication, mediated by
400 PGD2/DP1 signaling, is essential for the correct initiation of sexual maturation, reciprocal
401 signaling by astrocytes of the GnRH neural network, and adult pattern of estrous cyclicity.

402

403 **DISCUSSION**

404 The ability to reproduce is acquired through a long and complex process that relies on
405 the fine regulation of GnRH neuronal network activity during the postnatal period. This
406 process involves intrinsic and extrinsic modifications to functional gene networks^{4, 27} and
407 neuronal circuits converging onto GnRH neurons^{28, 29}, as well as the increased stimulatory
408 influence of glial cells⁶. Our data provide the first neuroanatomical and physiological
409 evidence that GnRH neurons build their own glial environment during a circumscribed period
410 after birth via a specific cell-cell communication pathway with neural progenitors born during
411 a wave of astrogenesis, and that this reciprocal communication is essential for the maturation
412 of the hypothalamic neuroendocrine system controlling the survival of the species.

413 Astrocytes have long been known to play a role in the control of GnRH neuronal
414 function, particularly during postnatal sexual maturation²⁵, being sophisticated sensors⁸ and
415 regulators of neuronal activity via both the timely trafficking of energy metabolites³⁰ and
416 gliotransmission³¹. They communicate with GnRH neurons by releasing bioactive molecules
417 such as PGE2^{32, 33}, known to be released at active synapses in response to glutamate^{34, 35}.
418 Glutamate-stimulated PGE2 release within the GnRH neural network may involve the
419 activation of the ErbB signaling pathway³⁶, also important for puberty onset^{25, 37}, in
420 hypothalamic astrocytes. Here, we pinpoint the production of another prostaglandin, PGD2,
421 by GnRH neurons themselves as a potent communication signal used by these neurons to
422 attract infantile astroglial progenitors to their vicinity. Our findings demonstrate that inhibiting

423 cell proliferation or impairing PGD2/DP1 signaling in the preoptic region during this period
424 significantly alters sexual maturation, indicating that astrogenesis in the vicinity of GnRH
425 neurons during postnatal development is an important component of the homeostatic
426 mechanism required for GnRH neural network maturation. While recent advances in glial cell
427 biology have provided substantial insights into how astrocytes influence neuronal function
428 (see ³⁸ for review), the concept that neurons could stably recruit astrocytic progenitors as
429 companions or “escorts”, i.e. allow newborn neighboring astrocytes to become
430 structurally/functionally associated with them during development and maintain this
431 relationship into adulthood, has rarely been evoked.

432 Interestingly, the birth of new cells in the infantile preoptic region could also be
433 modulated by neuronal factors released by other components of the GnRH neuroglial
434 network. For example, some incoming fibers from the developing arcuate nucleus of the
435 hypothalamus release β -endorphin^{39, 40}, which has recently been shown to activate quiescent
436 neural stem cells in the adult brain⁴¹, and could thus be a candidate to regulate this infantile
437 proliferation. While we cannot rule out an indirect influence of the proliferation or signaling of
438 neural progenitors on reproductive function through similar mechanisms in other components
439 of the GnRH network, e.g. by altering astrocyte-kisspeptin-neuron interactions in the adjacent
440 AVPV, the fact that paclitaxel did not modify BrdU+ cells in this nucleus suggests that the
441 physiological changes we observed were selectively due to perturbed astrocyte-GnRH
442 neuron association, rather than alterations in the neuroglial interactions of afferent neurons.
443 Nevertheless, paclitaxel affected the reproductive phenotype more severely than BWA868C,
444 suggesting that the progenitor depletion might have been harder to compensate for than
445 simply inadequate GnRH neuron connectivity due to impaired association with glial
446 progenitors.

447 Interestingly, deficient DP1 signaling in the preoptic region also blunts the FSH surge
448 at P12, i.e. during minipuberty, the first centrally driven and gonad-independent activation of
449 the HPG axis⁵. This phenotype is highly reminiscent of that of mice in which astrocytic
450 function is selectively altered by expressing a dominant-negative ErbB4 receptor (DN-ErbB4)

451 under the control of the GFAP promoter³⁷. On the other hand, the disrupted estrous cycle
452 triggered by blocking preoptic DP1 signaling in rats phenocopies transgenic mice expressing
453 a dominant negative mutation of the adhesion molecule SynCAM1 under the GFAP
454 promoter⁴². SynCAM1 expression, required to maintain the physical contact between
455 astrocytes and GnRH neuronal cell bodies⁴³, is activated by astroglial ErbB4 signaling⁴².
456 Altogether, these findings evoke a scenario in which the birth of new astrocytes during
457 postnatal development promotes a selective increase in the expression of the PGD2-
458 producing enzyme, PGDS, in infantile GnRH neurons via an as yet unidentified signaling
459 pathway involving soluble factors. The choice of candidate signaling factors is large, with
460 astrocytes releasing not only gliotransmitters but numerous neuroactive substances,
461 including thrombospondin, TNF α and TGF β 1 (see for review ⁴⁴⁻⁴⁶). Regardless of its identity,
462 PGD2 released by GnRH neurons attracts DP1-expressing neural progenitors that eventually
463 differentiate into astrocytes and remain associated with these neurons until adulthood, an
464 “escort” function that could facilitate their integration into functional networks and sexual
465 maturation (**Extended Data Fig. 1**), and that could require other neuron-to-glia
466 communication processes similar to those involving infantile ErbB4 signaling^{42, 43}. While such
467 experiments in rats are beyond the technical scope of this study, preliminary experiments in
468 *Gfap::DN-ErbB4* mice indicate that newborn cells that are morphologically associated with
469 infantile GnRH neurons fail to accompany them into adulthood, in contrast to wild-type
470 littermates, despite the unaltered proliferation and long-term survival of these cells
471 (**Supplementary Note; Supplementary Fig. 10**).

472 Astrocytes also play key roles in the control of synapse formation and functional efficacy⁴⁷,
473 ⁴⁸, shaping synaptic properties to fit ongoing developmental and functional needs, thus
474 providing contextual guidance to the synapses^{49, 50}. During postnatal development, synaptic
475 punctae in the brain appear concurrently with astrogenesis⁴⁷. Remarkably, our results show
476 that preventing GnRH neurons from attracting glial progenitors in their immediate
477 neighborhood by blocking DP1 signaling markedly decreases the number of vGluT2
478 appositions on GnRH neurons and significantly attenuates GnRH neuron firing, primarily by

479 decreasing the rate of depolarizing postsynaptic potentials. Since the FSH surge at
480 minipuberty is also blunted in these pups, we can postulate that the birth of new astrocytes
481 near infantile GnRH neurons is required for the establishment of excitatory glutamatergic
482 inputs onto GnRH neurons, confirming the long-suspected notion that such afferents
483 contribute to the gonad-independent maturation of the HPG axis in infantile rats^{51, 52}. These
484 excitatory synaptic inputs are thought to develop after GABAergic inputs^{53, 54}, which,
485 contrarily, do not appear to require infantile gliogenesis for their establishment or
486 maintenance (**Fig. 7d**). Intriguingly, in the interconnected GnRH neuroglial network⁶,
487 glutamate spillover at the synaptic cleft by the co-activation of metabotropic and AMPA
488 glutamatergic receptors in hypothalamic astrocytes³⁶ or their progenitors may initiate a
489 signaling cascade that allows ErbB4 receptor recruitment³⁶, promotes astrogenesis⁵⁵ and
490 thus leads to the stabilization and maturation of glutamatergic synapses. This causes
491 increased PGE2 synthesis and release by astrocytes³⁶, which can then signal back to
492 GnRH-secreting neurons⁶. An infantile GnRH neuron-dependent modulation of astrogenesis
493 may therefore contribute to building a signaling network capable of delivering coordinated
494 trans-synaptic and astroglial inputs to GnRH neurons themselves for the initiation of puberty
495 and the timely acquisition of adult reproductive capacity (**Extended Data Fig. 1**)⁴.

496 Recent human and animal studies increasingly demonstrate that the GnRH neural
497 network is particularly sensitive to environmental pollutants, in particular endocrine
498 disruptors, during early postnatal development¹⁴. Exposure to EDCs such as the plasticizer
499 BPA during the critical perinatal period is indeed reported to be associated with early or late
500 puberty onset and subsequent reproductive impairments in both boys and girls (see for
501 review¹⁴), and to affect the central control of puberty, including glutamatergic and
502 GABAergic inputs to GnRH neurons, in preclinical animal models^{16, 56, 57}. While the underlying
503 mechanisms remain elusive, glial cells, which play a central role in GnRH neuronal network
504 development and function⁶, would be the obvious suspects to mediate this EDC-induced
505 pathophysiological process¹⁴. Our results intriguingly show that treating infantile rats with
506 very low but environmentally relevant doses of BPA¹⁵ known to cause alterations in sexual

507 maturation, phenocopying paclitaxel-treated rats¹⁶, alters the recruitment of newborn
508 neighboring cells by GnRH neurons at minipuberty, without appearing to affect overall cell
509 proliferation in the infantile preoptic region. This raises the possibility that early-life exposure
510 to chemicals in contact with food⁵⁸, such as BPA, may perturb puberty onset and durably
511 impact reproductive function at least partially by interfering with the capacity of GnRH
512 neurons to build a proper glial environment through altered PGDS expression⁵⁹ or ErbB
513 signaling⁶⁰, resulting in their miswiring during postnatal development.

514 To summarize, our findings shed light on a hitherto unsuspected neuron-to-neural-
515 progenitor communication process that shapes the glial environment of GnRH neurons
516 during postnatal development and is required for the proper wiring and function of these
517 neurons, which control the survival of the species in mammals.

518

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536

537 **AUTHOR CONTRIBUTIONS**

538 A.S., A.-S. P. and V.P. designed the experiments. G.P., M.M., C.A., T.L., S.G., D.F., V.M., M.
539 M.-L., A C.-R., V.D., D.M., A.L., M.T.S., P.C., A.S. and V.P. performed the experiments. A.S.
540 and V.P. analyzed the data. J.S., G.C. and F.P. contributed material. All authors discussed
541 the results and made edits to the manuscript. A.S., S.R., S.R.O. and V.P. wrote the
542 manuscript.

543

544 **COMPETING FINANCIAL INTERESTS**

545 The authors declare no competing financial interests.

546

547 **FIGURE LEGENDS**

548 **Figure 1. Postnatally-born astrocytes preferentially associate with GnRH neuronal cell**
549 **bodies. (a)** Experimental protocol. **(b)** Representative coronal section of the preoptic region
550 immunolabeled for GnRH (green) and BrdU (magenta), nuclei counterstained with Hoechst
551 (white), illustrating quantifications in **c**. Arrow, arrowheads: GnRH neuronal cell bodies. Inset:
552 higher magnification of the GnRH neuron indicated, showing physical proximity to a BrdU⁺
553 cell (yellow arrows). Scale bars: 100µm (main panel), 10 µm (inset). **(c)** Percentage of GnRH
554 neurons associated with BrdU⁺ cells 2h, 24h, 7d or 60d after a single BrdU injection at P1,
555 P5, P8, P12, P20 or P24 (*n*=9,6,7,6,8,5,10,5,5,6,9,3,3,6,9,3,4,9,5 rats/group) (two-way
556 ANOVA: age at BrdU injection, $F_{(5,73)}=44.92$, $P<0.0001$; survival time (2h, 7d), $F_{(1,73)}=3.116$,
557 $P=0.0817$; interaction, $F_{(5,73)}= 8.392$, $P< 0.0001$; Tukey's multiple comparison test, P8+2h
558 versus P8+7d: $q_{(73)} = 8.583$, $P<0.0001$; P8, Kruskal-Wallis one-way ANOVA on ranks:
559 $H_{(4)}=20.98$, $P=0.0001$; Dunn's multiple comparison: 2h versus 24h, $P=0.0274$; 2h versus 60d,
560 $P>0.9999$). **(d)** Percentage of GnRH- or HuC/D-expressing neurons associated with BrdU⁺
561 cells 2h or 7d after a single BrdU injection at P8 (% of GnRH neurons versus % of HuC/D⁺

562 neurons associated with BrdU⁺ cells: Mann-Whitney test, 2h: $U=0$, $P=0.004$; $n=8,4$
563 rats/group, respectively; 7d: $U=0$, $P=0.002$; $n=10,4$ rats/group).**(e,f,i-k)** Representative triple
564 immunofluorescence labeling for GnRH (green), BrdU (magenta) and Sox2 (**e**, white), GFAP
565 (**f,i**, white), APC (**j**, white) or HuC/D (**k**, white) in the female rat preoptic region injected with
566 BrdU at P8 and sacrificed 2h (**e,f**) or 7d later (**i-k**), illustrating quantifications in **g**. Yellow
567 arrows: BrdU⁺ cells associated with GnRH neurons co-expressing the indicated markers.
568 Scale bars: 20 μ m. **(g)** Percentage of BrdU⁺ cells associated with GnRH neurons co-
569 expressing Sox2, GFAP, APC or HuC/D in animals injected with BrdU at P8 and sacrificed
570 2h or 7d later (GFAP, Mann-Whitney test: $U=0$, $P=0.0012$, $n=6,7$ rats/group). **(h)** Co-
571 immunolabeling for GnRH (green) and BrdU (magenta), nuclei counterstained with Hoechst
572 (white), in female rats injected with BrdU thrice daily from P6 to P8 and sacrificed at P15,
573 showing two GnRH neurons with companion BrdU⁺ cells (yellow arrows). $n=4$ animals. Scale
574 bar: 20 μ m. **(c,d,g)** Values: means \pm s.e.m.; * $P<0.05$; ** $P<0.01$; i.p., intraperitoneal; neo,
575 neonatal period; OVLT, organum vasculosum laminae terminalis.

576

577 **Figure 2. Inhibiting GnRH neuron association with newborn glia delays the onset of**
578 **puberty and impairs mature estrous cyclicity.** **(a)** Diagram of the experimental protocol,
579 with a coronal view of the rat brain showing the anatomical localization of the microparticle
580 implantation site (red line) in the preoptic region (dashed area). **(b, c)** Coronal sections from
581 adult animals injected with blank- (b) or paclitaxel-loaded microparticles (c) at P6,
582 immunolabeled for GnRH (green) and counterstained with the nuclear marker Hoechst
583 (white). The region delimited by the dashed white line in **c** shows the hypocellular region
584 around the injection site (asterisk). ac, anterior commissure. Scale bars: 200 μ m.**(d-g)** Cell
585 nuclear density at the injection site (**d**: two-tailed unpaired t -test, $t_{(10)}=4.366$, $P=0.0014$, $n=6$
586 rats/group), number of BrdU⁺ cells at the injection site (**e**: two-tailed unpaired t -test, $t_{(11)}=4.2$,
587 $P=0.0015$; $n=7,6$ rats/group), percentage of GnRH neurons associated with BrdU⁺ cells (**f**:
588 two-tailed unpaired t -test with Welch's correction, $t_{(6,9)}=4.24$, $P=0.0040$; $n=7$ rats/group) and
589 total number of GnRH neurons in the preoptic region (**g**: two-tailed unpaired t -test,

590 $t_{(15)}=2.075$, $P=0.0556$; $n=7,10$ rats/group) of control and paclitaxel-treated rats. (h-i)
591 Cumulative percentage (h) and age of vaginal opening (i: Mann-Whitney test: $U=69.5$,
592 $P=0.0428$, $n=17,14$ rats/group) in control and paclitaxel-treated rats. (j-k) Cumulative
593 percentage (j) and age of first estrus (k: Mann-Whitney test: $U=32$, $P=0.0002$, $n=17,14$
594 rats/group) in control and paclitaxel-treated rats. (l) Representative estrous cycle profiles of
595 control and paclitaxel-implanted animals showing the day of vaginal opening (vo) and first
596 estrus (red dot). The first paclitaxel-treated animal has a normal estrous cycle while the next
597 three are examples of altered estrous cyclicity. D, diestrus; E, estrus; P, proestrus. (m)
598 Difference between age at vaginal opening and at first estrus in control and paclitaxel-treated
599 animals (Mann-Whitney test: $U=28.5$, $P<0.0001$, $n=17,14$ rats/group). (n) Percentage of
600 complete estrous cycles in control and paclitaxel-treated animals (Mann-Whitney test:
601 $U=80.5$, $P=0.0156$, $n=17,18$ rats/group). (d-g,i,k,m-n) Values: means \pm s.e.m.* $P<0.05$;
602 ** $P<0.01$; *** $P<0.001$.

603

604 **Figure 3. Early exposure to bisphenol A inhibits infantile GnRH neuron-progenitor**
605 **association.** (a) Schematic of the protocol used to evaluate the effect of bisphenol A (BPA)
606 on the association between GnRH neurons and progenitors. (b) Percentage of GnRH
607 neurons associated with BrdU⁺ cells in control and BPA-treated animals (one-way ANOVA:
608 $F_{(3,26)}=16.6$, $P<0.0001$, $n=11,4, 8,7$ rats/group; Tukey's multiple comparisons test: 2h control
609 versus 7d control, $q_{(26)}=7.739$, $P<0.0001$; 2h BPA versus 7d BPA, $q_{(26)}=4.46$, $P=0.0198$; 2h
610 control versus 2h BPA, $q_{(26)}=1.412$, $P=0.7517$). (c) Total number of BrdU⁺ cells 2h and 7d
611 after a single injection of BrdU at P8 in animals treated with BPA from P1 to P15 (two-tailed
612 unpaired t -test, $t_{(11)}=0.4553$, $P=0.6578$; $n=6,7$ rats/group). (b,c) Values: means \pm
613 s.e.m.* $P<0.05$; *** $P<0.001$.

614

615 **Figure 4. PGD2 is a chemoattractant for preoptic progenitor cells *in vitro*.** (a)
616 Immunofluorescence labeling for Sox2, nestin and BLBP in one primary progenitor culture
617 representative of three independent cultures. Cell nuclei were stained with Hoechst (blue).

618 Insets: higher magnification views of cells co-expressing the three markers (arrows). Scale
619 bars: 50 μ m. **(b)** Quantification of transwell assays showing the effect of different factors on
620 the migration of preoptic progenitor cells across a porous membrane. Epidermal growth
621 factor (EGF) was used as a positive control⁶¹ (Kruskal-Wallis one-way ANOVA on ranks:
622 $H_{(6)}=25.731$, $P<0.001$; Dunn's multiple comparison method: control versus treatment: PGD2,
623 $Q_{(6)}=4.255$, $P<0.001$; EGF, $Q_{(6)}=3.925$, $P=0.0018$; $n=18,19,12,17,10,10,12$ wells/condition;
624 $N=5$ independent experiments). **(c)** Strategy used to identify astrocyte-regulated gene
625 expression in GnRH neurons (See **Supplementary Data 1**). **(d)** Real-time PCR analysis of
626 *Ptgds* mRNA levels in GnV3 cells treated with hypothalamic astrocyte-conditioned medium
627 (ACM) or non-conditioned medium (control) (Mann-Whitney test: $U=195$, $P<0.001$; $n=13,15$
628 replicates/condition; $N=3$ independent experiments). ACM values are expressed relative to
629 control values, arbitrarily set at 1. **(e)** Percentage of preoptic progenitor cells expressing the
630 proliferation marker Ki67 after *in vitro* treatment with the same factors as in **b**. Fibroblast
631 growth factor 2 (FGF2) was used as a positive control⁶² (Kruskal-Wallis one-way ANOVA on
632 ranks: $H_{(6)}=38.576$, $P<0.001$; Dunn's multiple comparison method: control versus FGF2,
633 $Q_{(6)}=4.798$, $P<0.001$; $n=23,22,16,17,15,16,16$ wells/condition; $N = 3$ independent
634 experiments). **(f)** Real-time PCR analysis of *Ptgdl* and *Gpr44* mRNA levels in primary
635 preoptic progenitor cell cultures ($n=4$ cultures). Values expressed relative to mRNA levels in
636 the total brain, arbitrarily set at 1 (blue line). **(g)** Representative photomicrographs of the
637 Hoechst-stained lower side of transwell assay membranes, illustrating quantifications in **h**.
638 Scale bar: 100 μ m. **(h)** Quantification of transwell assays (Kruskal-Wallis one-way ANOVA on
639 ranks: $H_{(5)}=25.995$, $P<0.001$; Dunn's multiple comparison method: control versus PGD2,
640 $Q_{(5)}=3.277$, $P=0.0157$; PGD2 versus PGD2 + BWA 10nM, $Q_{(5)}=3.321$, $P=0.0135$; PGD2
641 versus PGD2 + BWA 1 μ M, $Q_{(5)}=4.548$, $P<0.001$; $n=11,11,12,12,11,10$ wells/condition; $N=3$
642 independent experiments). **(i)** ERK1/2 phosphorylation (pERK) in progenitors treated with
643 PGD2 (1 μ M) and/or the DP1 receptor antagonist BWA868C (BWA). Representative
644 immunoblot of three independent experiments. tERK, total ERK. **(b,d-f,h)** Values: means \pm
645 s.e.m.* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

646

647 **Figure 5. *In vivo* expression of *Ptgds* and *Ptgdl* in the preoptic region of infantile**
648 **female rats. (a)** Procedure used to isolate GnRH neurons from the preoptic region (POA) of
649 GnRH-EGFP transgenic rats. FACS, fluorescence-activated cell sorting; Pos, EGFP-positive
650 fraction; Neg, EGFP-negative fraction. **(b,c)** Real-time PCR analysis of the expression of
651 *Ptgds* mRNA in EGFP-positive- (b) or negative cells (c) at different postnatal ages **(b:**
652 Kruskal-Wallis one-way ANOVA on ranks: $H_{(2)}=7.476$, $P=0.024$, $n=10$ rats/group; Dunn's
653 multiple comparisons method: P8 versus P20, $Q_{(2)}=3.844$, $P=0.0191$; **c:** Kruskal-Wallis one-
654 way ANOVA on ranks: $H_{(2)}=5.280$, $P=0.071$, $n=10$ rats/group). Values expressed relative to
655 P8 values, arbitrarily set at 1. n.s., non-significant. **(d)** Representative confocal images of
656 fluorescent *in situ* hybridization for *Gnrh*, *Sox2*, *Ptgds* and *Ptgdl* mRNAs in the preoptic
657 region of P8 female rats, illustrating quantifications in **e-h**. (Upper row) Arrow: a GnRH
658 neuron containing *Ptgds* mRNA (magenta dots) and surrounded by *Sox2*-expressing cells
659 (arrowheads, white dots). (Lower row) Arrow: a GnRH neuron surrounded by *Sox2*-
660 expressing cells (white arrowheads). Purple arrowheads: two *Sox2*-expressing cells next to
661 the GnRH neuron, co-expressing *Ptgdl* mRNA (magenta dots). No such dots could be seen
662 in any section of the negative controls (see methods). Sections were counterstained with the
663 nuclear marker DAPI (blue). Scale bar: 5 μ m. **(e,f)** Percentage of GnRH neurons that contain
664 *Ptgds* mRNA **(e:** one-way ANOVA: $F_{(2,16)}=10.96$, $P=0.001$, $n=7,6,6$ rats/group; Tukey's
665 multiple comparison test: P8 versus P12, $q_{(16)}=5.185$, $P=0.0056$; P8 versus P20, $q_{(16)}=6.046$,
666 $P=0.0016$) and of the average number of *Ptgds* dots per *Ptgds*-expressing GnRH neuron **(f:**
667 Kruskal-Wallis one-way ANOVA on ranks: $H_{(3)}=5.484$, $P=0.0585$, $n=7,6,6$ rats/group)
668 determined from fluorescent *in situ* hybridization experiments. **(g,h)** Percentage of *Sox2*-
669 expressing cells that contain *Ptgdl* mRNAs **(g:** one-way ANOVA: $F_{(2,15)}=1.528$, $P=0.2489$,
670 $n=6$ rats/group) and average number of *Ptgdl* dots per *Sox2/Ptgdl*-co-expressing cell **(h:**
671 Kruskal-Wallis one-way ANOVA on ranks: $H_{(3)}=3.029$, $P=0.2306$, $n=6$ rats/group) determined
672 from fluorescent *in situ* hybridization experiments. **(b-c,e-h)** Values: means \pm s.e.m.* $P<0.05$;
673 ** $P<0.01$.

674

675 **Figure 6. Activation of DP1 signaling in the infantile preoptic region controls GnRH**
676 **neuron-progenitor association, and is involved in sexual maturation.** (a) Protocol used
677 to evaluate the involvement of DP1 signaling in the recruitment of progenitors by GnRH
678 neurons. Drawing: coronal view of the rat brain showing the anatomical localization of the
679 injection site, i.e. the preoptic region (dashed area). i.c., intracerebral; i.p., intraperitoneal. (b)
680 Percentage of GnRH neurons associated with BrdU⁺ cells 24h after a single BrdU injection at
681 P7 in the absence (control) or presence of the DP1 antagonist BWA868C (BWA) (one-way
682 ANOVA: $F_{(2,11)}=12.933$, $P=0.001$, $n=4,5,5$ rats/group; Tukey's multiple comparison test: 2h
683 versus 24h groups: 24h-control, $q_{(11)}=6.778$, $P=0.002$; 24h-BWA, $q_{(11)}=1.763$, $P=0.452$; 24h-
684 control versus 24h-BWA, $q_{(11)}=5.319$, $P=0.008$). (c) Total number of BrdU⁺ cells in the
685 preoptic region of control or BWA-treated animals (one-way ANOVA: $F_{(2,11)}=20.887$, $P<0.001$,
686 $n=4,5,5$ rats/group; Tukey's multiple comparison test: 2h versus 24h-control, $q_{(11)}=8.019$,
687 $P<0.001$; 2h versus 24h-BWA, $q_{(11)}=8.102$, $P<0.001$; 24h-control versus 24h-BWA,
688 $q_{(11)}=0.0883$, $P=0.998$). (d) Protocol used to evaluate the involvement of DP1 signaling in
689 sexual maturation. (e) Percentage of GnRH neurons associated with BrdU⁺ cells 7d after a
690 single BrdU injection at P8 in the absence or presence of BWA868C (two-tailed unpaired t -
691 test, $t_{(6)}=2.532$, $P=0.045$; $n=3,5$ rats/group). (f) Total number of BrdU⁺ cells in the preoptic
692 region of control or BWA-treated animals (two-tailed unpaired t -test, $t_{(6)}=0.771$, $P=0.470$;
693 $n=3,5$ rats/group). (g) Circulating FSH levels at P12 in BWA868C (BWA)-injected- and
694 control animals (two-tailed unpaired t -test, $t_{(20)}=3.075$, $P=0.006$; $n=8,14$ rats/group). Values:
695 means \pm s.e.m.; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

696

697 **Figure 7. Blocking DP1 signaling in the infantile period impairs GnRH neuron**
698 **maturation and functional activation.** (a,c) RT-PCR in control versus BWA868C-treated
699 FACS-sorted GnRH-EGFP neurons for (a) *Gnrh* and upstream regulators (Mann-Whitney
700 tests: *Gnrh*, $U=27$, $P=0.7925$; *Cebpb*, $U=8$, $P=0.0426$; *Kiss1r*, $U=27$, $P>0.9999$; two-tailed
701 unpaired t -tests: *Dicer*, $t_{(13)}=0.6232$, $P=0.5439$; *Otx2*, $t_{(11)}=0.7906$, $P=0.4459$; *Zeb1*,

702 $t_{(14)}=0.2605$, $P=0.7983$; $n=6,10,6,8,6,9,6,9,5,8,6,10$ rats/group) and (c) prostaglandin
703 pathway genes (Mann-Whitney tests: *Ptgs-2*, $U=18$, $P=0.3277$; *Ptges*, $U=19$, $P=0.3884$;
704 *Ptger1*, $U=4$, $P=0.0140$; *Ptger2*, $U=16$, $P=0.2238$; *Ptger3*, $U=4$, $P=0.0048$; two-tailed
705 unpaired t -tests: *Pla2g4a*, $t_{(14)}=0.1837$, $P=0.8569$; *Ptgs-1*, $t_{(13)}=0.1135$, $P=0.9113$; *Ptgds*,
706 $t_{(11)}=2.671$, $P=0.0218$; *Ptger4*, $t_{(14)}=1.656$, $P=0.12$; $n=6,10,6,9,6,9,6,7,6,9,6,7,6,9,6,9,6,10$
707 rats/group). Values expressed relative to control values, set at 1. (b) Prostaglandin
708 biosynthesis. (d) Representative GnRH neuronal soma (red) with apposed vGluT2- (left,
709 green) and vGAT- (right, green) immunoreactive (ir) punctae (arrowheads) in control (top)
710 and BWA868C-treated animals (bottom). Scale bar: 10 μ m. Bar graphs: vGluT2- (two-tailed
711 unpaired t -test, $t_{(12)}=3.057$, $P=0.0099$; $n=7$ rats/group) and vGAT-immunoreactive punctae
712 (two-tailed unpaired t -test, $t_{(10)}=1.61$, $P=0.1384$; $n=6$ rats/group) per GnRH soma. (e)
713 Representative fluorescent (top) and infrared-differential-interference-contrast (IR-DIC)
714 (bottom) photomicrographs of acute brain slice from a *Gnrh::Egfp* infantile female rat,
715 showing a patched preoptic GnRH neuron (green). Scale bar: 25 μ m. (f) Left: Whole-cell
716 recording of spontaneous activity in representative GnRH neurons from control (top) and
717 BWA868C-treated (bottom) animals. Arrowhead bottom left: resting membrane potential.
718 Right: Expansion of action potentials (APs) indicated at left. Note: Control cell APs (top) were
719 driven by temporal summation of small-amplitude depolarizing events (oblique lines in
720 expansion), while APs from BWA868C-treated animals (bottom) were preceded by a smooth,
721 relatively fast depolarization without synaptic events, except in recording #3. Threshold for
722 AP generation: dashed line. (g-j) Electrophysiological cell properties: mean resting
723 membrane potential (V_m) (g: Mann-Whitney test: $U=602$, $P=0.760$; $n=37$ cells, 21 control
724 rats; $n=34$ cells, 14 BWA868C-treated rats), membrane resistance (R_m) (h: Mann-Whitney
725 test: $U=157$, $P=0.589$; $n=16$ cells, 8 control rats; $n=22$ cells, 7 BWA868C-treated rats),
726 voltage threshold for AP generation (VTH) (i: two-tailed unpaired t -test, $t_{(18)}=0.963$, $P=0.348$;
727 $n=10$ cells, 8 control rats; $n=10$ cells, 8 BWA868C-treated rats), spontaneous firing rate (j:
728 Mann-Whitney test: $U=285.5$, $P<0.0001$; $n=37$ cells, 21 control rats; $n=34$ cells, 14
729 BWA868C-treated rats). Values: means \pm s.e.m.; * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

730

731 **Figure 8. Inhibition of DP1 signaling in the infantile period leads to perturbed estrous**

732 **cyclicity. (a)** Examples of estrous cycles in two control (*upper panels*) and two BWA-treated

733 animals (*lower panels*). Red dots: first estrus. D, diestrus; E, estrus; P, proestrus; vo, vaginal

734 opening. **(b)** Age at vaginal opening in BWA868C (BWA)-treated and control animals (two-

735 tailed unpaired *t*-test, $t_{(17)}=0.837$, $P=0.414$; $n=9,10$ rats/group). **(c)** Quantification of time

736 spent in the different phases of the estrous cycle during the first 15 days after vaginal

737 opening in control and BWA-treated animals (Diestrus, two-tailed unpaired *t*-test, $t_{(17)}=-1.160$,

738 $P=0.262$; Proestrus, Mann-Whitney test: $U=12.5$, $P=0.006$; Estrus, Mann-Whitney test:

739 $U=71.5$, $P=0.023$; $n=9,10$ rats/group). **(b-c)** Values: means \pm s.e.m. * $P<0.05$; ** $P<0.01$.

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- 898
- 899

900 METHODS

901

902 **Animals.** Sprague-Dawley rats were obtained from Janvier Laboratories (Saint Berthevin,
903 France). GnRH-EGFP transgenic rats, which express the enhanced green fluorescent
904 protein (EGFP) under the control of the rat GnRH promoter (NBRP Rat No: 0469, The

905 National BioResource Project for the Rat, Japan)^{24, 63} was a kind gift from Dr. Masakatsu
906 Kato (University of Tokyo Health Sciences, Tokyo, Japan). Wild-type and transgenic mice
907 expressing a dominant-negative form of the ErbB4 receptor in astrocytes (*GFAP::DN-ErbB4*)
908 were generated as described previously³⁷. The animals were maintained at 23-25°C on a
909 12-h light-dark cycle, with food and water available *ad libitum*. Animal studies were approved
910 by the Institutional Ethics Committee for the Care and Use of Experimental Animals of the
911 University of Lille (APAFIS#2617-2015110517317420 v5). The bisphenol A treatment
912 experiment was performed on Wistar rats purchased from the University of Liège and carried
913 out with the approval of the Belgian Ministry of Agriculture and the Ethical Committee at the
914 University of Liège. Animals were raised in polypropylene cages (Ref 1291H006, Tecnilab,
915 323 Netherlands), fed with low phytoestrogen chow (V135 R/Z low phytoestrogen pellets,
916 SSNIFF 324 Diet, Netherlands). Water was supplied in glass bottles to reduce EDC
917 contamination. All experiments were performed in accordance with the guidelines for animal
918 use specified by the European Union Council Directive of September 22, 2010 (2010/63/EU).
919 All experiments were performed in female animals, except the preparation of primary cultures
920 of preoptic progenitors, for which both sexes were used.

921

922 **BrdU injections.** Unless otherwise stated, animals received a single intraperitoneal (i.p.)
923 injection of BrdU (Sigma) at a dose of 300 mg/kg body weight at different postnatal ages and
924 were allowed to survive for 2h, 24h, 7 days or \geq 60 days. A group of rats received 3 i.p.
925 injections of BrdU (150 mg/kg) per day for 3 consecutive days (P6-P8) and were sacrificed at
926 P15.

927

928 **Stereotactic brain injection of paclitaxel-loaded microparticles.** The preparation,
929 physicochemical characterization and drug release kinetics of paclitaxel-loaded, poly(D,L-
930 lactic-co-glycolic acid) (PLGA)-based microparticles have been described¹¹. Briefly,
931 Paclitaxel (Zyo Pharma Trade, Hamburg, Germany) was encapsulated into PLGA-based

932 microparticles at a drug loading of 40% (w/w) (Carlina Technologies, Angers, France). Drug-
933 free microparticles were prepared accordingly without adding paclitaxel. Female rats at P6 or
934 P7 ($n = 35$) were anesthetized with an i.p. dose of a mixture of ketamine hydrochloride
935 (50 mg/ml; 60 mg/kg) and xylazine hydrochloride (2%; 10 mg/kg), secured in a stereotaxic
936 apparatus, and injected medially with 1 μ l of a suspension of 50 μ g paclitaxel-loaded or drug-
937 free microparticles in sterile water using a microsyringe with a 21 gauge needle (Hamilton,
938 Reno, NV) into the preoptic region of the hypothalamus, dorsal to the MePO (bregma:
939 0.0 mm, 6 mm deep from the skull)⁶⁴. All animals received an i.p. injection of BrdU at P8 and
940 were sacrificed at adult age. Brains were processed for immunofluorescent labeling of BrdU,
941 GnRH and GFAP, and cell nuclei counter-staining using the nuclear marker Hoechst or DAPI
942 to determine the implantation site of microparticles. Animals with an implantation site located
943 outside the preoptic region were excluded from the analysis.

944

945 **Bisphenol-A treatment.** Exposure to bisphenol A (BPA) was performed as described
946 previously¹⁶. Briefly, female pups were subcutaneously injected (0.05 mL) with either vehicle
947 (corn oil) or BPA (Ref 23,9658; Sigma-Aldrich) at a dose of 25 ng/kg.d. Injections were given
948 every 24 hours from P1 to P15. All animals received an i.p. injection of BrdU at P8 and were
949 sacrificed 2 hours or 7 days later. Brains were extracted and processed for
950 immunofluorescent staining of BrdU and GnRH.

951

952 **Stereotaxic brain infusions of BWA868C.**

953 *Acute injections.* Female rats received an i.p. injection of BrdU at P7. A group of animals ($n =$
954 4) was sacrificed 2h after the injection. The other animals were placed in a stereotaxic frame
955 (Kopf® Instruments, California) under anesthesia (isoflurane), and 2 burr holes were drilled
956 0.5 mm apart from the bregma, according to a rat brain atlas⁶⁴. A 10 μ l Hamilton syringe was
957 slowly inserted into the preoptic region (5.2 mm deep relative to the dura), and 1 μ l of a
958 solution containing 10 nM BWA868C (Cayman Chemical, Ann Arbor, Michigan, USA) ($n = 5$)
959 or vehicle (saline solution 0.9%, $n = 5$) was injected bilaterally using an infusion pump (KD

960 Scientific, Holliston, MA) over 5 min. Animals were killed 24h later and their brains processed
961 for immunofluorescent labeling of BrdU and GnRH.

962 *BWA868C implants.* Female rats were placed in a stereotaxic frame under anesthesia, and
963 one burr hole was drilled at the level of the bregma as detailed above. A stainless steel 5 mm
964 cannula filled at its tip with BWA868C (10 nM) or vehicle mixed with melted cocoa butter was
965 stereotaxically implanted in the preoptic region at the level of the bregma, as previously
966 described⁶⁵. After implantation, the cannulas were fixed to the skull with superglue, and the
967 skin wound sutured. Blood was collected via submandibular bleeding from animals at P12 for
968 plasma FSH measurements using a procedure described previously by others⁶⁶.

969

970 **Physiological measurements.**

971 *Puberty onset and estrous cyclicity.* Animals were monitored daily for imperforation of the
972 vaginal membrane (i.e. vaginal opening). After vaginal opening, vaginal smears were
973 performed daily and analyzed under an inverted microscope to identify the specific day of the
974 estrous cycle. The percentage of complete estrus cycles was calculated as the number of
975 occurrences of the sequence “Diestrus-Diestrus-Proestrus-Estrus”, defined as a complete
976 estrus cycle, divided by the theoretical maximum possible number of complete estrus cycles
977 over the recorded period of time for each animal (> 2 weeks).

978 *Follicle-stimulating hormone level measurements.* Serum FSH levels were measured
979 using radioimmunoassay as previously described⁴. The accuracy of hormone measurements
980 was confirmed by the assessment of rodent serum samples of known concentration (external
981 controls).

982

983

984 **Electrophysiology.**

985 *Brain slices preparation.* Electrophysiological recordings were performed on living brain
986 slices containing the preoptic region from 10- to 15-days-old GnRH-EGFP transgenic rats.
987 Rats were anesthetized with isoflurane and decapitated. The brain was removed and rapidly
988 placed in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 120 NaCl, 3.2 KCl,

989 1 NaH₂PO₄, 26 NaHCO₃, 1 MgCl₂, 2 CaCl₂, 10 glucose (300 mOsm, pH 7.4) and bubbled
990 with 95% O₂-5% CO₂. 200- μ m coronal slices were cut using a vibratome (VT1200; Leica).
991 Slices were incubated at 34°C in oxygenated aCSF for a recovery period of 1 hour, and then
992 at room temperature until recording.

993 *Patch-clamp recording.* For patch-clamp recording, individual brain slices were placed in a
994 submerged recording chamber (Warner Instruments), immobilized by a nylon grid and
995 continuously perfused at 3 ml/min with oxygenated aCSF maintained at 32.8°C by a heater
996 controller (TC-344C; Warner Instruments). GnRH neurons were visualized and identified
997 under 10x and 40x magnification using an upright fluorescent microscope with infrared
998 differential interference contrast (Leica DM-LFSA) and an ORCA-Flash 4.0 digital CMOS
999 camera (Hamamatsu). Recording pipettes were pulled from borosilicate glass capillaries (1.5
1000 mm outer diameter; 1.12 mm inner diameter; World Precision Instruments) using P1000
1001 Flaming Brown puller (Sutter Instruments Co) and had resistance from 7 to 9 M Ω when filled
1002 with an internal solution containing (in mM): 140 K-gluconate, 10 KCl, 1 EGTA, 2 Na₂-ATP
1003 and 10 HEPES, pH 7.3 with KOH. Pipettes were placed in contact with GnRH neurons using
1004 a PCS-5400 micromanipulator (Thorlabs). Whole-cell patch-clamp recordings were
1005 performed in current-clamp mode using a Multiclamp 700B Amplifier, digitized with the
1006 Digidata 1322A interface and acquired with pClamp 10.2 software (Molecular Devices). Data
1007 were filtered at 1 kHz and sampled at 5 kHz. Recordings were analyzed with Clampfit 10.2
1008 from pClamp software (Molecular Devices). Resting membrane potential, spontaneous firing
1009 rate, membrane resistance, voltage threshold for action potential generation, full amplitude
1010 and half width duration of action potentials, after-hyperpolarizing potential amplitude and
1011 duration, and time constant were determined as previously described^{32, 67}. Only cells which
1012 showed less than 20% change in access resistance throughout the recording were included
1013 in this study. Junction potential was corrected in the data analysis.

1014 *Biocytin labeling of preoptic astrocytes.* Slices were incubated during 30 minutes in 100 nM
1015 sulforhodamine 101 (SR101, Sigma), a fluorescent dye that is selectively taken up by
1016 astrocytes, and were pretreated for 20 minutes with 50 μ M carbenoxolone (Sigma) to inhibit

1017 gap junctions. Biocytin (2 mg/ml, Sigma) was added to the internal solution and astrocytes
1018 were patched in whole-cell configuration for 20 minutes. Electrical membrane properties of
1019 astrocytes were measured in voltage-clamp mode by applying a series of voltage pulses
1020 from -100 mV to +100 mV (300 ms, 10 mV increments) from a holding potential of -80 mV as
1021 previously described³⁰. Astrocytes showed typical glial cell membrane properties including a
1022 highly polarized membrane potential and a linear current-voltage (I-V) relationship³⁰. Slices
1023 were fixed with 4% paraformaldehyde overnight at 4°C, incubated with Streptavidin Alexa
1024 Fluor 647 (1:250, Invitrogen) in PBS containing 0.5% Triton X-100 overnight at 4°C,
1025 counterstained with DAPI (1:1000) and mounted on glass slides with Mowiol.

1026

1027 **Preoptic neurosphere cultures.** The preoptic region of female rats aged 10 or 19 days was
1028 dissected and minced on 80 µm nylon mesh (Buisine, Clermont de l'Oise, France). The
1029 dissected region corresponded to a 1-mm-thick triangular tissue located behind the diagonal
1030 band of Broca just before the OVLT, i.e., the beginning of the third ventricle, and the optic
1031 chiasm. We checked that our dissection excluded the subventricular zone of the lateral
1032 ventricle. The cell suspension was transferred to a culture flask (BD Biosciences, San Jose,
1033 CA) and cultured in neurosphere medium composed of Dulbecco's Modified Eagle's Medium
1034 (DMEM)/F-12 with L-glutamine, 15 mM HEPES (#31330-038) supplemented with 2% (v/v) B-
1035 27 (#17504-044), 20 ng/ml EGF (human recombinant; #PHG0311), 20 ng/ml FGF2 (human
1036 recombinant; #13256-029), 2 mM L-glutamine (25030-024), and 100 units of penicillin and
1037 100 µg of streptomycin/ml (#15140-122) (all from Gibco, Carlsbad, CA) under a humid
1038 atmosphere of 5% CO₂-95% air at 37°C. Cells were grown in hypoxic conditions by covering
1039 flask caps with parafilm. Neurospheres were formed after ~1 week. Thereafter, the
1040 neurospheres were collected twice a week through centrifugation, dissociated by mechanical
1041 trituration and passaged in half-renewed neurosphere medium to form further generations of
1042 neurospheres. Neurospheres were kept for up to 31 passages, the longest time examined.

1043 *Clonogenicity.* To evaluate the generation of secondary neurospheres from single cells,
1044 primary neurospheres were dissociated into single cells by either mechanical dissociation

1045 through 20 µm nylon mesh (Buisine) or enzymatic digestion using StemProAccutase Cell
1046 Dissociation Reagent (Gibco) for 10 min at room temperature. Cells were plated in 96-well
1047 culture plates at a density of 50, 75 or 100 cells per well in 50 µl of neurosphere medium.
1048 Wells containing cell aggregates 24h after plating were excluded from the analysis. Cultures
1049 were fed with 100 % (v/v) fresh neurosphere medium every 3 days until day 10, when wells
1050 were examined for the presence of neurospheres.

1051 *Differentiation experiments.* To evaluate their differentiation potential, neurospheres were
1052 transferred to 24-well plates on poly-L-lysine (0.01%; #P4832, Sigma)-coated glass
1053 coverslips and grown in DMEM/F12 with L-glutamine, 15 mM HEPES (#31330-038)
1054 supplemented with 10% (v/v) fetal bovine serum (#10270106), 2 mM L-glutamine, and 100
1055 units of penicillin and 100 µg of streptomycin/ml (all from Gibco) for 7 days.

1056

1057 **Adherent cultures of preoptic progenitors.** The preoptic region of rats aged 1 or 2 days
1058 was dissected as described above and minced on 20 µm nylon mesh (Buisine). The cell
1059 suspension was transferred to a culture flask (BD Biosciences) coated with poly-D-lysine (10
1060 µg/ml, P7405, Sigma) and laminin (10 µg/ml, L6274, Sigma) and cultured in neurosphere
1061 medium under a humid atmosphere of 5% CO₂-95% air at 37°C. The medium was changed
1062 every 3 to 4 days. Cells were passaged once, when 70-80% confluence was reached, and
1063 were then used for migration assays, snap frozen in dry ice for gene expression analysis or
1064 seeded on 24-well plates glass coverslips for proliferation assays or immunocytochemical
1065 characterization.

1066

1067 **GnV3 cell culture and treatment.** Cells were grown in Neurobasal-A medium (10888-022)
1068 supplemented with 2% (v/v) B-27, 100 units of penicillin and 100 µg of streptomycin/ml, 25
1069 mM GlutaMAX (35050-038), 1% fetal bovine serum, 50 µg/ml FGF2 (all from Gibco) and 1
1070 µg/µl doxycycline (D-9891, Sigma) as described⁶⁸. All experiments were performed on GnV3
1071 cells at their 13th or 14th passage.

1072 *Preparation of hypothalamic astrocyte-conditioned medium.* Primary astrocyte cultures
1073 were prepared from the hypothalamus of 1-2-day-old rat pups and grown in DMEM/F12
1074 medium (#31330-038) supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine,
1075 and 100 units of penicillin and 100 µg of streptomycin/ml (all from Gibco) as previously
1076 described³⁶. After reaching confluence (i.e., after ~7-10 days), contaminants were removed
1077 by overnight shaking. The growth medium was replaced by astrocyte-defined medium (ADM)
1078 composed of DMEM (41965-039) supplemented with 2 mM L-glutamine, 5 µg/ml insulin (I-
1079 5500, Sigma) and 100 µM putrescine (P-5780, Sigma). After two days, the culture medium
1080 (now ACM, astrocyte-conditioned medium) was collected, centrifuged at 1,000 rpm and the
1081 supernatant was kept at -80°C until use.

1082 *Treatment of GnV3 cells with hypothalamic astrocyte-conditioned medium.* One day
1083 before treatment, GnV3 cells were plated at a density of 2×10^5 cells/cm² in their growth
1084 medium. Cells were treated with ADM (control condition) or ACM for a total of 4 days, with a
1085 culture medium renewal after every 2 days. At the end of the 4-day treatment, GnV3 cells
1086 were collected in Trizol (Invitrogen) for microarray analysis or lysis buffer (Qiagen) for RT-
1087 qPCR analysis, and stored at -80°C until use. Treatment of GnV3 cells was performed in
1088 three experiments using ACM from three different primary astrocyte cultures.

1089

1090 ***In vitro migration assay.*** Boyden microchemotaxis chambers were used according to the
1091 manufacturer's instructions (Neuro Probe, Gaithersburg MD, USA). In brief, primary preoptic
1092 progenitors were harvested, re-suspended in control medium (Neurobasal-A medium
1093 supplemented with 2% (v/v) B-27, 2 mM L-glutamine, and 100 units of penicillin and 100 µg
1094 of streptomycin/ml (all from Gibco)) and plated in 24-well plate chambers at a density of
1095 150,000 cells per well on the open-bottom wells of the upper compartment, previously coated
1096 with poly-D-lysine and laminin. The two compartments of each well were separated by a
1097 polyvinylpyrrolidone-free polycarbonate porous membrane (8 µm pore size). The lower
1098 chamber was filled with control medium containing GnRH (100 ng/ml, GeneCust, Ellange,
1099 Luxembourg), PGD2 (1 µM, Sigma), galanin (2 µM, Tocris Bioscience, Bristol, UK),

1100 glutamate (10 μ M, Sigma), GABA (2 μ M, Sigma) or EGF (100 ng/ml, Gibco). BWA868C (10
1101 nM or 1 μ M) was added to the cell suspension in the upper compartment. After 18h of
1102 incubation in 5% CO₂, 95% air at 37°C, cells attached to the upper side of the filter were
1103 mechanically removed with a cotton swab. Cells that had migrated to the lower side were
1104 fixed with 4% paraformaldehyde (30 minutes at room temperature) and stained with Hoechst
1105 (1:10,000). Cells were photographed with a 20x objective and counted using ImageJ 1.52p
1106 software (NIH, Bethesda).

1107

1108 ***In vitro* proliferation assay.** Primary preoptic progenitors were transferred to 24-well plates
1109 on poly-D-lysine/laminin-coated glass coverslips and grown in neurosphere medium. Once
1110 cells were 70-80% confluent, they were transferred to neurosphere medium devoid of FGF2
1111 (starvation medium) for 24h prior to treatment with GnRH, PGD2, galanin, glutamate, GABA
1112 (same concentrations as in migration assays) or FGF2 (100 ng/ml) in starvation medium for
1113 24h. Coverslips were fixed and processed for Ki67 immunolabeling.

1114

1115 **Immunofluorescence labeling**

1116 *Tissue preparation.* Animals were anesthetized by i.p. injection of pentobarbital (70
1117 mg/kg). They were perfused transcardially with a rinse of saline solution (0.9% NaCl),
1118 followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer(PB) (pH 7.4). The
1119 brains were removed and immersed in the same fixative for 2-3h at 4°C. They were then
1120 transferred to PB containing 20% sucrose until they had sunk, embedded in Tissue Tek®
1121 (Sakura Finetek, Villeneuve d'Ascq, France), and frozen in liquid nitrogen. Frozen 14- μ m
1122 coronal sections containing the preoptic region (0.20 mm to -0.30 mm relative to bregma)⁶⁴
1123 were prepared using a cryostat (CM3050S, Leica, Nussloch, Germany) and mounted on
1124 chrome-alum-gelatin coated slides, air-dried and subjected to immunolabeling.

1125 *Immunohistofluorescence.* The sections were subjected to microwave pretreatment in a
1126 solution of sodium citrate 0.01 M (pH 6) for 4 minutes at 800 W followed by 2 cycles 5
1127 minutes each at 400 W for antigen retrieval. After cooling at room temperature and washing

1128 in PB, sections were incubated with primary antibodies (**Supplementary Table 1**) diluted in
1129 PB containing 0.9% sodium chloride, 0.3% Triton X-100 and 2% normal donkey serum
1130 (PBSTS) overnight at 4°C. Sections were then incubated with appropriate secondary
1131 antibodies at room temperature for 1h (**Supplementary Table 2**). After nuclei staining with
1132 Hoechst or DAPI (1/1,000), sections were coverslipped in Mowiol mounting medium (20%
1133 Mowiol, 2.5% DABCO in Tris 0.2 M pH 8.5). Control sections were incubated in the absence
1134 of primary antibody. Omission of the primary antibody resulted in no staining.

1135 *Immunocytofluorescence.* Coverslips were fixed in 4% paraformaldehyde (15 min at room
1136 temperature), incubated with PBS 0.1 M containing 0.1% Triton X-100 and 0.3% normal
1137 donkey serum for 30 minutes at room temperature, and subjected to immunofluorescent
1138 labeling following the same procedure as for tissue sections. For O4 immunodetection, the
1139 anti-O4 antibody (**Supplementary Table 1**) was applied to living cells for 2h at 37°C before
1140 fixation.

1141

1142 **Fluorescent *in situ* hybridization.** FISH was performed on frozen brain sections of the
1143 preoptic region of female rats at P8, P12 and P20 with the RNAscope® Multiplex Fluorescent
1144 Kit v2 according to the manufacturer's protocol (Advanced Cell Diagnostics, Inc., Newark,
1145 CA, USA). Specific probes were used to detect *Ptgds* (486051, NM_013015.2, target region
1146 2 - 702), *Ptgdl* (486041, NM_022241.1, target region 224 - 1157), *Gnrh1* (502431-C2,
1147 NM_012767.2, target region 2 - 454) and *Sox2* (502441-C3, NM_001109181.1, target region
1148 51 - 2079) mRNAs. Hybridization with a probe against the *Bacillus subtilis* dihydrodipicolinate
1149 reductase (*dapB*) gene (320871) was used as negative control.

1150

1151 **Microscopy**

1152 Analysis of sections and acquisition of images were done using an Axio Imager Z2 Apo-
1153 Tome microscope equipped with a motorized stage (Zeiss, Germany) and an ORCA-Flash
1154 4.0 V2 camera (Hamamatsu, Japan) driven by the Zen 2.3 (blue edition) imaging software
1155 (Zeiss). For FISH experiments and immunofluorescence labeling for vGluT2 and vGAT,

1156 acquisition of images was performed using an inverted confocal microscope (LSM 710,
1157 Zeiss, Jena, Germany). High magnification photomicrographs were acquired with a 63x
1158 objective (NA 1.4) using the Airyscan detector (Zeiss). Images to be used for figures were
1159 pseudocolored, adjusted for brightness and contrast and merged using Adobe Photoshop
1160 22.1.0 (Adobe Systems, San Jose, CA).

1161

1162 **Microscopic analyses.** All analyses were done in the preoptic region, as defined in the
1163 Swanson stereotaxic rat brain atlas, between plate 17 (i.e., beginning of the OVLT) and plate
1164 20 (i.e., crossing of the anterior commissure) ⁶⁹, with a particular attention to the MePO,
1165 where most GnRH neurons that stimulate the LH surge are located ⁷⁰. Analyses were made
1166 on one out of every 5 sections. Estimation of total cell populations in the preoptic region was
1167 obtained by multiplying the number of cells per section by the total number of sections per
1168 preoptic region, and then correcting for split nuclei ⁷¹.

1169 *Morphological association between GnRH neurons and BrdU⁺ cells.* Quantification was
1170 made under the fluorescent microscope at the 40x objective. A BrdU⁺ cell was considered
1171 morphologically associated with a GnRH neuron when it was located at a distance \leq the size
1172 of the BrdU⁺ nucleus (i.e., $\leq 10 \mu\text{m}$) from the GnRH neuron cell body with no interposed cell
1173 nucleus in between (**Supplementary Fig. 3**).

1174 *Number of cell nuclei at the injection site of microparticle-injected rats.* The total number of
1175 Hoechst-stained nuclei was automatically quantified using the ImageJ software on
1176 microphotographs of the injection site taken at the 20x objective. Quantification represents
1177 the mean number of Hoechst-stained nuclei in a 20x microscopic field, corresponding to a
1178 surface area of 0.5 mm^2 , counted on 3 sections per animal ($n = 12$).

1179 *Number of BrdU⁺ cells at the injection site of microparticle-injected rats.* Analysis was
1180 performed on photomontages of the preoptic region taken at the 20x objective. Quantification
1181 represents the mean number of BrdU⁺ cells in a 1 mm square centered on the injection site,
1182 counted on 3 to 4 sections per animal ($n = 13$).

1183 *Quantification of vGluT2 and vGaT appositions to GnRH neuron cell bodies.* Analysis was
1184 performed on high magnification images of GnRH neuron somata located at the level of the
1185 OVLT using the IMARIS 9.3 software (Bitplane) as described before ⁷². Briefly, the number of
1186 vGluT2- or vGaT-immunoreactive punctae closely apposed with the GnRH neuron soma was
1187 quantified on 3D reconstructions from raw confocal image z-stacks using a channel of
1188 colocalization. A punctum was considered apposed when no black pixels were visible
1189 between the GnRH and the vGluT2 or vGaT fluorescence. The value was normalized to the
1190 number of optical sections through the z-depth of the neuron and the surface area of the
1191 neuronal soma. The resulting value was multiplied by the average number of optical sections
1192 and the average surface area of all neurons analyzed to provide a number of punctae per
1193 GnRH soma.

1194

1195 **Western blot analyses.** Preoptic primary progenitors were starved for 24h in DMEM/F-12
1196 with L-glutamine, 15 mM HEPES supplemented with 2 mM L-glutamine, 100 units of
1197 penicillin and 100 µg of streptomycin/ml, 5 µg/ml insulin (Sigma) and 16 µg/ml putrescine
1198 (Sigma). The cells were treated with PGD2 1 µM and/or BWA868C (10 nM or 1 µM) in fresh
1199 medium for 7 minutes and snap-frozen on dry ice. Proteins were extracted and subjected to
1200 size-fractionation as previously described ⁴. The proteins were then transferred onto 0.2 µm
1201 pore-size nitrocellulose membranes (Invitrogen) using the NuPAGE system (Invitrogen).
1202 Membranes were blocked for 1 h in blocking buffer (Tris buffer saline (0.05 M Tris, pH 7.4,
1203 0.15 M NaCl) with 0.05% Tween 20 (TBST) and 5% nonfat milk) at room temperature and
1204 incubated overnight at 4°C with the appropriate primary antibody diluted in blocking buffer
1205 (rabbit polyclonal anti-phospho-p44/42 MAPK (Thr202/Tyr204) (pErk) #9101, 1:1,000; rabbit
1206 polyclonal anti-p44/42 MAPK (Erk) #9102, 1:1,000, Cell Signaling Technology, Beverly, MA,
1207 USA). After washing with TBST, membranes were exposed to horseradish peroxidase-
1208 conjugated secondary antibodies (goat anti-rabbit IgG, PI1000, 1:2000, Vector) diluted in
1209 blocking buffer for 1 h at room temperature. Immunoreactions were visualized using the ECL

1210 detection kit (NEL101; PerkinElmer, Boston, MA). Immunoblots were scanned using a
1211 desktop scanner (Epson Expression 1680 PRO) and Adobe Photoshop.

1212

1213 **Isolation of hypothalamic GnRH neurons using fluorescence-activated cell sorting.**

1214 The preoptic regions of *Gnrh::Egfp* transgenic rats were microdissected and enzymatically
1215 dissociated using a Papain Dissociation System (Worthington, Lakewood, NJ) to obtain
1216 single-cell suspensions. FACS was performed using an ARIA SORP cell sorter cytometer
1217 and FACSDiva 8.0 software (BD Biosciences). Data were analyzed using the Kaluza 2.0
1218 software (Beckman Coulter). The sort decision was based on measurements of EGFP
1219 fluorescence (excitation: 488 nm, 50 mW; detection: GFP bandpass 530/30 nm,
1220 autofluorescence bandpass 695/40 nm) by comparing cell suspensions from the preoptic
1221 region and from the cerebral cortex of *Gnrh::Egfp* animals. A total of 174 ± 20 (range, 44 –
1222 700) EGFP-positive cells were sorted per animal and directly lysed into 10 μ l of extraction
1223 buffer containing 0.1% Triton[®] X-100 (Sigma) and 0.4 unit/ μ l RNaseOUT[™] (Life
1224 technologies).

1225

1226 **Quantitative RT-PCR analyses.** Gene expression analyses were performed on FACS-
1227 sorted GnRH neurons, primary preoptic progenitor cultures and GnV3 cells. For primary
1228 preoptic progenitors, RNAs were extracted as follows: 100 μ l of Trizol (Life Scientific,
1229 Carlsbad CA, USA) were used to lyse cells before adding 100 μ l of chloroform (Merck,
1230 Darmstadt, Germany) and centrifuging at 12,000 g for 15 minutes at 4°C. The aqueous
1231 phase was carefully collected and added to isopropanol at 1:1 before centrifuging at 12,000 g
1232 for 10 minutes at 4°C. The aqueous phase was then discarded and the pellet washed in 70%
1233 ethanol. After centrifugation at 12,000 g for 5 minutes at 4°C, the pellet was left to air-dry and
1234 then diluted in 10 μ l DEPC water. The purity and quantity of RNA were determined by UV
1235 spectroscopy (Nanodrop 1000, Thermo Scientific, Waltham MA, USA). Messenger RNAs
1236 obtained from FACS-sorted GnRH neurons or primary progenitors were reverse transcribed
1237 using SuperScript[®] III Reverse Transcriptase (Life Technologies). For sorted cells, a linear

1238 preamplification step was performed using the TaqMan®PreAmp Master Mix Kit protocol
1239 (Applied Biosystems). Real-time PCR was carried out on Applied Biosystems 7900HT Fast
1240 Real-Time PCR System using exon-boundary-specific TaqMan® Gene Expression Assays
1241 (Applied Biosystems) (**Supplementary Table 3**). Gene expression data were analyzed using
1242 SDS 2.4.1 and Data Assist 3.0.1 software (Applied Biosystems) with R45S and actin as
1243 control housekeeping mRNAs following a standardized procedure ⁷³. For GnV3 cells, RNAs
1244 were purified using the RNeasy Mini Kit (Qiagen, Basel, Switzerland) according to the
1245 manufacturer's instructions. The solution was treated with DNase (RNase-Free DNase Set,
1246 Qiagen, Basel, Switzerland) and eluted with 30 µl of water. RNA concentrations were
1247 determined with a Nanodrop® (ND-1000 Spectrophotometer, Witec, Luzern, Switzerland).
1248 Two hundred ng of RNA was reverse transcribed using the RT High Capacity RNA-to-cDNA
1249 Kit (Applied Biosystems, Rotkreuz, Switzerland). Real-Time PCR analysis was performed on
1250 2 µl of cDNA using Power SYBR Green Taq polymerase master mix (Applied Biosystems,
1251 Rotkreuz, Switzerland). The primer sequences used were: Ptgds forward: 5'-
1252 TGGGTCTCTTGGGATTCCA-3', Ptgds reverse: 5'-CCCCAGGAACTTGTCTTGTTG-3';
1253 polymerase 2a (Polr2a, as endogenous control) forward: 5'-
1254 GTAAGGGCCACTATCTTCATCATCA-3', Polr2a reverse: 5'-
1255 CCCTCATCATACTGGTCACATC-3'.

1256

1257 **Microarray hybridization and data analysis.** RNA purification and microarray hybridization
1258 were performed as previously described ²⁰. Total RNA was extracted from the cells using
1259 Trizol (Invitrogen), according to the manufacturer's instructions. Only RNA samples with RNA
1260 integrity numbers greater than 9.0 were accepted for microarray analysis. Microarray
1261 hybridization was performed with Affymetrix GeneChip Rat Gene 1.0 ST arrays according to
1262 the manufacturer's instructions and scanned with the Gene Array scanner (Affymetrix). The
1263 fluorescence images obtained were processed using the BioConductoraffy package (DAFL,
1264 Lausanne). Expression values were measured using the Robust Multi-array Average (RMA)
1265 algorithm generating expression values on a log₂ scale. RMA includes background

1266 correction, quantile normalization and probe set summary by robust regression. GnV3
1267 signature of 22,336 transcripts was generated and annotated with Gene Symbol. Genes
1268 without an Entrez annotation were filtered out. The fold change was calculated individually
1269 for each gene. Differential hybridized features were identified using Bioconductor package
1270 “limma”⁷⁴. Additionally, the false discovery rate, i.e. the expected proportion of rejected null
1271 hypotheses that are wrongly rejected, was estimated by a resampling procedure⁷⁵. Genes
1272 that showed substantial up- or down-regulation by log fold changes (FC) > 1 with a FDR <
1273 0.05 were considered as differentially expressed. For each condition (ADM or ACM),
1274 microarray analyses were performed using RNA samples derived from three wells per
1275 condition. Only the list of genes showing significant up and down-regulation is provided in the
1276 **Supplementary Data 1**.

1277

1278 **Statistics.** No statistical methods were used to pre-determine sample sizes but our sample
1279 sizes are similar to those reported in previous publications^{4, 25, 26, 37, 76}. Studies were not
1280 formally randomized and investigators were not blind to treatment group. All analyses were
1281 performed using the SigmaStat 3.5 (Systat) or GraphPad Prism 7.05 (GraphPad) software.
1282 Outliers were identified using the ROUT (Q=1%) method. Statistical differences were
1283 evaluated using unpaired two-sided Student’s *t*-tests for comparison of two groups and one-
1284 or two-way analysis of variance (ANOVA) with Tukey’s *post hoc* tests for comparison of more
1285 than two groups. When the criterion for normality or equal variances was not met, the Mann-
1286 Whitney Rank Sum Test or the Kruskal-Wallis One Way ANOVA on Ranks Test was used to
1287 compare two or more groups, respectively. $P < 0.05$ was considered significant.

1288

1289 **Data availability.** Uncropped Western blots and Source Data Files for transcriptomic
1290 analysis of GnV3 cells are provided as Extended data. Additional data that support the
1291 findings of this study are available from the corresponding author upon request.

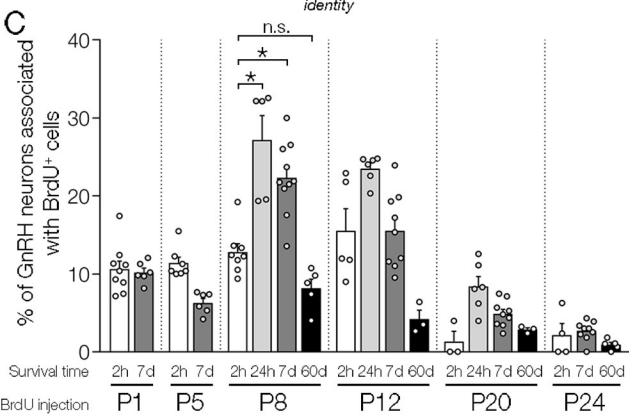
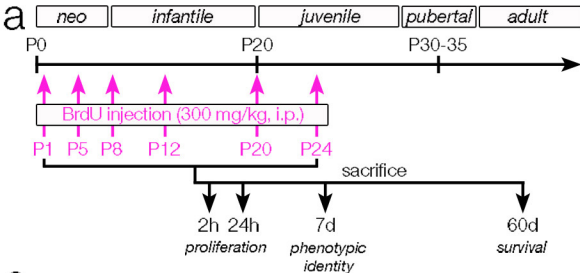
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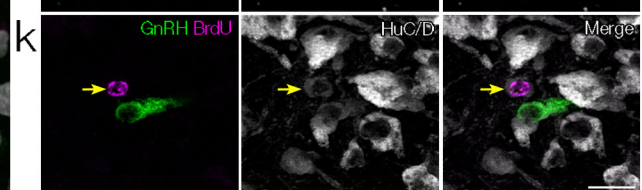
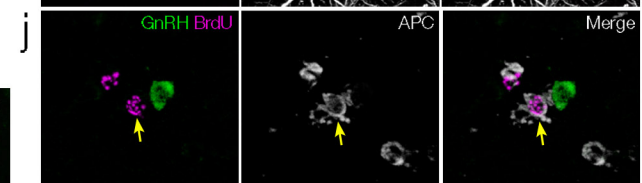
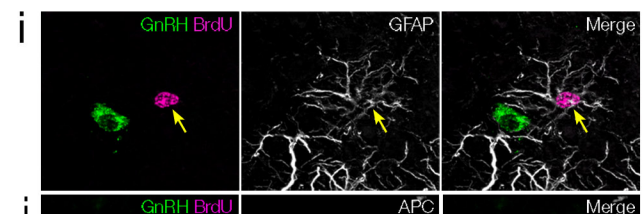
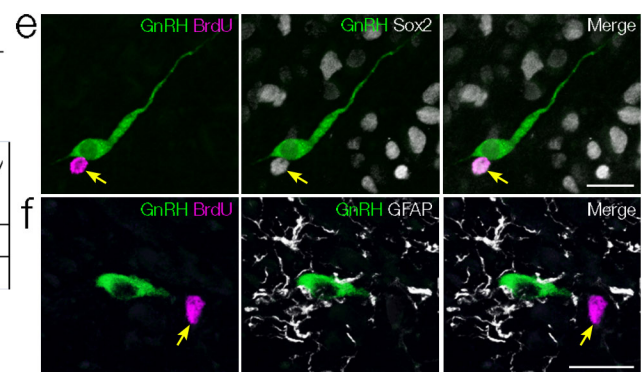
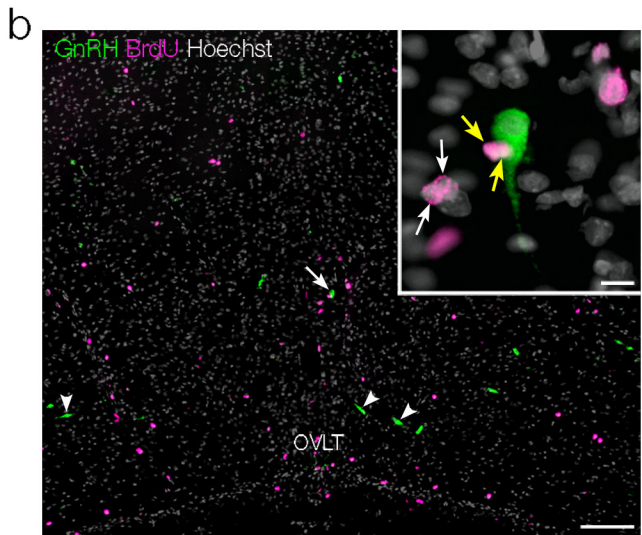
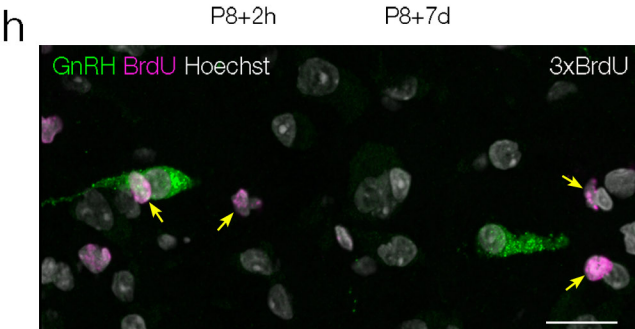
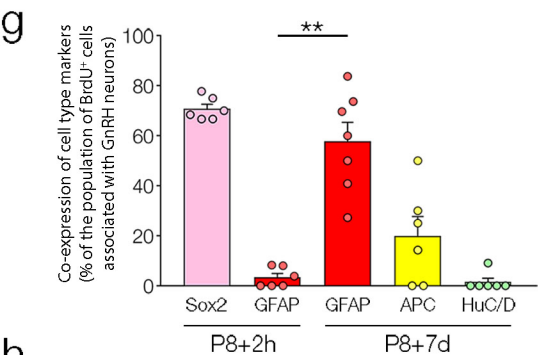
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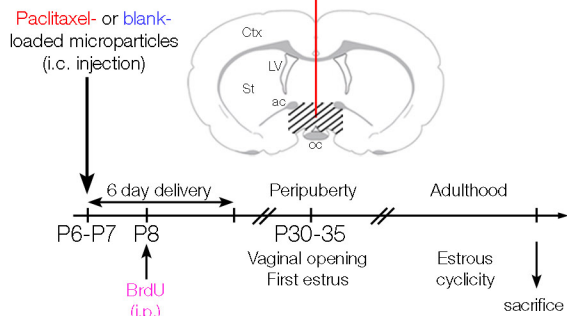


d

	% of GnRH neurons associated with BrdU ⁺ cells	% of HuC/D ⁺ neurons associated with BrdU ⁺ cells	Ratio % GnRH/% HuC/D ⁺
P8 + 2h	12.8 ± 1.0%	3.6 ± 0.2%	3.56
P8 + 7d	22.3 ± 1.5%	5.0 ± 0.2%	4.46

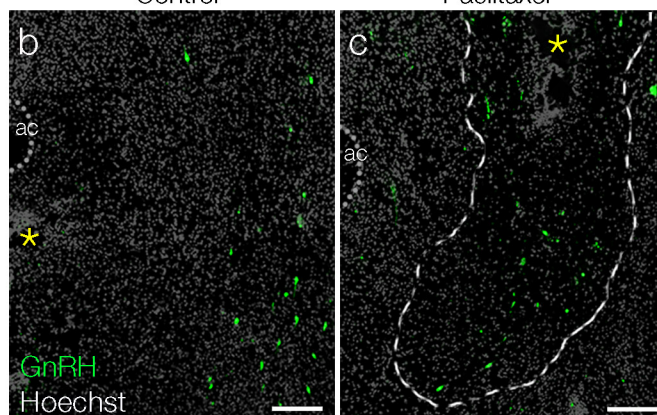


a

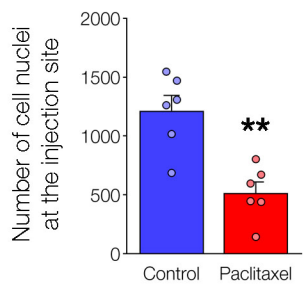


Control

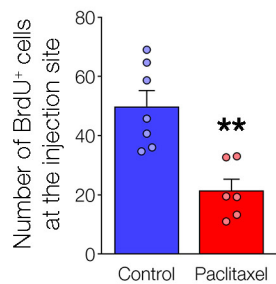
Paclitaxel



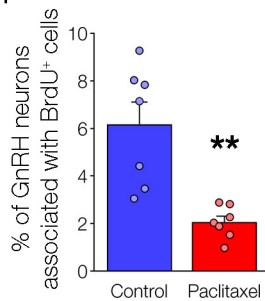
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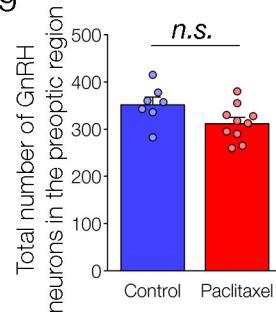
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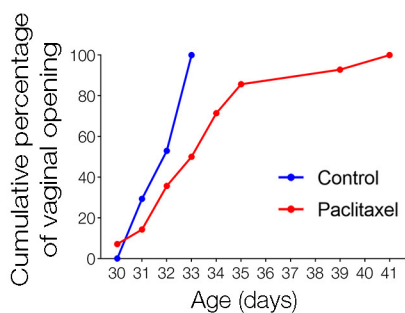
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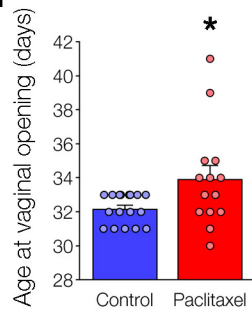
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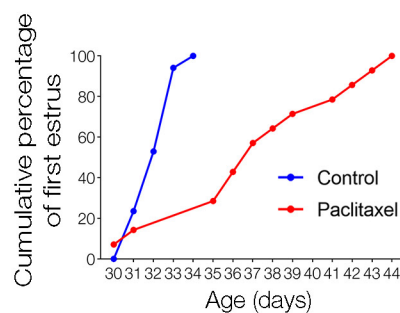
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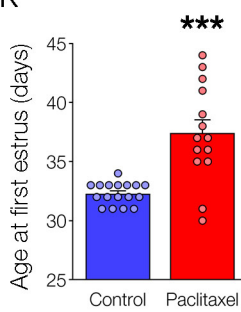
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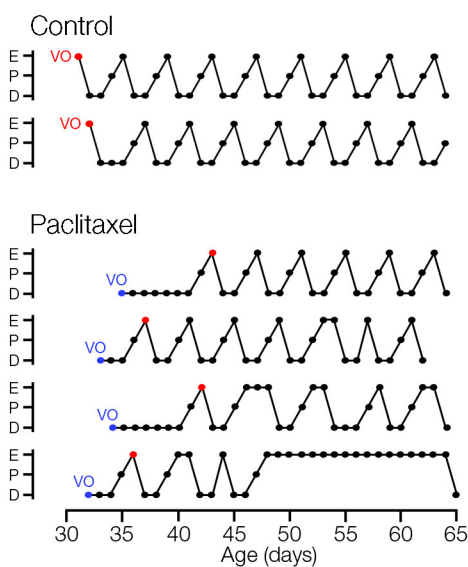
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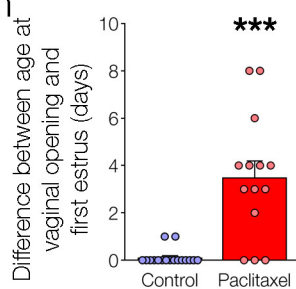
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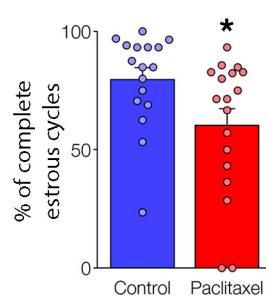
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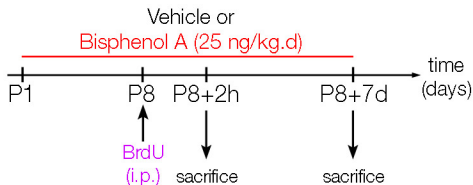
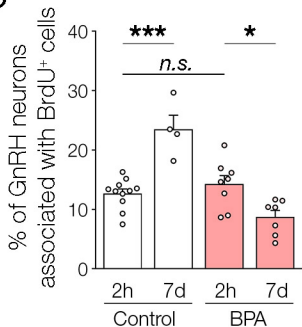
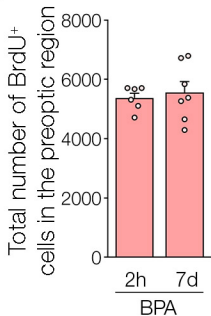


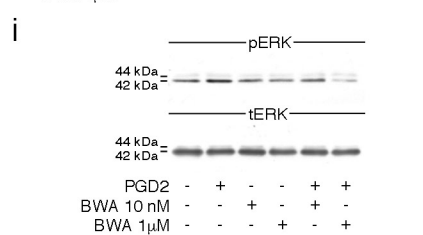
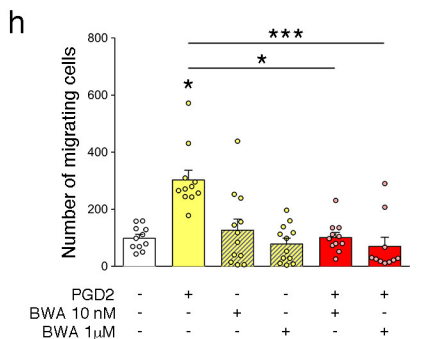
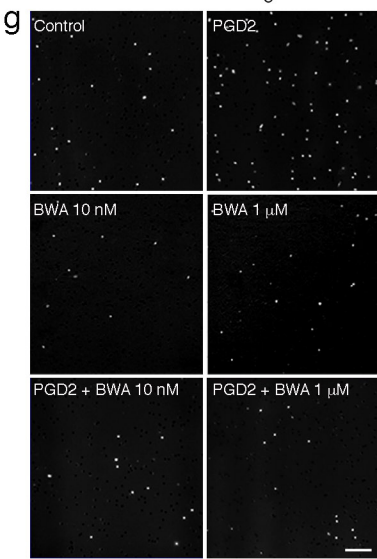
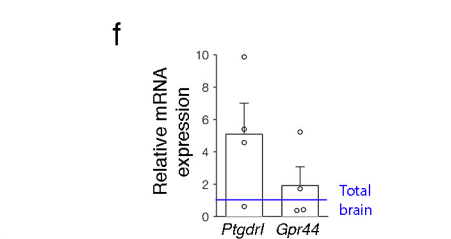
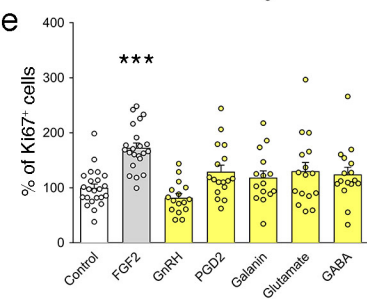
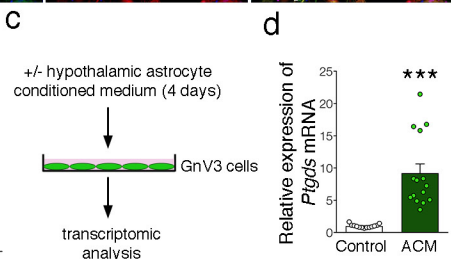
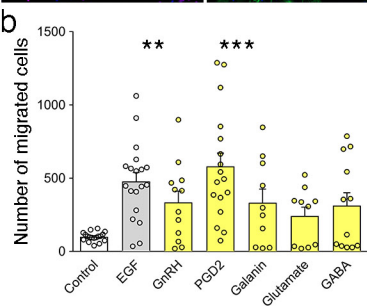
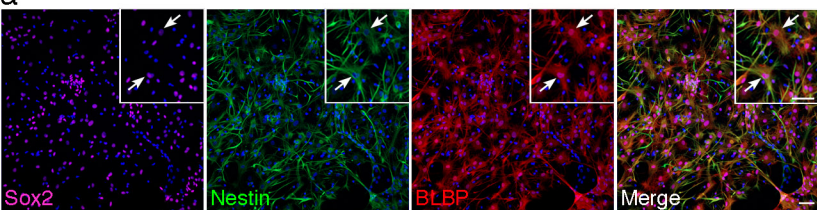
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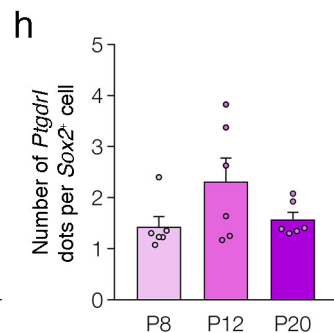
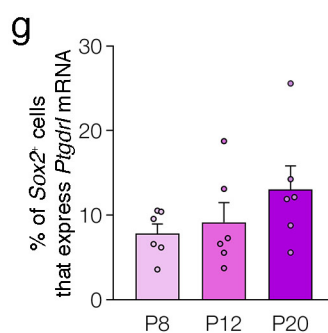
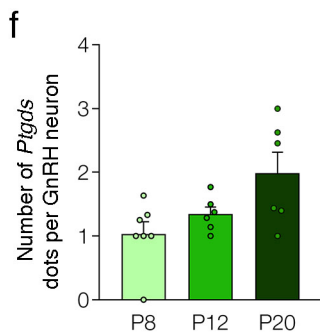
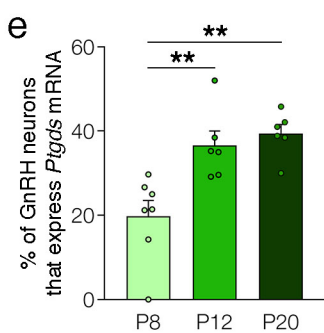
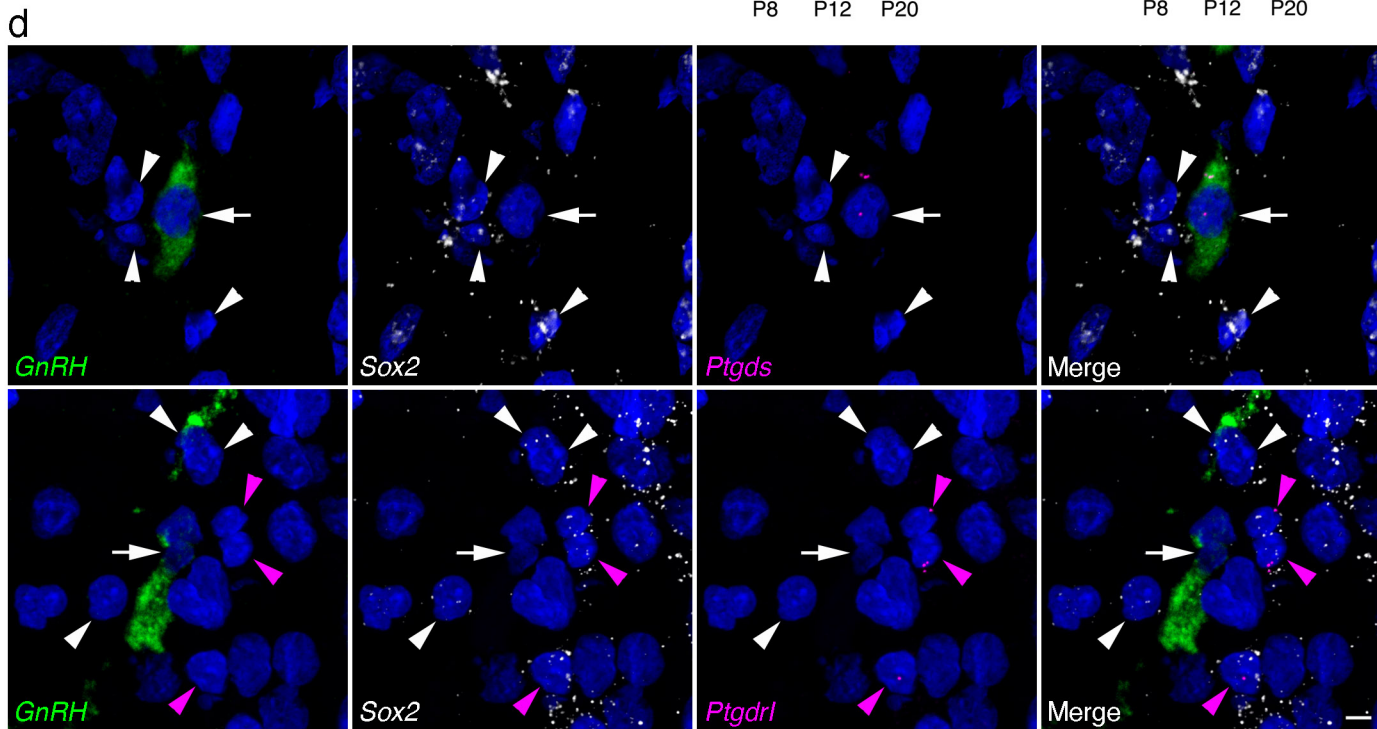
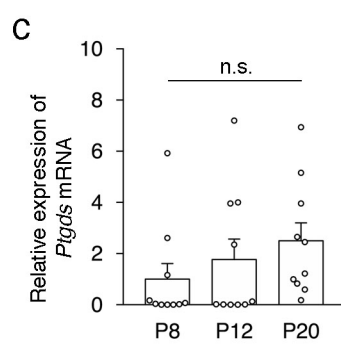
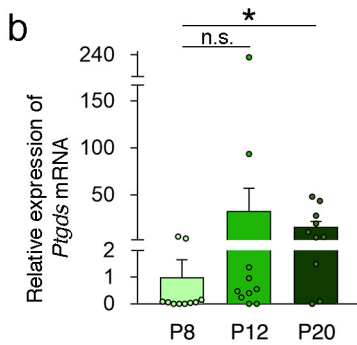
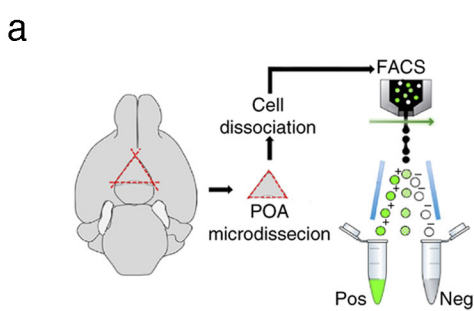


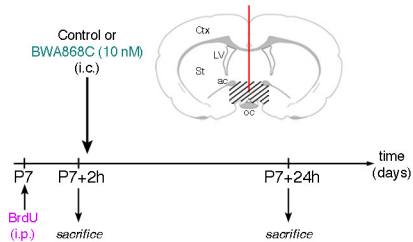
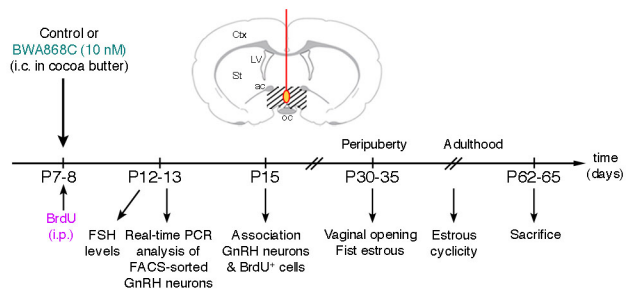
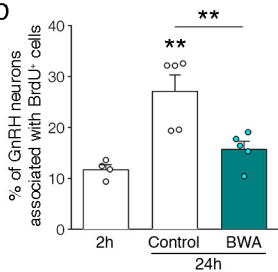
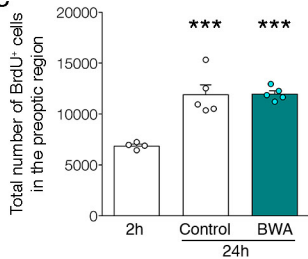
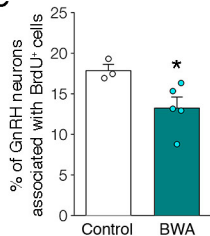
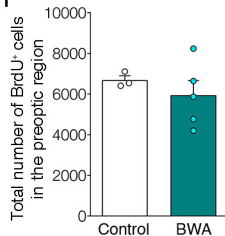
n



a**b****c**





a**d****b****c****e****f****g**