Prostaglandin E_2 release from astrocytes triggers gonadotropin-releasing hormone (GnRH) neuron firing via EP2 receptor activation

Jerome Clasadonte[a,b,c], Pierre Poulain[a,b,c], Naresh K. Hanchate[a,b,c], Gabriel Corfas[d,e,f], Sergio R. Ojeda[g,1], and Vincent Prevot[a,b,c,1]

[a]Institut National de la Santé et de la Recherche Médicale, Jean-Pierre Aubert Research Center, U837, Development and Plasticity of the Postnatal Brain, F-59000 Lille, France; [b]Université Lille Nord de France, Institut Fédératif de Recherche 114, F-59000 Lille, France; [c]UDSL, School of Medicine, F-59000 Lille, France; [d]F. M. Kirby Neurobiology Center, Children's Hospital Boston, Boston, MA 02115; Departments of [e]Neurology and [f]Otology and Laryngology, Harvard Medical School, Boston, MA 02115; and [g]Division of Neuroscience, Oregon National Primate Research Center/Oregon Health and Science University, Beaverton, OR 97006

Edited* by Donald W. Pfaff, The Rockefeller University, New York, NY, and approved August 17, 2011 (received for review May 12, 2011)

Astrocytes in the hypothalamus release prostaglandin E_2 (PGE_2) in response to cell–cell signaling initiated by neurons and glial cells. Upon release, PGE_2 stimulates the secretion of gonadotropin-releasing hormone (GnRH), the neuropeptide that controls reproduction, from hypothalamic neuroendocrine neurons. Whether this effect on GnRH secretion is accompanied by changes in the firing behavior of these neurons is unknown. Using patch-clamp recording we demonstrate that PGE_2 exerts a dose-dependent postsynaptic excitatory effect on GnRH neurons. These effects are mimicked by an EP2 receptor agonist and attenuated by protein kinase A (PKA) inhibitors. The acute blockade of prostaglandin synthesis by indomethacin (INDO) or the selective inhibition of astrocyte metabolism by fluoroacetate (FA) suppresses the spontaneous firing activity of GnRH neurons in brain slices. Similarly, GnRH neuronal activity is reduced in mice with impaired astrocytic PGE_2 release due to defective erbB signaling in astrocytes. These results indicate that astrocyte-to-neuron communication in the hypothalamus is essential for the activity of GnRH neurons and suggest that PGE_2 acts as a gliotransmitter within the GnRH neurosecretory system.

cyclooxygenase | glia-to-neuron signaling | luteinizing hormone-releasing hormone | preoptic region | fertility

It is increasingly clear that astrocytes play an important role in maintaining central nervous system function (1–3) and controlling key bodily processes, such as breathing (4), sleep (5), and reproduction (6). Because of their perivascular and interneuronal localization, astrocytes are well positioned to sense afferent neuronal and blood-borne signals and ideally suited for the temporal and spatial propagation of these signals (7–9). The activation of astrocytes leads to the release of gliotransmitters (8, 10) that trigger rapid responses in neighboring cells and thus contribute to the region-specific homeostatic regulation of neuronal function.

In the hypothalamus, astrocytes regulate the secretory activity of neuroendocrine neurons (11–14). A subset of such neurons secretes the decapeptide gonadotropin-releasing hormone (GnRH), which controls both the initiation of puberty and adult reproductive function. In rodents, GnRH neurons are mostly located in the preoptic region of the ventral forebrain. They project to the median eminence of the hypothalamus, where GnRH is released into the pituitary portal blood for delivery to the anterior pituitary. In the pituitary, GnRH elicits the secretion of luteinizing hormone and follicle-stimulating hormone, which stimulate gametogenesis and gonadal hormone secretion and thus support reproductive function. It is now clear that the secretory activity of GnRH neurons is controlled by both neuronal and glial input (12, 13, 15, 16). Whereas glutamate is a key neurotransmitter involved in the transsynaptic activation of GnRH neurons (17, 18), prostaglandin E_2 (PGE_2) mediates cell–cell communication between astrocytes and GnRH neurons (6, 19, 20). A functional connection between the two systems is demonstrated by the ability of glutamate to elicit PGE_2 release from astrocytes (21–23). In the hypothalamus, the glutamate-dependent activation of PGE_2 release involves astroglial erbB signaling (22), a crucial component of the cell–cell communication process used by astrocytes to facilitate neuroendocrine reproductive development and adult function (6, 24). Whether PGE_2 affects the electrical activity of GnRH neurons is, however, unknown. Here we report a remarkably potent postsynaptic excitatory effect of PGE_2 on GnRH neurons that requires the activation of the EP2 subclass of PGE_2 receptors and of cAMP/protein kinase A (PKA)-mediated downstream signaling pathways.

Results

PGE_2 Activates Adult GnRH Neurons in a Potent and Reversible Manner. We used whole-cell patch-clamp recording to study the electrical activity of 193 GnRH neurons in brain slices containing the preoptic region from 175 adult transgenic mice (61 males and 114 females) expressing green fluorescent protein (GFP) under the control of the GnRH promoter (25). Pipette solution 1 (ps1) resulted in an average resting potential of -75.54 ± 0.38 mV ($n = 151$) and an input resistance of 1417.76 ± 43.07 MΩ ($n = 97$). In this configuration, 87% of the neurons remained silent at the resting potential (*SI Results* and Fig. S1). Bath application of PGE_2 resulted in a striking, dose-dependent depolarizing effect on GnRH neurons (Fig. 1 *A–C*), with an EC_{50} of 0.018 μM (Fig. 1 *C* and *D*). Among all of the GnRH neurons treated with 1 μM PGE_2, 78% (91 out of 116) depolarized rapidly (within 10–190 s; mean 41.00 ± 3.21 s) (Fig. 1 *A–C*). PGE_2-induced (1 μM) depolarization had a mean amplitude of 8.95 ± 0.32 mV ($n = 91$) and was accompanied by the sustained generation of action potentials (Fig. 1 *A–C*) and a decrease in membrane resistance (Fig. 1*C*). Interestingly, 33 cells out of 91 responded to PGE_2 with bursting activity (Fig. 1*B*) similar to that which occurred spontaneously in some neurons (Fig. S1 *B* and *C*) and which is thought to be essential for neuropeptide secretion from neuroendocrine cells (26). The PGE_2 excitatory effect was fully reversible in 73 neurons (Fig.1 *A–C*), whereas 18 neurons did not recover their basal membrane potential after cessation of the PGE_2 treatment. No difference in the response of GnRH neurons to PGE_2 was detected between animals of different sex or stages of the estrous cycle (Table S1). Because PGE_2 has previously been shown to stimulate glutamate release from astrocytes (21), we next examined the effects of PGE_2 on GnRH-GFP neurons in the presence of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 100 μM DL-2-amino-5-phosphonopentanoic acid (DL-AP5) (*Materials and Methods*), to block

Author contributions: J.C., P.P., S.R.O., and V.P. designed research; J.C., P.P., and N.K.H. performed research; G.C. contributed new reagents/analytic tools; J.C., P.P., and V.P. analyzed data; and J.C., P.P., G.C., S.R.O., and V.P. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence may be addressed. E-mail: vincent.prevot@inserm.fr or ojedas@ohsu.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1107533108/-/DCSupplemental.

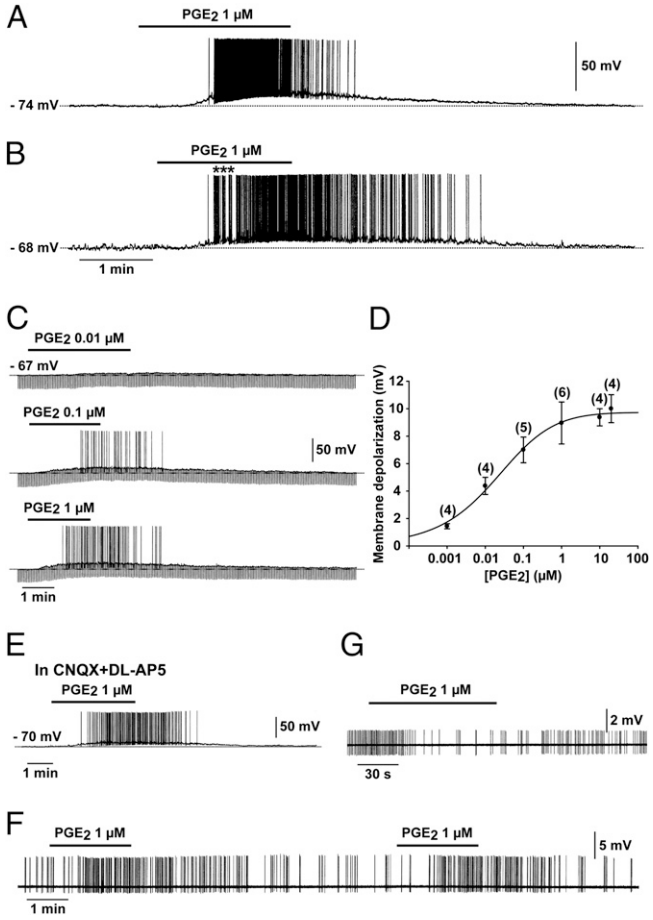

Fig. 1. PGE_2 powerfully activates GnRH neurons. (*A* and *B*) Whole-cell current-clamp recordings showing the effect of bath application of PGE_2 on two GnRH neurons. Note that in these two silent GnRH neurons, which had a resting membrane potential of −74 mV (*A*) and −68 mV (*B*), respectively, PGE_2 induced a reversible membrane depolarization that led to the initiation of spike firing. The effect was short-lived in *A* and long-lasting in *B*. Note that in *B*, the spike firing started with a bursting pattern (*). (*C*) Whole-cell current-clamp recording of a single GnRH neuron showing that PGE_2 (0.01–1 μM) depolarized the membrane in a dose-dependent manner. Note that in this cell, 0.01 μM of PGE_2 did not trigger spike firing. In this and the following figures, the downward deflections correspond to voltage responses to 300 ms hyperpolarizing current pulses used to test the membrane input resistance. (*D*) Dose–response curve of the PGE_2-induced membrane depolarization. The numbers of neurons tested at each dose is given in parentheses. Error bars indicate SEM. The EC_{50} for the PGE_2-induced membrane depolarization was 0.018 μM, based on a logistic equation fitted to the data points. (*E*) The excitatory effect of PGE_2 on GnRH neurons persisted in the presence of the AMPA/kainate and NMDA receptor antagonists CNQX (20 μM) and DL-AP5 (100 μM), respectively. (*F*) Loose patch-clamp recording showing the excitatory effect of PGE_2 on a GnRH neuron, characterized by a reversible acceleration of firing. Note that the effect was reproduced by a second application of PGE_2 to the same neuron. (*G*) Loose patch-clamp recording showing the inhibitory effect of PGE_2 on a non-GnRH neuron located in the vicinity of GnRH-GFP neurons. The effect was characterized by a reversible slowing down of firing.

AMPA/kainate and NMDA receptors, respectively. Under these conditions, the membrane depolarization induced by 1 μM PGE_2 was not altered (10.62 ± 1.20 mV, $n = 4$; Fig. 1*E*), indicating that glutamate is not an intermediary in the excitatory effects of PGE_2 on GnRH neuronal activity.

PGE_2 maintained its potent stimulatory effect on the firing behavior of GnRH neurons recorded using the loose patch-clamp configuration (Fig. 1*F*). This indicates that the dilution of the intracellular compartment by the patch electrode medium in the whole-cell configuration does not alter the response of GnRH neurons to PGE_2. In the loose patch-clamp configuration, GnRH neurons were spontaneously active and all responded to the addition of 1 μM PGE_2 to the perfusion medium by a transient acceleration of the action potential discharge (0.51 ± 0.15 Hz before treatment vs. 1.35 ± 0.12 Hz with bath application of PGE_2, *t* test; $P < 0.05$; $n = 5$; Fig. 1*F*). To investigate whether PGE_2 had similarly consistent excitatory effects on the electrical activity of non-GnRH neurons in the preoptic region, 14 non-GFP–labeled neurons located near GnRH-GFP neurons were recorded. Whereas 10 neurons responded to the bath application of 1 μM PGE_2 with either an acceleration ($n = 6$) or an inhibition ($n = 4$, Fig. 1*G*) of their firing pattern, 4 were insensitive to the prostaglandin.

PGE_2 Elicits Membrane Depolarization of GnRH Neurons via a Postsynaptic Effect Involving Activation of an Inward Current. The effect of PGE_2 was further investigated in the presence of tetrodotoxin (TTX; 0.5–1 μM) to block action potential-dependent synaptic transmission, in addition to the glutamate and $GABA_A$ receptor antagonists DL-AP5 (100 μM), CNQX (20 μM), and bicuculline (BIC) (20 μM) to block ionotropic receptor-mediated presynaptic inputs. Under these conditions, PGE_2 (1 μM) consistently depolarized the membrane potential of GnRH neurons (Fig. 2*A*) with an average depolarization of 7.72 ± 0.48 mV ($n = 10$). The current–voltage relationship before and after applying PGE_2 (1 μM) was obtained by injecting a series of square wave currents from −70 to +70 pA (Fig. 2 *B1* and *B2*). The input resistance was calculated on the basis of the linear part of the current–voltage curve (Fig. 2 *B3*). PGE_2 (1 μM) decreased the input resistance by 27.21 ± 2.38% ($n = 10$), indicating an increase in conductance. These results suggest that PGE_2 triggers firing in GnRH neurons via a direct postsynaptic mechanism.

To identify the conductance involved in the PGE_2-induced membrane depolarization, we performed voltage-clamp recordings at a holding potential of −70 mV and observed that 1 μM PGE_2 elicited an inward current of 22.92 ± 5.57 pA ($n = 8$) in the presence of TTX (0.5 μM), DL-AP5 (100 μM), CNQX (20 μM), and bicuculline (20 μM). This current appeared 40.00 ± 13.89 s (10–130 s, $n = 8$) after the initiation of PGE_2 treatment and ended 193.75 ± 32.07 s ($n = 8$) after the removal of PGE_2 from the bath solution (Fig. 2*C*). We then investigated the current evoked by PGE_2 using a ramp voltage-clamp protocol from −120 mV to −30 mV and a holding potential of −70 mV, in the presence of TTX (0.5 μM), DL-AP5 (100 μM), CNQX (20 μM), and bicuculline (20 μM; Fig. 2 *D1*). PGE_2 (1 μM) increased the current evoked by this protocol (Fig. 2 *D1*). After subtracting control values from the current recorded after applying PGE_2, a PGE_2-induced inward current was obtained (Fig. 2 *D2*). This inward current had a linear voltage dependence from −120 to −40 mV and was suppressed at a mean value of −41.12 ± 3.54 mV ($n = 4$; Fig. 2 *D2*), suggesting the activation of a nonselective cation conductance (27, 28). This value was also close to the membrane potential measured in current-clamp mode (−45.66 ± 1.68 mV, *t* test; $P > 0.05$; $n = 7$; Fig. 2 *B3*), for which the two current–voltage curves obtained in the presence and absence of PGE_2 converged.

EP2 Receptors Are Expressed in GnRH Neurons and Their Activation Mimics the Effects of PGE_2. To gain insight into the nature of the receptors that mediate the potent postsynaptic excitatory action of PGE_2 on GnRH neurons, we tested the effects of 17-phenyl trinor PGE_2 (17PT-PGE_2), sulprostone, and butaprost, which are EP1, EP1/EP3, and EP2 receptor agonists (29), respectively, on GnRH neuronal activity. The bath application of 17PT-PGE_2 or sulprostone at 10 μM, a concentration previously shown to promote GnRH release in GnRH-secreting cell lines (20), had no effect on GnRH neuronal activity (Fig. 3 *A* and *B*). However, 10 μM butaprost resulted in membrane depolarization (Fig. 3 *A* and *B*). Analysis of the voltage-clamp ramps in the presence or absence of butaprost (Fig. 3 *C1*) revealed that this EP2 receptor agonist activated an inward current that was suppressed at a mean value of −48.33 ± 6.23 mV ($n = 3$, Fig. 3 *C2*), close to the value obtained with PGE_2 (Fig. 2 *D2*; *t* test, $P > 0.05$). Perfusion of the slices with 30 μM AH 23848, an antagonist of the EP4

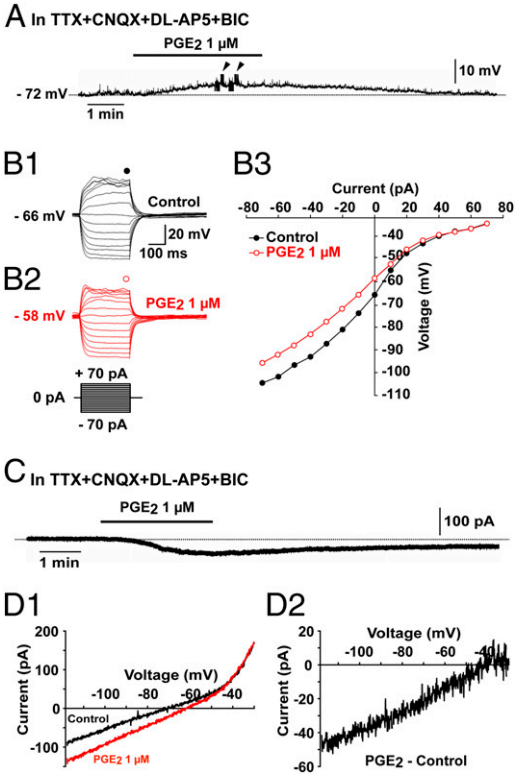

Fig. 2. The PGE_2-induced activation of GnRH neurons is direct and involves an inward current. (*A*) Whole-cell current-clamp recording showing that PGE_2 depolarized GnRH neurons in the presence of TTX (0.5 μM), CNQX (20 μM), DL-AP5 (100 μM), and bicuculline (BIC, 20 μM). Arrowheads indicate the time of application of hyperpolarizing and depolarizing current pulses to trace current–voltage relationships. (*B1–B3*) Responses of a GnRH neuron to current injection from −70 pA to +70 pA before (*B1*, control) and during application of PGE_2 (*B2*). The current–voltage relationship (*B3*) for the corresponding neuron obtained before (control) and during the application of PGE_2 indicate that PGE_2 decreased the slope of the linear part of the curve, indicating a decrease in the membrane input resistance. Note that the two curves converged at −40 mV in this example. (*C*) Whole-cell voltage-clamp recording showing that PGE_2 evoked an inward current in GnRH neurons in the presence of TTX (0.5 μM), CNQX (20 μM), DL-AP5 (100 μM), and BIC (20 μM). The inward current was recorded at a holding potential of −70 mV. (*D1* and *D2*) Current traces evoked by voltage ramps (duration, 12.5 s) from −120 mV to −30 mV at a holding potential of −70 mV in GnRH neurons. (*D1*) Traces showing the current responses to the voltage ramp in GnRH neurons before (control) and during application of PGE_2. (*D2*) PGE2-induced current obtained after subtracting the control from the PGE_2 curve. Note that the PGE2-induced current is suppressed at −40 mV.

receptor (29), did not modify the response of GnRH neurons to PGE_2 treatment ($n = 3$). Taken together, these observations strongly suggest that the excitatory effects of PGE_2 on GnRH neuronal activity are mediated by EP2 receptor activation. To confirm the presence of EP2 receptors in GnRH neurons, we used immunohistochemistry. The EP2 receptor was abundantly expressed in the preoptic region; among the 128 GnRH-GFP neurons analyzed, 72 (56%) displayed EP2 receptor immunostaining ($n = 4$ animals) (Fig. 3*D*).

PGE_2-Mediated Membrane Depolarization in GnRH Neurons Requires Protein Kinase A Activation. Because EP2 receptors are linked to the Gs-cAMP/PKA pathway (29, 30), we used PKA inhibitors to determine whether the excitatory effect of PGE_2 on GnRH neurons could be inhibited or attenuated. In the presence of 0.5 μM TTX, the bath application of the PKA inhibitors H89 (10 μM, $n = 5$) and KT 5720 (10 μM, $n = 3$) or the competitive PKA antagonist Rp-cAMP (20 μM, $n = 3$) for 30 min significantly attenuated the stimulatory effect of PGE_2 on membrane depolarization in GnRH neurons (Fig. 3 *E* and *F*). The activation of EP1 and EP3 receptors is coupled to the mobilization of intracellular calcium stores (29), but did not cause membrane depolarization in GnRH neurons (Fig. 3 *A* and *B*). Consistent with this observation, the depletion of intracellular calcium stores with thapsigargin (2 μM) did not block the depolarizing effects of PGE_2 on GnRH neurons ($n = 2$). Taken together, our data suggest that the excitatory effects of PGE_2 on GnRH neuronal activity are exerted via an EP2-Gs-cAMP/PKA signaling pathway.

Blockade of Endogenous Cyclooxygenase Activity Inhibits Spontaneous Firing of GnRH Neurons. To monitor spontaneous GnRH neuronal activity, whole-cell patch-clamp recordings were performed using a pipette solution 2 (ps2) that conferred the cells with an average resting potential of −61.50 ± 0.62 mV ($n = 10$) and an input resistance of 1384.27 ± 73.71 MΩ ($n = 10$), as shown previously (31). At this resting potential, all neurons exhibited spontaneous activity with a mean discharge of 0.45 ± 0.07 Hz ($n = 10$). To explore the contribution of PGE_2 to this spontaneous activity, we bath applied indomethacin (INDO), an inhibitor of cyclooxygenase, the rate-limiting enzyme in prostaglandin synthesis, to slices of the preoptic region during recording from GnRH neurons (Fig. 4). Bath application of this inhibitor at 50–100 μM either greatly reduced (by 95%; $n = 3$; Fig. 4*A*) or fully suppressed ($n = 5$; Fig. 4*B*) the spontaneous discharge of GnRH neurons (Fig. 4*C*). These effects were either reversible ($n = 2$) or irreversible ($n = 6$). Lower concentrations of indomethacin (5–10 μM) had no effect on GnRH neuronal activity, except in the case of one cell that exhibited a reversible reduction of its basal firing rate by 57%. At 100 μM indomethacin, the suppression of firing was accompanied by a membrane hyperpolarization (4.5 ± 0.6 mV, $n = 6$) (Fig. 4*B*). However, the bath application of PGE_2 (1 μM) remained capable of triggering membrane depolarization (8.12 ± 2.39 mV) and action potentials ($n = 4$) (Fig. 4*B*). These experiments suggest that the endogenous production of PGE_2 contributes to the maintenance of the activity of GnRH neurons in the preoptic region.

Astrocytic PGE_2 Regulates GnRH Neuronal Activity in Situ. Hypothalamic astrocytes are a source of PGE_2 (19), which is required for the normal release of GnRH (6, 24). To determine whether astrocytes contribute to the regulation of GnRH neuronal firing, we pretreated hypothalamic slices with the glial toxin fluoroacetate (5 mM; 60–120 min) (2, 32–34). Fluoroacetate significantly impaired astrocyte function as assessed by the inability of astrocytes to take up sulforhodamine 101 (SR101) (Fig. 4*D*), a fluorescent dye that is selectively taken up by astrocytes both in vivo (35) and in living brain slices (36). The number of astrocytes capable of taking up the dye in the vicinity of GnRH neurons was reduced by 78 ± 1% compared with untreated slices ($n = 4$; *t* test, $P < 0.01$). The spontaneous firing of GnRH neurons (detected using ps2 conditions) was strikingly reduced in fluoroacetate-treated slices (9 out of 12) (Fig. 4 *C* and *E*). The remaining neurons (3 out of 12) either exhibited a sporadic pattern of action potential discharge (0.01 Hz, $n = 2$) or fired in repetitive bursts (0.15 Hz, $n = 1$). In contrast, fluoroacetate treatment did not affect the basal membrane properties of GnRH neurons (resting potential of −74.29 ± 1.44 mV and input resistance of 1123.50 ± 130.18 MΩ in ps1, $n = 7$; *t* test for treated vs. control slices; $P > 0.05$). Importantly, in most GnRH neurons, fluoroacetate treatment did not affect the membrane depolarizing effect of PGE_2 (1 μM; 6 out of 9 neurons; 7.92 ± 0.42 mV; Fig. 4*E*). The treatment also failed to affect the latency of the membrane depolarization upon initiation of PGE_2 treatment (50.00 ± 13.36 s in artificial cerebrospinal fluid (ACSF) vs. 31.25 ± 6.10 s in FA, $n = 8$ for each treatment, *t* test, $P > 0.05$), the duration of the effect (234.28 ± 36.70 s in ACSF vs. 377.14 ± 74.08 s in FA, $n = 7$ for each treatment, *t* test, $P > 0.05$) or the ability of the prostaglandin to trigger action potentials (5 out of 6 neurons, Fig. S2).

We next sought to investigate the firing activity of GnRH neurons in mice with deficient astrocytic PGE_2 production. We used transgenic mice expressing a dominant-negative form of the erbB4 receptor (DN-erbB4) specifically targeted to astrocytes by means of the human GFAP promoter (6). This mutated receptor

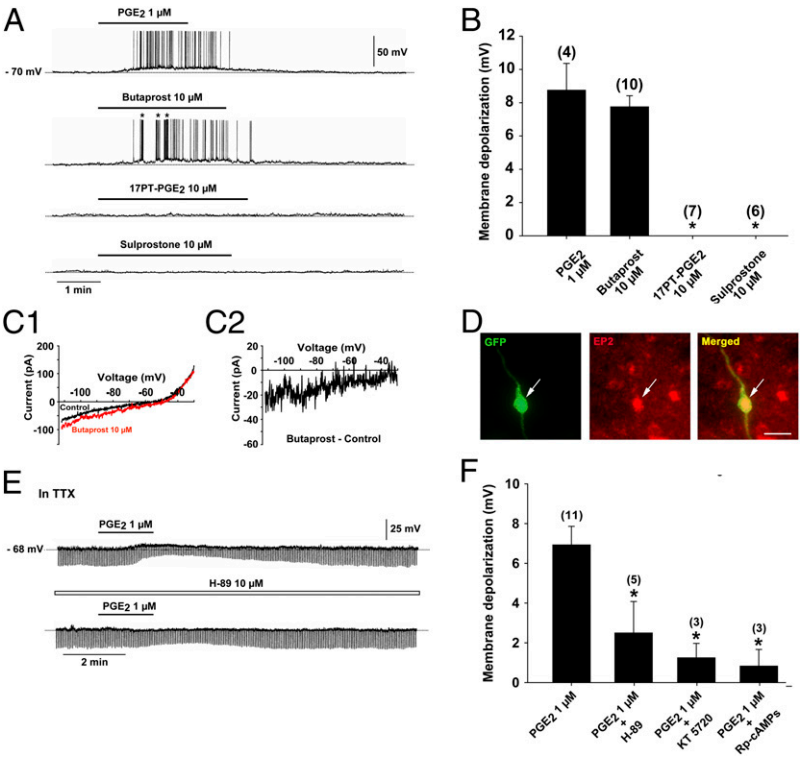

Fig. 3. The PGE_2-induced activation of GnRH neurons is mediated by the EP2 receptor and requires the cAMP/PKA pathway. (*A*) In a single GnRH neuron recorded with a whole-cell current clamp, the EP2 receptor agonist, butaprost, evoked a membrane depolarization similar to that induced by PGE_2, whereas the EP1 receptor agonist 17-phenyl trinor PGE_2 (17PT-PGE_2) and the EP1–3 receptor agonist, sulprostone, had no effect. Note that the discharge elicited by the butaprost-induced membrane depolarization led to bursts of action potentials (*). (*B*) Bar graph illustrating the membrane depolarization in GnRH neurons induced by PGE_2, butaprost, 17PT-PGE_2, and sulprostone (*$P < 0.05$ compared with the membrane depolarization induced by PGE_2, one-way ANOVA; $n = 4$–10 neurons). Error bars indicate SEM. (*C1* and *C2*) Current traces evoked by voltage ramps (duration, 12.5 s) from −120 mV to −30 mV at a holding potential of −70 mV in GnRH neurons. (*C1*) Traces showing the current response to the voltage ramp in GnRH neurons before (control) and during application of butaprost, a selective EP2 receptor agonist. (*C2*) Butaprost-induced current obtained after subtracting the control from the butraprost curve. Note that the butraprost-induced current was suppressed at −35 mV in this example. (*D*) EP2 receptor immunoreactivity (red) was detected in the cell body of GnRH-GFP neurons (green, arrow). (Scale bar, 20 μm.) (*E*) Whole-cell current-clamp recording of a single GnRH neuron in the presence of TTX (0.5 μM) showing the effect of PGE_2 in the absence (*Upper*) and presence (*Lower*) of the membrane-permeable PKA inhibitor H89. Note that H89 attenuated the membrane depolarization and the decrease in membrane input resistance (downward deflections) induced by PGE_2. (*F*) Bar graph illustrating the membrane depolarization induced by PGE_2 alone or in the presence of H89 and two other membrane-permeable compounds, the more selective PKA inhibitor KT 5720 and the cAMP antagonist Rp-cAMPs (*$P < 0.05$ compared with the membrane depolarization induced by PGE_2 alone, one-way ANOVA; $n = 3$–11 neurons). Error bars indicate SEM.

blocks the ligand-dependent activation of erbB2 and erbB4 receptors without affecting signaling through other receptors, such as erbB1 or Notch1 (6, 37). GnRH secretion is deficient in GFAP–DN-erbB4 mice due to the inability of astrocytes to respond to erbB4 activation by producing PGE_2 (6). To visualize GnRH neurons in these animals we crossed GFAP–DN-erbB4 mice with GnRH-GFP animals. GnRH neurons recorded from four double-transgenic mice under ps2 conditions were either silent ($n = 5$; Fig. 4*F*) or displayed a sporadic low-frequency (0.02 Hz) pattern of action potential discharge ($n = 1$), in contrast to GnRH-GFP neurons recorded from wild-type controls (Fig. 4*C*). These results suggest that the excitability of GnRH neurons is reduced in GFAP–DN-erbB4 mice due to the failure of PGE_2 production by erbB4-deficient astrocytes. We tested this assumption by determining whether exogenous PGE_2 could restore GnRH membrane depolarization in GFAP–DN-erbB4 mice. The bath application of PGE_2 (1 μM) elicited membrane depolarization equally strongly in hypothalamic slices from wild-type and GFAP–DN-erbB4 mice (8.67 ± 1.32 mV in DN-erbB4 mutant mice vs. 8.72 ± 067 mV in wild-type controls, $n = 6$ and 10, respectively, *t* test, $P > 0.05$) (Fig. 4*F*).

Discussion

The disruption of astrocytic erbB4 receptor signaling by the overexpression of a dominant-negative erbB4 receptor leads to diminished astrocytic PGE_2 release in response to ligand-dependent erbB4 activation, leading in turn to reduced GnRH release, delayed puberty, and disrupted adult reproductive function (6, 24). The present results show that GnRH neuronal activity is decreased in these animals and that this deficiency is mimicked by the bath application of either fluoroacetate, an inhibitor of astrocyte metabolism (2, 32, 34), or the cyclooxygenase blocker indomethacin, to slices of the preoptic region from wild-type animals. Our findings that GnRH neurons respond to PGE_2 with an enhancement of the firing rate and that PGE_2 rescues GnRH neuronal activity in brain slices with reduced astrocytic PGE_2 output indicate that glial PGE_2 is an important component of the homeostatic mechanism controlling GnRH neuronal activity.

The presence of EP2 receptors in GnRH neurons (ref. 38 and present study) and the finding that the selective EP2 receptor agonist butaprost mimics the effect of PGE_2 suggest that the excitatory effect of PGE_2 on GnRH neuronal activity involves the activation of EP2 receptors. This inference is supported by an earlier report showing that the stimulation of GnRH-producing GT1–7 cells with butaprost results in GnRH release (20). In addition, the attenuation of the effect of PGE_2 by the bath application of PKA inhibitors or biologically inactive cAMP is consistent with earlier findings demonstrating the coupling of the EP2 receptor to the cAMP/PKA pathway (29) and the involvement of cAMP in the intracellular mechanism underlying the stimulatory effect of PGE_2 on GnRH secretion (39). A previous study has reported that native GnRH neurons also express EP1 receptors (20), which are coupled to the mobilization of intracellular calcium stores (29) and to GnRH release (20). Our electrophysiological data show that the EP1 receptor agonists 17PT-PGE_2 and sulprostone had no effect on GnRH neuronal activity and that the depletion of intracellular calcium stores with thapsigargin did not alter the depolarizing response of GnRH neurons to PGE_2. These results, together with earlier findings demonstrating that PGE_2-induced GnRH release from median eminence explants requires the mobilization of intracellular calcium stores (40), suggest that the EP1 receptor-dependent stimulation of GnRH release is exerted at the level of GnRH nerve terminals rather than GnRH cell bodies. Thus, PGE_2 may stimulate both GnRH neuron firing and GnRH release by acting at the cell soma and nerve terminal levels, respectively.

An intriguing finding in this study is that the cyclooxygensase (COX) inhibitor indomethacin failed to affect spontaneous GnRH neuronal activity at doses lower than 50 μM, which is >1,700 times the EC_{50} of COX-1 (0.028 μM), but only ~25 times the EC_{50} of COX-2 (1.68 μM) (41). This finding suggests that eicosanoids sustaining spontaneous GnRH neuronal activity are mostly COX-2–derived products, a conclusion consistent with evidence that COX-2 is the most abundant COX form expressed in astrocytes (42, 43). Because endocannabinoids, such as 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (AEA or anandamide) were re-

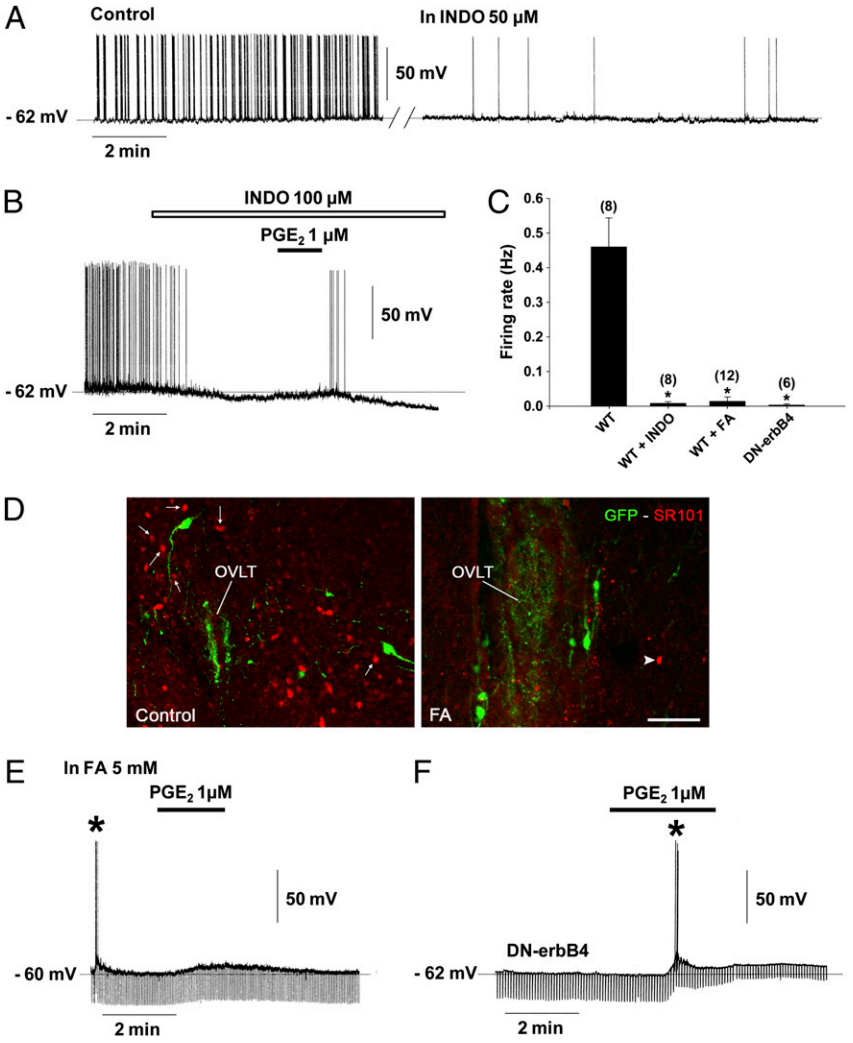

Fig. 4. Astrocytic prostaglandin production sustains the electrical activity of GnRH neurons. Recordings of GnRH neurons were performed under whole-cell current-clamp using pipette solution 2 (ps2) (*Materials and Methods*) to obtain a background of spontaneous activity from GnRH neurons. (*A*) GnRH neurons showed spontaneous activity (control), which was reduced after 10 min of perfusion with indomethacin (INDO; 50 μM), an inhibitor of the cyclooxygenases, enzymes responsible for prostaglandin production. (*B*) INDO at a higher concentration (100 μM) strongly attenuated the spontaneous activity of GnRH neurons, accompanied by membrane hyperpolarization. Note that PGE_2 reversed the inhibitory effect of INDO. (*C*) Bar graph illustrating the firing rate of GnRH neurons recorded in brain slices from wild-type mice (WT) exposed to INDO or fluoroacetate (FA) and from DN-erbB4 mice. (*$P < 0.05$ compared with the firing rate of GnRH neurons recorded in brain slices from WT mice, one-way ANOVA; $n = 6–12$ neurons). Error bars indicate SEM. (*D*) Pretreatment of brain slices with fluoroacetate (FA, 5 mM, 60–120 min), a glial toxin, impaired the astrocytic uptake of sulforhodamine101 (SR101, red). Arrows show cells that took up SR101 in the vicinity of GnRH neurons (GFP, green) under control conditions (*Left*). After FA treatment, very few cells were labeled with SR101 (*Right*, arrowhead). Images were acquired at the level of the organum vasculosum of the lamina terminalis (OVLT). (Scale bar, 100 μm.) (*E*) FA strongly reduced the firing rate of GnRH neurons. In this example, the slice was pretreated with FA for 60 min and action potentials could be driven by a brief injection of a depolarizing current (*). Note that PGE_2 retained its depolarizing effect. (*F*) In DN-erbB4 mice, in which astrocytic PGE_2 release is diminished, most GnRH neurons were silent. In this recording, action potentials could be driven by a brief injection of depolarizing current (*). Note that PGE_2 retained its depolarizing effect and reduced the membrane input resistance (downward deflections).

cently shown to be substrates for COX-2 (44) and because endocannabinoids modulate GABAergic excitatory inputs to GnRH neurons (45), we cannot rule out that part of the effects of indomethacin on the spontaneous activity of GnRH neurons may be due to inhibition of endocannabinoid oxygenation by COX-2 (46).

Equally intriguing is the apparent lack of sex and estrous cycle effects of PGE_2 on GnRH neuronal activity. Previous studies have shown that GnRH firing activity is modified by estradiol in a diurnal-dependent manner (47), and that COX-2 expression in the preoptic region is greater in males than females (48). Perhaps the lack of changes in GnRH firing activity is related to the fact that all recordings were made at the same time of day and using a saturating dose of PGE_2. In addition to its postsynaptic effect on GnRH neurons, PGE_2 may also modulate the activity of steroid-sensitive afferent neuronal populations (49), including kisspeptin neurons that ensure coordinated progression of neuroendocrine events sustaining ovulatory cyclicity (50). The recent development of a mouse model that enables identification of kisspeptin neurons using fluorescent reporter genes (51) now renders these neurons amenable to scrutiny.

PGE_2 has long been known to play a role in the control of GnRH neuronal function (52, 53). More recent work points to PGE_2 as a mediator of astrocyte-to-GnRH-neuron communication initiated by the activation of erbB signaling in astrocytes (6, 19, 24, 54). Astrocytes, which are sophisticated sensors of neuronal activity (7, 8), release PGE_2 at active synapses in response to glutamate (21, 23, 55). Interestingly, a ligand-dependent increase in erbB signaling appears to mediate glutamate-stimulated PGE_2 release in hypothalamic astrocytes (22). Within the hypothalamus, both glutamate and glial erbB signaling play physiological roles in promoting GnRH release at the onset of puberty (6, 18, 54, 56) and during adult reproductive life (17, 24). The present results identify PGE_2 as a potent excitatory regulator of GnRH neuronal activity and indicate that the production of PGE_2 by astroglial cells plays a hitherto unappreciated role in the homeostatic regulation of GnRH neuronal excitability.

Materials and Methods

Animals. Electrophysiological recordings were performed on adult GnRH-GFP and GFAP–DN-erbB4/GnRH-GFP transgenic mice. The generation of GFAP–DN-erbB4 mice has been described previously (6).

Electrophysiological Experiments. GnRH-GFP neurons were recorded as described in a previous study (31). Pipette solution 1 (ps1) contained (in mM) K-gluconate, 125; Hepes, 10; $CaCl_2$, 1; $MgCl_2$, 1; ATP-Mg, 2; EGTA, 11; GTP, 0.3; and NaCl, 15 (pH 7.3 with KOH; osmolarity, 270–280 mOsm). Pipette solution 2 (ps2) contained (in mM): K-gluconate, 140; Hepes, 10; ATP-Mg, 2; EGTA, 1; and KCl, 10 (pH 7.3 with KOH; osmolarity, 270–290 mOsm). Drugs were applied to the perfusing system (bath application) to obtain the final concentrations indicated. The drugs used were: PGE_2, butaprost, sulprostone, 17-phenyl trinor PGE_2 (Cayman Chemical), CNQX, DL-AP5, TTX, thapsigargin (Tocris), sodium fluoroacetate, indomethacin, AH 23848, H-89, KT 5720, Rp-cAMPs, and bicuculline methiodide (Sigma). Detailed methods are provided in *SI Materials and Methods*.

Fluorescent Staining. Detection of EP2 receptor (rabbit polyclonal antibody, Cayman Chemical) and SR101 uptake were performed using protocols de-

scribed previously in refs. 6 and 36, respectively. Detailed methods are provided in *SI Materials and Methods*.

Data Analysis. Statistical analysis was performed using Sigma-Stat software (Jandel). Differences between several groups were analyzed by one-way ANOVA, followed by a Student-Newman-Keuls multiple comparison test. The Student *t* test was used to compare two groups. The data points of the dose–response curve were fitted with a four-parameter logistic curve using Sigma Plot 2001 (SPSS). A P value of <0.05 was considered to indicate a statistically significant difference. Values are reported as the mean ± SEM.

ACKNOWLEDGMENTS. We thank Julien Devassine (animal facility, Institut Fédératif de Recherche, IFR 114) for his brilliant management of our mouse colony. This research was supported by the Agence National pour la Recherche Grants ANR-07-NEURO-026-03 and ANR-09-BLAN-0267, the Fondation pour la Recherche Médicale (equipe FRM 2005), and the IFR 114 electrophysiological platform (to V.P.), in addition to National Institutes of Health Grants HD25123 and RR000163 for the operation of the Oregon National Primate Research Center (to S.R.O.). J.C. was supported by a doctoral fellowship from the Institut National de la Santé et de la Recherche Médicale and the Région Nord Pas de Calais.

1. Gordon GR, et al. (2009) Astrocyte-mediated distributed plasticity at hypothalamic glutamate synapses. *Neuron* 64:391–403.
2. Henneberger C, Papouin T, Oliet SH, Rusakov DA (2010) Long-term potentiation depends on release of D-serine from astrocytes. *Nature* 463:232–236.
3. Panatier A, et al. (2006) Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* 125:775–784.
4. Gourine AV, et al. (2010) Astrocytes control breathing through pH-dependent release of ATP. *Science* 329:571–575.
5. Halassa MM, et al. (2009) Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron* 61:213–219.
6. Prevot V, et al. (2003) Normal female sexual development requires neuregulin-erbB receptor signaling in hypothalamic astrocytes. *J Neurosci* 23:230–239.
7. Barres BA (2008) The mystery and magic of glia: A perspective on their roles in health and disease. *Neuron* 60:430–440.
8. Haydon PG, Carmignoto G (2006) Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev* 86:1009–1031.
9. Iadecola C, Nedergaard M (2007) Glial regulation of the cerebral microvasculature. *Nat Neurosci* 10:1369–1376.
10. Parpura V, Zorec R (2010) Gliotransmission: Exocytotic release from astrocytes. *Brain Res Brain Res Rev* 63:83–92.
11. Hatton GI (1997) Function-related plasticity in hypothalamus. *Annu Rev Neurosci* 20: 375–397.
12. Ojeda SR, et al. (2003) Glia-to-neuron signaling and the neuroendocrine control of female puberty. *Ann Med* 35:244–255.
13. Prevot V, et al. (2010) Function-related structural plasticity of the GnRH system: A role for neuronal-glial-endothelial interactions. *Front Neuroendocrinol* 31:241–258.
14. Theodosis DT, Poulain DA, Oliet SH (2008) Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. *Physiol Rev* 88:983–1008.
15. Herbison AE, Neill JD (2006) Physiology of the gonadotropin-releasing hormone neuronal network. *Knobil and Neill's Physiology of Reproduction*, eds Knobil E, Neill JD (Elsevier, New York), 3rd Ed, pp 1415–1482.
16. Moenter SM, DeFazio AR, Pitts GR, Nunemaker CS (2003) Mechanisms underlying episodic gonadotropin-releasing hormone secretion. *Front Neuroendocrinol* 24:79–93.
17. Brann DW, Mahesh VB (1997) Excitatory amino acids: evidence for a role in the control of reproduction and anterior pituitary hormone secretion. *Endocr Rev* 18:678–700.
18. Urbanski HF, Ojeda SR (1990) A role for N-methyl-D-aspartate (NMDA) receptors in the control of LH secretion and initiation of female puberty. *Endocrinology* 126:1774–1776.
19. Ma YJ, Berg-von der Emde K, Rage F, Wetsel WC, Ojeda SR (1997) Hypothalamic astrocytes respond to transforming growth factor-alpha with the secretion of neuroactive substances that stimulate the release of luteinizing hormone-releasing hormone. *Endocrinology* 138:19–25.
20. Rage F, Lee BJ, Ma YJ, Ojeda SR (1997) Estradiol enhances prostaglandin E2 receptor gene expression in luteinizing hormone-releasing hormone (LHRH) neurons and facilitates the LHRH response to PGE2 by activating a glia-to-neuron signaling pathway. *J Neurosci* 17:9145–9156.
21. Bezzi P, et al. (1998) Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature* 391:281–285.
22. Dziedzic B, et al. (2003) Neuron-to-glia signaling mediated by excitatory amino acid receptors regulates ErbB receptor function in astroglial cells of the neuroendocrine brain. *J Neurosci* 23:915–926.
23. Zonta M, et al. (2003) Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci* 6:43–50.
24. Prevot V, Lomniczi A, Corfas G, Ojeda SR (2005) erbB-1 and erbB-4 receptors act in concert to facilitate female sexual development and mature reproductive function. *Endocrinology* 146:1465–1472.
25. Spergel DJ, Krüth U, Hanley DF, Sprengel R, Seeburg PH (1999) GABA- and glutamate-activated channels in green fluorescent protein-tagged gonadotropin-releasing hormone neurons in transgenic mice. *J Neurosci* 19:2037–2050.
26. Leng G, Brown D (1997) The origins and significance of pulsatility in hormone secretion from the pituitary. *J Neuroendocrinol* 9:493–513.
27. Zhang C, Roepke TA, Kelly MJ, Rønnekleiv OK (2008) Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. *J Neurosci* 28:4423–4434.
28. Roland AV, Moenter SM (2011) Glucosensing by GnRH neurons: Inhibition by androgens and involvement of AMP-activated protein kinase. *Mol Endocrinol* 25:847–858.
29. Coleman RA, Smith WL, Narumiya S (1994) International Union of Pharmacology classification of prostanoid receptors: Properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* 46:205–229.
30. Sang N, Zhang J, Marcheselli V, Bazan NG, Chen C (2005) Postsynaptically synthesized prostaglandin E2 (PGE2) modulates hippocampal synaptic transmission via a presynaptic PGE2 EP2 receptor. *J Neurosci* 25:9858–9870.
31. Clasadonte J, Poulain P, Beauvillain JC, Prevot V (2008) Activation of neuronal nitric oxide release inhibits spontaneous firing in adult gonadotropin-releasing hormone neurons: A possible local synchronizing signal. *Endocrinology* 149:587–596.
32. Fonnum F, Johnsen A, Hassel B (1997) Use of fluorocitrate and fluoroacetate in the study of brain metabolism. *Glia* 21:106–113.
33. Okada-Ogawa A, et al. (2009) Astroglia in medullary dorsal horn (trigeminal spinal subnucleus caudalis) are involved in trigeminal neuropathic pain mechanisms. *J Neurosci* 29:11161–11171.
34. Parsons MP, Hirasawa M (2010) ATP-sensitive potassium channel-mediated lactate effect on orexin neurons: Implications for brain energetics during arousal. *J Neurosci* 30:8061–8070.
35. Nimmerjahn A, Kirchhoff F, Kerr JN, Helmchen F (2004) Sulforhodamine 101 as a specific marker of astroglia in the neocortex in vivo. *Nat Methods* 1:31–37.
36. Kafitz KW, Meier SD, Stephan J, Rose CR (2008) Developmental profile and properties of sulforhodamine 101—Labeled glial cells in acute brain slices of rat hippocampus. *J Neurosci Methods* 169:84–92.
37. Patten BA, Sardi SP, Koirala S, Nakafuku M, Corfas G (2006) Notch1 signaling regulates radial glia differentiation through multiple transcriptional mechanisms. *J Neurosci* 26:3102–3108.
38. Jasoni CL, Todman MG, Han SK, Herbison AE (2005) Expression of mRNAs encoding receptors that mediate stress signals in gonadotropin-releasing hormone neurons of the mouse. *Neuroendocrinology* 82:320–328.
39. Ojeda SR, Urbanski HF, Katz KH, Costa ME (1985) Stimulation of cyclic adenosine 3′,5′-monophosphate production enhances hypothalamic luteinizing hormone-releasing hormone release without increasing prostaglandin E2 synthesis: Studies in prepubertal female rats. *Endocrinology* 117:1175–1178.
40. Ojeda SR, Negro-Vilar A (1985) Prostaglandin E2-induced luteinizing hormone-releasing hormone release involves mobilization of intracellular Ca+2. *Endocrinology* 116:1763–1770.
41. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR (1993) Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci USA* 90:11693–11697.
42. Koyama Y, et al. (1999) Endothelins stimulate expression of cyclooxygenase 2 in rat cultured astrocytes. *J Neurochem* 73:1004–1011.
43. Luo J, Lang JA, Miller MW (1998) Transforming growth factor beta1 regulates the expression of cyclooxygenase in cultured cortical astrocytes and neurons. *J Neurochem* 71:526–534.
44. Yang H, Chen C (2008) Cyclooxygenase-2 in synaptic signaling. *Curr Pharm Des* 14: 1443–1451.
45. Farkas I, et al. (2010) Retrograde endocannabinoid signaling reduces GABAergic synaptic transmission to gonadotropin-releasing hormone neurons. *Endocrinology* 151:5818–5829.
46. Prusakiewicz JJ, Duggan KC, Rouzer CA, Marnett LJ (2009) Differential sensitivity and mechanism of inhibition of COX-2 oxygenation of arachidonic acid and 2-arachidonoylglycerol by ibuprofen and mefenamic acid. *Biochemistry* 48:7353–7355.
47. Christian CA, Mobley JL, Moenter SM (2005) Diurnal and estradiol-dependent changes in gonadotropin-releasing hormone neuron firing activity. *Proc Natl Acad Sci USA* 102:15682–15687.
48. Amateau SK, McCarthy MM (2004) Induction of PGE2 by estradiol mediates developmental masculinization of sex behavior. *Nat Neurosci* 7:643–650.
49. Christian CA, Moenter SM (2010) The neurobiology of preovulatory and estradiol-induced gonadotropin-releasing hormone surges. *Endocr Rev* 31:544–577.
50. Mayer C, et al. (2010) Timing and completion of puberty in female mice depend on estrogen receptor alpha-signaling in kisspeptin neurons. *Proc Natl Acad Sci USA* 107: 22693–22698.
51. Cravo RM, et al. (2011) Characterization of Kiss1 neurons using transgenic mouse models. *Neuroscience* 173:37–56.
52. Ojeda SR, Harms PG, McCann SM (1975) Effect of inhibitors of prostaglandin synthesis on gonadotropin release in the rat. *Endocrinology* 97:843–854.
53. Ojeda SR, Aguado LI (1985) Prostaglandins in reproductive neuroendocrinology. *Handbook of Pharmacologic Methodologies for the Study of the Neuroendocrine System*, eds Steger RW, Johns A (CRC, Boca Raton, FL), pp 205–243.
54. Ma YJ, et al. (1999) Neuregulins signaling via a glial erbB-2-erbB-4 receptor complex contribute to the neuroendocrine control of mammalian sexual development. *J Neurosci* 19:9913–9927.
55. Zonta M, et al. (2003) Glutamate-mediated cytosolic calcium oscillations regulate a pulsatile prostaglandin release from cultured rat astrocytes. *J Physiol* 553:407–414.
56. Ojeda SR, Urbanski HF, Costa ME, Hill DF, Moholt-Siebert M (1990) Involvement of transforming growth factor alpha in the release of luteinizing hormone-releasing hormone from the developing female hypothalamus. *Proc Natl Acad Sci USA* 87: 9698–9702.