



HAL
open science

Morphological evidence for direct interaction between gonadotrophin-releasing hormone neurones and astroglial cells in the human hypothalamus.

Marc Baroncini, Cécile Allet, Daniel Leroy, Jean-Claude Beauvillain, Jean-Paul Francke, Vincent Prevot

► To cite this version:

Marc Baroncini, Cécile Allet, Daniel Leroy, Jean-Claude Beauvillain, Jean-Paul Francke, et al.. Morphological evidence for direct interaction between gonadotrophin-releasing hormone neurones and astroglial cells in the human hypothalamus.: Neuroglial interactions for GnRH neurones in the human hypothalamus. *Journal of Neuroendocrinology*, 2007, 19 (9), pp.691-702. 10.1111/j.1365-2826.2007.01576.x . inserm-00363976

HAL Id: inserm-00363976

<https://inserm.hal.science/inserm-00363976>

Submitted on 24 Feb 2009

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Morphological Evidence for Direct Interaction Between Gonadotrophin-Releasing Hormone Neurones and Astroglial Cells in the Human Hypothalamus

M. Baroncini,*†‡ C. Allet,* D. Leroy,* J.-C. Beauvillain,* J.-P. Francke*† and V. Prevot*

*Inserm, Jean-Pierre Aubert Research Center, U837, Development and Plasticity of the Postnatal Brain, Lille, France.

†Université de Lille 2, Faculté de Médecine, Laboratoire d'Anatomie, Institut de médecine prédictive et recherche thérapeutique, Lille, France.

‡CHU Lille, Clinique de Neurochirurgie, Lille, France.

Journal of Neuroendocrinology

In rodents, there is compelling evidence indicating that dynamic cell-to-cell communications involving cross talk between astroglial cells (such as astrocytes and specialised ependymogial cells known as tanocytes) and neurones are important in regulating the secretion of gonadotrophin-releasing hormone (GnRH), the neurohormone that controls both sexual maturation and adult reproductive function. However, whether such astroglial cell–GnRH neurone interactions occur in the human brain is not known. In the present study, we used immunofluorescence to examine the anatomical relationship between GnRH neurones and glial cells within the hypothalamus of five women. Double-staining experiments demonstrated the ensheathment of GnRH neurone perikarya by glial fibrillary acidic protein (GFAP)-immunoreactive astrocyte processes in the periventricular zone of the tuberal region of the hypothalamus. GFAP immunoreactivity did not overlap that of GnRH at the GnRH neurone's projection site (i.e. the median eminence of the hypothalamus). Rather, human GnRH neuroendocrine fibres were found to be closely associated with vimentin or nestin-immunopositive radial glial processes likely belonging to tanocytes. In line with these light microscopy data, ultrastructural examination of GnRH-immunoreactive neurones showed numerous glial cells in direct apposition to pre-embedding-labelled GnRH cell bodies and/or dendrites in the infundibular nucleus, whereas postembedding immunogold-labelled GnRH nerve terminals were often seen to be enwrapped by glial cell processes in the median eminence. GnRH nerve button were sometimes visualised in close proximity to fenestrated pituitary portal blood capillaries and/or evaginations of the basal lamina that delineate the pericapillary space. In summary, these data demonstrate that GnRH neurones morphologically interact with astrocytes and tanocytes in the human brain and provide evidence that glial cells may contribute physiologically to the process by which the neuroendocrine brain controls the function of GnRH neurones in humans.

Correspondence to:

Vincent Prevot, Inserm U837, Place de Verdun, 59045 Lille, Cedex, France
(e-mail: prevot@lille.inserm.fr).

Key words: LHRH, astrocytes, tanocytes, neuroendocrine brain, reproduction, human.

doi: 10.1111/j.1365-2826.2007.01576.x

Gonadotrophin-releasing hormone (GnRH) is the neurohormone controlling sexual maturation and adult reproductive function (1–3). In rodents, the cell bodies of GnRH neurones are diffusely distributed in the preoptic region; in primates, including humans, they are also present in the tuberal region of the hypothalamus. The neuroendocrine fraction of GnRH neurones sends axons to the median eminence of the hypothalamus, where they release their neurohormone into the pituitary portal vasculature. On reaching

the anterior, pituitary GnRH elicits the secretion of the gonadotrophins luteinising hormone and follicle-stimulating hormone that stimulate gametogenesis and gonadal steroids secretion, and thus support reproductive physiology.

Because GnRH neurones are the final common pathway for the central control of reproduction, their activity is regulated by a complex array of excitatory and inhibitory transsynaptic inputs (4–9). Noticeably, both GnRH neurones and the multiple neuronal

networks involved in the control of GnRH secretion can be subjected to the direct modulatory influence of gonadal steroids (10, 11). However, it is becoming increasingly clear from experiments conducted in rodents that, in addition to these transsynaptic regulatory mechanisms, cell-cell interactions involving non-neuronal cells, such as astrocytes and specialised ependymoglia cells known as tanycytes, might be of critical importance for the regulation of GnRH secretion in females (12–16).

Glial cell processes abundantly appose the GnRH cell membrane in both rodents and nonhuman primates and at both GnRH perikarya and GnRH axon terminals (17–23). Whether GnRH neurones exhibit strong associations with astroglial cells in the human brain is not known. To provide an anatomical basis for the potential direct interaction of glial cells with GnRH neurones in the human hypothalamus, morphological studies were conducted using post-mortem human material.

In the present study, double-immunofluorescence was used to examine whether astroglia processes from the tuberal region of the hypothalamus make close appositions to GnRH perikarya, as well as to its nerve terminals. In a second experiment, the glial ensheathment of GnRH neurones was examined by electron microscopy.

Materials and methods

Tissue

Brains of five women were obtained from autopsies at 6–48 h postmortem (Table 1). A review of medical records indicated that specimens were obtained from individuals with no neurological or neuroendocrinological disorder (Table 1). The brain samples were taken from patients that donated bodies to Science in compliance with the French laws on bioethics. Structures inside and outside the human brain were identified by reference to an atlas of the human brain (24).

Fluorescent immunostaining

After whole brain removal, blocks of 20 mm per side encompassing the hypothalamus were harvested with the optic chiasm as the anterior limit and the mamillary bodies as the posterior limit. Hypothalami were immersion fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) (PB) for 1 week, cryoprotected in 20% sucrose in PB containing 0.9% sodium chloride (PBS) for 48 h, embedded in Tissue Tek (Miles, Elkhart, IN, USA) and

Table 1. Clinicopathologic Data.

Number	Sex	Age (years)	Postmortem delay (h)	Cause of death
Ha07	Female	67	24	Ano-rectal cancer with hepatic metastases
Ha08	Female	76	36	Renal insufficiency on peritonitis by duodenal perforation
Ha011	Female	89	32	Cardio-respiratory failure
Ha012	Female	88	48	Coronary thrombosis
Ha150	Female	74	6	Cardio-respiratory failure

Table 2. Primary Antibodies Used in the Study.

Antigen	Host	Code	Dilution	References	Specificity
GnRH	Rabbit	–	1 : 1000	(29)	(29)
GFAP	Mouse	Clone G-A-5	1 : 500	Sigma, France	(71)
GFAP	Rabbit	Nr. Z 0334	1 : 500	DAKO Cytomation, Denmark	(72)
Vimentin	Mouse	Nr. M 0725	1 : 1000	DAKO Cytomation, Denmark	(73)
Nestin	Mouse	MAB1259	1 : 250	R&D Systems, Germany	R&D Systems, Germany

GnRH, gonadotrophin-releasing hormone; GFAP, glial fibrillary acidic protein.

frozen in liquid nitrogen. Coronal cryostat sections (14 μ m) were mounted on chrome-alum-gelatin coated slides, air-dried, and subjected to immunofluorescent stainings using a procedure described previously (25, 26). Briefly, sections were incubated for 10 min in LKPBS (KPBS 0.02 M with 0.3% of triton 100X and 2% of normal goat serum) to block the nonspecific sites. Sections were then incubated for one night at 4 °C with 250 μ l of primary antibodies diluted in LKPBS. The characteristics of the primary antibodies used are shown in Table 2. After rinsing, secondary antibodies (250 μ l) were deposited on sections and incubated at room temperature (RT) for 1 h. Goat antirabbit Alexa Fluor 488 (1 : 400) and goat antimouse Alexa Fluor 546 (1 : 400) were obtained from Molecular Probes (Eugene, OR, USA). Cell nuclei were stained with 0.02% Hoechst 33258 bi-benzimidazole (Molecular Probes). Importantly, to avoid the strong autofluorescence caused by lipofuscin granules usually present in adult human brain tissue, sections were immersed in a solution of 0.3% Sudan Black B (Sigma, St Quentin Fallavier, France) in 70% ethanol for 10 min (27). This treatment completely blocked autofluorescence. Sections were then coverslipped in PermaFluor medium (Immunon, Pittsburg, PA, USA). Control sections were incubated in the absence of primary antibody.

Immunofluorescent images were acquired using a DC300FX camera (Leica, Nussloch, Germany) attached to a DMRB microscope (Leica) through FW4000 software (Leica) with $\times 20$ (numerical aperture 0.5) or $\times 40$, 0.7 Plan Fluotar objectives. Confocal images were captured using a TCS SP confocal system (Leica). For illustration purposes, photomontages of the median eminence were prepared with the help of Photoshop CS2 (Adobe Systems, San Jose, CA, USA) using 30–60 digitised images acquired with a $\times 20$ objective.

Electron microscopy

A female brain with a 6-h postmortem delay was subjected to intracerebroventricular injection of 10 ml of 2% paraformaldehyde, 0.2% picric acid and 0.1% glutaraldehyde in 0.1 M PB pH 7.4 to prior dissection. The median eminence and the periventricular zone of the tuberal region were then harvested and immersed overnight in the same fixative solution.

Pre-embedding immunostaining

The periventricular zone of the tuberal region was sliced coronally (60 μ m) with a vibratome in 0.1 M PBS. Floating sections were subsequently processed for diaminobenzidine immunohistochemistry, slightly modified from a previously described method (28). Briefly, sections were blocked in PBS with 0.05% Triton X-100 (Sigma), 1% bovine serum albumin (BSA) and 10% normal goat serum (NGS) for 1 h at RT before incubation with rabbit anti-GnRH (1 : 500) (29) in PBS with 1% BSA and 10% NGS overnight at 4 °C with gentle rocking. Sections were extensively washed in PBS and exposed

to biotinylated goat IgGs anti-rabbit IgGs (1 : 200, Jackson Immunoresearch Laboratories, West Grove, PA, USA) in PBS with 1% BSA and 10% NGS for 2 h at RT and then with avidin-peroxidase (Vector laboratories, Burlingame, CA, USA) for 1 h at RT. Finally, 5 mg/ml of 3,3'-diaminobenzidine tetrahydrochloride (Research Organics Inc., Cleveland, OH, USA) was added on sections in the presence of 0.01% H₂O₂ in 0.1 M Tris buffer pH 7.6 for 10 min at RT to create an electron-dense enzymatic reaction product. Slices were then subjected to 1% OsO₄ in phosphate buffer for 20 min at RT, dehydrated and flat embedded in Araldite (Huntsman, Everberg, Belgium). Semithin-sections (1–2 µm thick) were used to progressively approach GnRH-immunoreactive neurones. Ultrathin sections (80–90 nm thick) were collected on Parlodion 0.8% isoamylacetate-coated 100 mesh nickel grids (Electron Microscopy Sciences, Fort Washington, PA, USA) and counterstained with uranyl acetate and lead citrate before observation on a Zeiss transmission electron microscope 902 (Leo, Rueil-Malmaison, France).

Postembedding immunostaining

Subsequent to the aforementioned prefixation, the piece of tissue containing the median eminence was postfixed for 1 h at room temperature with 1% OsO₄ in PB. After dehydration, pieces of tissue were embedded in Araldite. Semithin sections (1–2 µm thick) were used to progressively approach and identify the portion of the median eminence where the ultrastructural studies were performed. To detect GnRH immunoreactivity, ultrathin sections (80–90 nm thick) collected onto nickel grids were treated using an immunogold procedure described previously (21, 25). Briefly, after a treatment with H₂O₂ (10%; 8 min) and a blocking step in TBS (0.1 M Tris; pH 7.4, 0.15 M NaCl; TBS) containing 1% normal goat serum and 1% bovine albumin serum (TBSB, 10 min at RT), the grids were floated on a drop of the following reagents and washing solutions: (i) rabbit anti-GnRH (1 : 5000) in TBSB for 60 h at 4 °C ; (ii) TBS to remove excess antibodies (3 × 10 min) ; (iii) colloidal gold (18 nm)-labelled goat anti-rabbit immunoglobulins (Jackson Immunoresearch Laboratories) 1 : 20 in TBS (0.1 M Tris; pH 7.4, 0.5 M NaCl) for 90 min at RT ; (iv) TBS (3 × 10 min); and (v) distilled water (3 × 10 min). The sections were also counterstained with uranyl acetate and lead citrate before observation.

Photographs were taken at an original magnification of ×7000; for illustration purposes, negatives were digitised with a Epson Expression 1680 Pro (Epson France S.A., Levallois Perret, France) and microphotograph montages were made using Photoshop CS2.

Results

Localisation of GnRH cell bodies and axon terminals in the periventricular zone of the tuberal region of the human hypothalamus

The localisation of GnRH expression was examined in the tuberal region of the human hypothalamus (Fig. 1A) using immunohistofluorescence. Neuronal perikarya immunoreactive for GnRH were not confined to specific nuclei, but were dispersed in the periventricular zone of the tuberal region, as previously described (30–34). Ten to 30 GnRH neurones were analysed per hypothalamus. GnRH-immunoreactive perikarya were often found within the infundibular nucleus (inf) of the hypothalamus (Fig. 1B–D). These neurones were predominantly bipolar (Fig. 1B,E) and both their soma and dendrites were noticeably surrounded by numerous cells bearing small round nuclei (Fig. 1F–G). Within the median eminence of the hypothalamus, GnRH-immunoreactive fibres abundantly innervated the external zone where they densely outlined capillary loops (Fig. 1B–D).

GnRH neurones are morphologically associated with GFAP-immunoreactive astrocytes at their cell body but not at their nerve terminals

Using double immunostaining, GnRH neurones and GFAP-immunoreactive astrocytes in the periventricular zone of the tuberal region were examined for possible close appositions. As shown in Fig. 2(A–C), GnRH cell bodies are enwrapped in GFAP-immunoreactive processes emanating from cells that exhibit small and round nuclei. By contrast, the distribution of GFAP immunoreactivity within the median eminence did not overlap that of GnRH neuroendocrine fibres and/or neurovascular terminals (Fig. 2D–F).

GnRH neurones are morphologically associated with vimentin-immunoreactive glial cells both at their cell body and at their nerve terminals

To determine whether in the human median eminence, as in the rodent brain, GnRH axon fibres are tightly associated with glial processes belonging to specialised ependymogial cells, known as tanycytes, we examined using a procedure similar to that described above immunoreactivities for GnRH and vimentin, which is an intermediate filament expressed by astrocytes in immature or dynamic conditions (35–37) and tanycytes (38). Figure 3(D–F) shows that GnRH fibres travel in close association with vimentin-immunoreactive tanycytic processes within the external layer of the median eminence. Unexpectedly, GnRH perikarya were also found in close apposition to vimentin-immunoreactive processes (Fig. 3A–C).

Vimentin colocalise with GFAP in some hypothalamic astrocytes, but not in tanycytic endfeets

To determine whether GFAP-immunoreactive hypothalamic glial cells also colocalise vimentin, we performed additional double immunofluorescent studies. As shown in Fig. 4(A–C), only a very few hypothalamic astrocytes express both GFAP and vimentin in the periventricular zone of the tuberal region. Within the median eminence, superimposition of the GFAP and vimentin stainings revealed that expression of these cytoskeletal proteins overlapped in the internal layer (Fig. 4D–F). By contrast, the glial cell processes contacting the pial surface in the external zone of the median eminence were only immunoreactive for vimentin (Fig. 4D–F).

GnRH axon terminals are in close association with nestin-immunoreactive tanycytic processes

Because, unlike GFAP, vimentin cannot form intermediate filaments on its own (39), we next investigated whether nestin, another intermediate filament protein expressed in immature glial cells (40) was expressed in the human hypothalamus. Within the periventricular zone of the tuberal region, nestin-immunoreactivity was strikingly confined to the median eminence. In particular, antinestin antibody stained ependymal cells lining the floor of the third ventricle and projecting to the ventral surface of the brain (Fig. 5B) (i.e. tanycytes). Nestin-positive tanycytes had tapering processes that

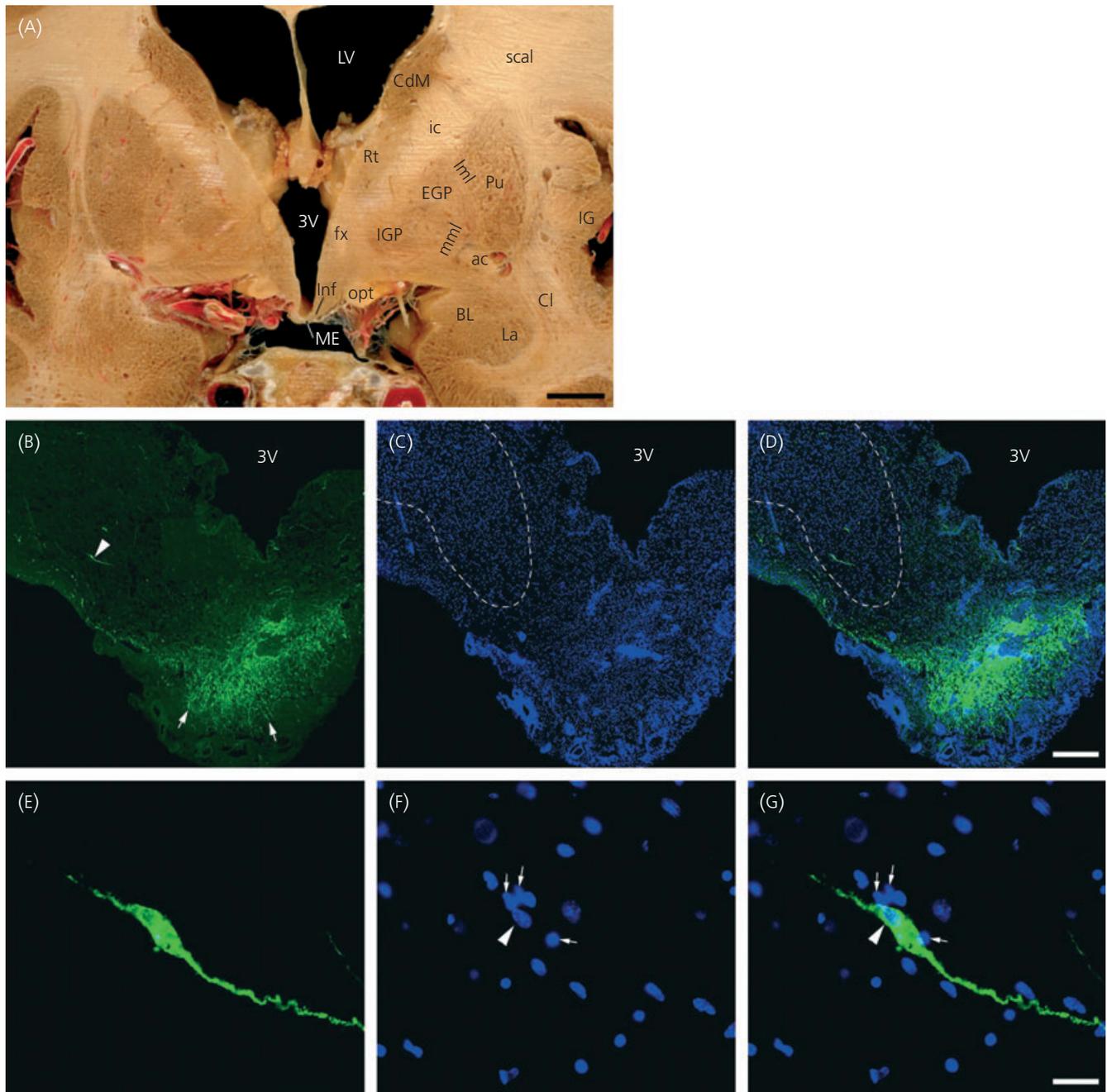


Fig. 1. Localisation of gonadotrophin-releasing hormone (GnRH) immunoreactivity by fluorescent microscopy in the tuberal region of the human hypothalamus. (A) Macroscopic coronal section of the female brain containing the infundibular nucleus (inf) and the median eminence (ME). 3V, Third ventricle; ac, anterior commissure; BL, basolateral amygdaloid nucleus; CdM, medial caudate nucleus; Cl, claustrum; EGP, external globus pallidus; fx, fornix; ic, internal capsule; IG, insular gyrus; IGP, internal globus pallidus; Iml, internal medullary lamina of thalamus; La, lateral amygdaloid nucleus; LV, lateral ventricle; mml, medial medullary lamina of the globus pallidus; opt, optic tract; Pu, putamen; Rt, reticular thalamic nucleus; scal, subcallosal bundle. (B–D) Representative micrograph montages of GnRH immunoreactivity (green) in the tuberal region of the hypothalamus. Note the presence of a GnRH cell body (arrowhead) within the infundibular nucleus (dotted lines) and of GnRH neuroendocrine axons (arrows) within the external zone of the median eminence. Cell nuclei are stained with Hoechst (blue, C,D). (E–G) Higher magnification of the GnRH immunofluorescent neurone shown in (B, arrowhead). Note the presence of numerous small nuclei-bearing cells (arrows) in close apposition to GnRH neurone's cell body and dendrite. Scale bars = 8 mm (A); 300 μ m (B), 20 μ m (G).

traversed the median eminence with an arching trajectory with end feet terminating close to the portal capillaries, thereby linking the third ventricle with the hypophysial vessels (Fig. 5B,E). In addition, the middle and the external parts of the median eminence (Fig. 5E)

contained positive cell bodies with short processes (Fig. 5E), also ending close to portal vessels (Fig. 5E). In accordance with the results presented in Figs 3(D–F), double-label immunocytochemical studies for GnRH and nestin revealed numerous GnRH axons in

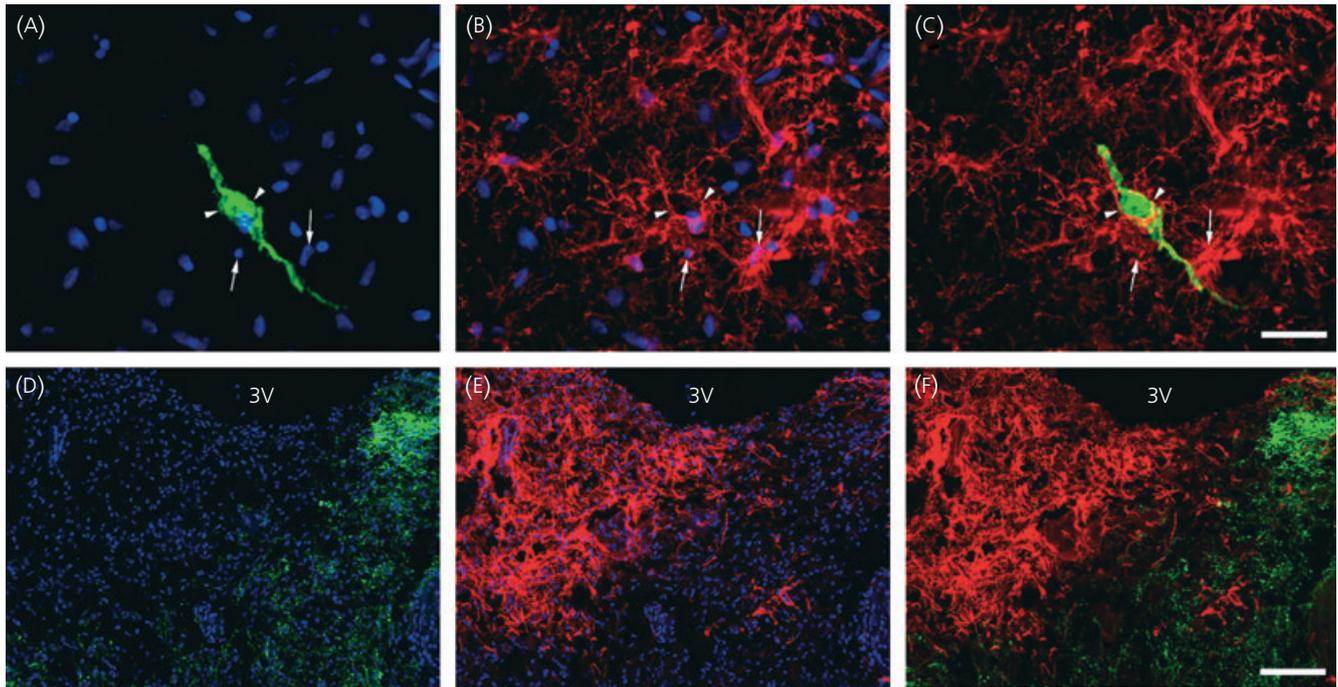


Fig. 2. Anatomical relationship between gonadotrophin-releasing hormone (GnRH)-immunoreactive neurones and glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes in the tuberal region of the human hypothalamus. (A–C) Representative micrographs of GFAP-immunoreactive astrocytes (red, arrows) enwrapping the cell body and the dendrites of a GnRH-immunofluorescent neurone (green, arrowheads). (D–E) Representative micrograph montages of the distribution of GFAP (red) and GnRH (green) immunoreactivities within the median eminence. (C,F) Merged images of GFAP and GnRH immunoreactivities. Cell nuclei are stained with Hoechst (blue). Scale bars = 20 μm (c), 100 μm (f).

close contact with tanyctic processes in the external layer of the median eminence (Fig. 5). No cells in close association with GnRH cell bodies were immunoreactive for nestin.

Ultrastructural analysis of the relationship between GnRH immunoreactivity and glial elements in the human hypothalamus

To refine our analysis of glia–GnRH neurone morphological interactions, we undertook pre-embedding and postembedding immunocytochemical studies to visualise GnRH perikarya and GnRH axon terminals at the ultrastructural level, respectively. Although the tissues were not perfectly preserved due to the postmortem delay, the examination of 1 μm -thick semithin sections with the light microscope showed that GnRH-immunopositive cell bodies were in direct contact with cells bearing small nuclei (Fig. 6A), which are seen to enwrap GnRH processes or immunoreactive sections of perikarya at the electron microscopic level (Fig. 6B). By contrast, in many cases, ultrastructural examination of the external zone of the median eminence showed that glial cell processes contacted immunogold-labelled GnRH fibres (Fig. 7B,D). Interestingly, GnRH nerve endings were sometimes visualised near to fenestrated pituitary portal blood capillaries (Fig. 7C,C'') and/or evaginations of the pericapillary space (Fig. 7E). Gold particles were exclusively found over nerve terminals (Fig. 7B–E). Within axon terminals, GnRH-immunogoldlabelling was not restricted to large dense-core secretory granules, but was also

seen in axoplasm and mitochondria, as shown previously in the monkey (17) and rat (41, 42).

Discussion

Astroglial cells morphologically interact with GnRH neurones in the human hypothalamus

The present study represents the first detailed morphological characterisation of neuro–glial interactions for GnRH neurones in the human brain. In keeping with previous observations demonstrating the ensheathment of both perikarya and neuroendocrine terminals of GnRH neurones by astroglia processes in rodents and nonhuman primates (17–23), our findings show that astrocytes and tanyctes are morphologically associated with GnRH neurones within the human hypothalamus. Previous work has established patterns of neuronal afferents to GnRH neurones (5) and documented action sites of oestrogen (43–45) within the human hypothalamus. However, we know remarkably little about how astroglia, which are key signalling components with the potential to modulate the way information is generated and disseminated within the brain (46), interact with GnRH neurones in the human hypothalamus. By using antibodies to intermediate filament proteins such as GFAP, we succeeded in visualising the anatomical relationship between GnRH neurones and astrocytes in the human hypothalamus. Our fluorescent microscopy results indicate that each individual GnRH cell

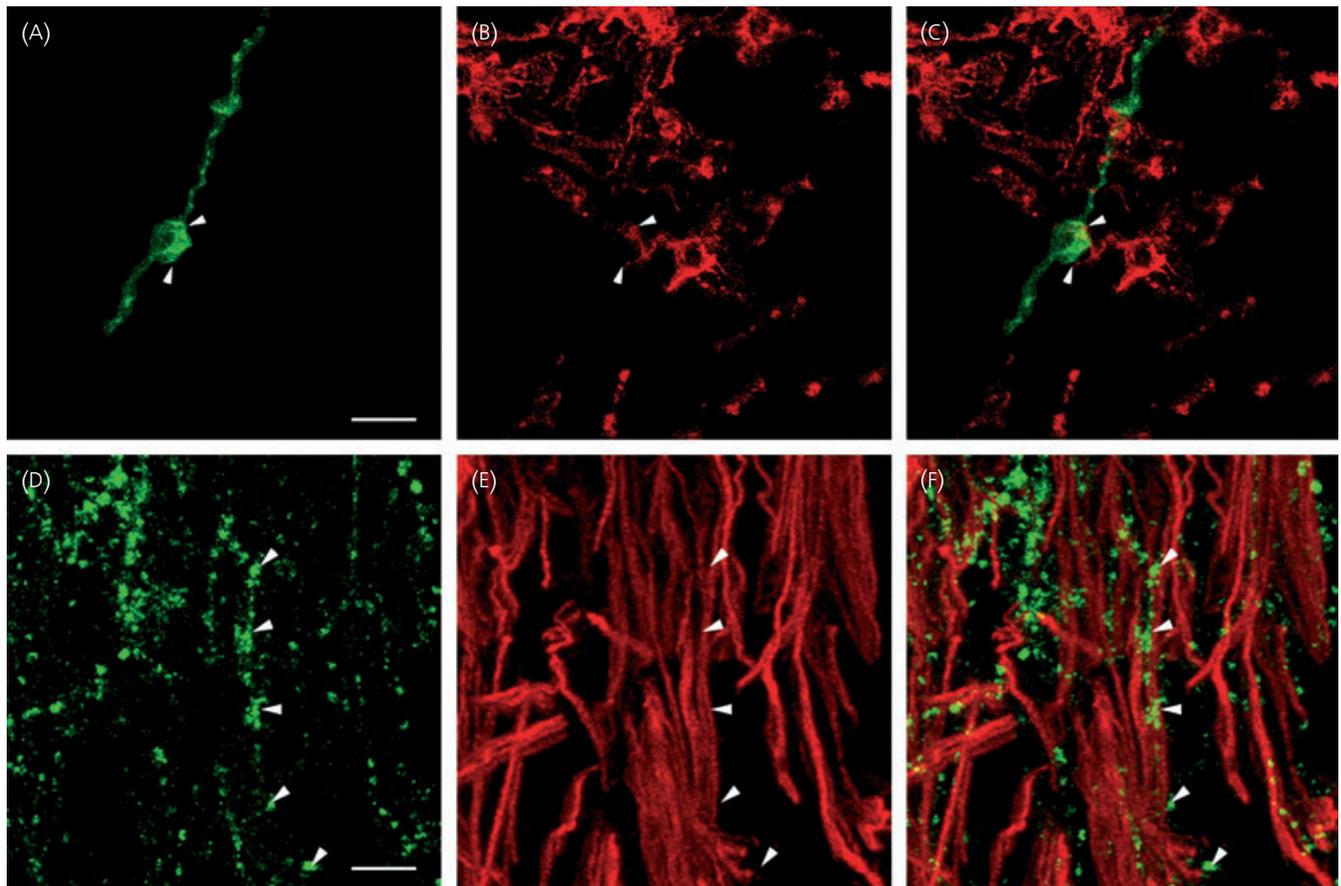


Fig. 3. Anatomical relationship between gonadotrophin-releasing hormone (GnRH)-immunoreactive neurones and vimentin-immunoreactive astrocytes and tancytes in the tuberal region of the human hypothalamus. (A–C) Representative micrographs of a vimentin-immunoreactive astrocyte (red) engulfing the cell body of a GnRH-immunofluorescent neurone (green, arrowheads). (D–F) Representative micrographs of the distribution of vimentin and GnRH (arrowheads) immunoreactivities in the external part of the median eminence. (C,F) Merged images of vimentin and GnRH immunoreactivities. Scale bars = 20 μm (A), 5 μm (D).

body is surrounded by several astrocytes that wrap themselves around their soma and dendrites. The existence of close contacts between GnRH perikarya and astrocytes was substantiated both by structural and ultrastructural analyses on semithin and ultrathin sections, respectively, in the human hypothalamus. At the GnRH neurone's projection site, astrocytes did not appear to interact with GnRH neuroendocrine terminals. Rather, our results show a spatial segregation of GnRH axons to GFAP-immunonegative territories in the median eminence that corresponds to its external part. By contrast, the external region of the median eminence was found to be strongly immunoreactive for vimentin, which is another intermediate filament protein only expressed in certain astroglial cells of the postnatal brain and, among them, modified ependymogial cells known as tancytes (38). Within the median eminence of the hypothalamus, tancytes line the ventral portion of the wall of the third ventricle and send radial cell processes reaching the external part of the median eminence, where they establish contact with the endothelial wall of the portal vessels, via 'end-feet' specialisations (47). Our data show that, as found in rodents (48, 49), vimentin-immunoreactive tancytic processes closely appose GnRH nerve terminals travelling down to the external layer of the median eminence in the human hypothalamus. Because, in contrast to

GFAP, vimentin cannot self-assemble into intermediate filament *in vivo* (39), we aimed to identify the expression of nestin, which is an additional intermediate filament associated protein expressed in immature astroglial cells (40) that requires heteropolymerisation with either GFAP or vimentin to form intermediate filament bundles *in vivo* (39), and which has recently been shown to be expressed in tancytes of the median eminence in rodents (50, 51). Our immunofluorescent data show that heavy glial cell processes are intensely labelled for nestin within the human median eminence. These results thus suggest that vimentin and nestin are coassembled in the intermediate filaments expressed in tancytes of the median eminence. As visualised with antibodies to vimentin, nestin immunoreactive radial fibres are tightly associated with GnRH neuroendocrine axons. The intimate relationship between GnRH axon terminals and glia processes was corroborated at the electron microscopy level using a postembedding immunogold labelling procedure in the human median eminence. Altogether, these results provide the exciting possibility that radial tancyte processes constitute glial elements which, in addition to serving as the scaffolding for GnRH neuroendocrine axons, may provide a regulatory role for those neuroendocrine nerve endings in the adult human median eminence.

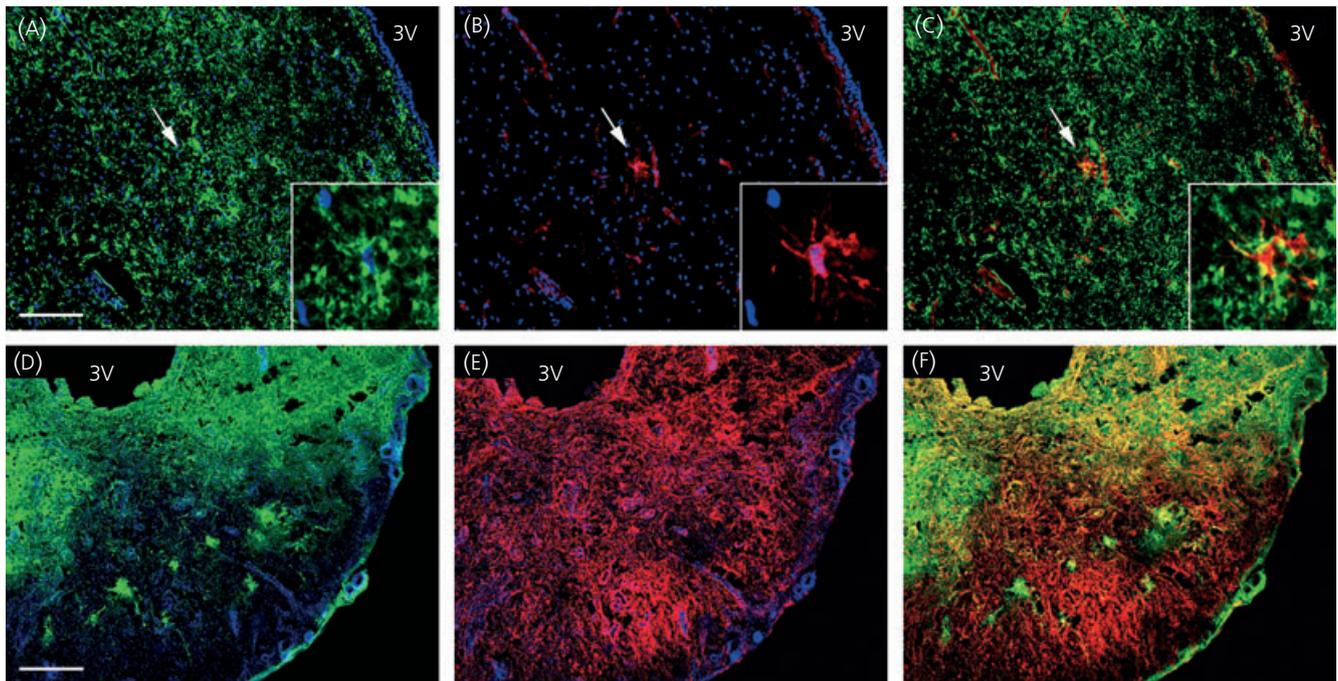


Fig. 4. Distribution of glial fibrillary acidic protein (GFAP) and vimentin immunoreactivities in the tuberal region of the human hypothalamus. (A–C) Representative micrographs of GFAP (green) and vimentin (red) fluorescent stainings in the periventricular zone. Note that among a wide GFAP-immunoreactive field only one astrocyte is immunoreactive for vimentin (arrow, inset). (D–F) Representative micrograph montages of GFAP and vimentin fluorescent stainings within the median eminence. (C,F) Merged images of vimentin and GFAP immunoreactivities. Cell nuclei are stained with Hoechst (blue). 3V, Third ventricle. Scale bars = 100 μm (A), 300 μm (D).

Hypothalamic neuroglial plasticity may modulate GnRH secretion

Hatton *et al.* (52) first demonstrated the importance of glial cells in the control of hypothalamic neurosecretion by documenting the plastic relationship that exists between astrocytes and vasopressin neurones. An increasing body of evidence suggests that astroglia are also functionally linked to GnRH neurones. In rodents, the direct access of GnRH neuroendocrine terminals to the vascular wall of the pituitary portal vessels in the external part of the median eminence is highly regulated by tancytic processes that enwrap GnRH axon terminals (18, 19, 21). At times of increased GnRH secretion (e.g. during the preovulatory surge of gonadotrophins), the terminals reach the endothelial wall due to, at least in part, retraction of the tancytic end feet and/or evagination of the pericapillary space into the parenchyma (21). Interestingly, the ultrastructural examination of the external zone of the human median eminence undertaken in the present study showed that evaginations of the basal lamina that delineates the pericapillary space occasionally appear in the nervous tissue in the immediate proximity of GnRH nerve terminals. Notwithstanding that these data were obtained from an aged female hypothalamus, our results raise the possibility that the aforementioned morphofunctional plasticity could take place within the human median eminence during the menstrual cycle.

At GnRH perikarya, neuronal–glial interactions may also play a critical role in determining specific patterns of synapse formation

during postnatal female sexual maturation, as well as participating in adult synaptic plasticity, as suggested by several nonhuman studies (22, 23, 53, 54). Intriguingly, our results show that, among those astrocytes ensheathing GnRH cell bodies, some of them are immunoreactive for vimentin. Because double-fluorescent staining experiments showed that vimentin-immunoreactive astrocytes within the tuberal region of the hypothalamus are only lightly labelled for GFAP, it appears unlikely that they correspond to reactive astrocytes which, in addition to a transient re-expression of vimentin, are known to dramatically increase their GFAP synthesis (55). Rather, the vimentin immunoreactive astrocytes could possibly serve a 'plastic' role by allowing changes in the apposition of astroglia processes to GnRH cell bodies, thus allowing synapses to form or to brake depending on environmental cues, as demonstrated for other neuroendocrine systems (56, 57). Intermediate filaments are highly polymorphic structures. This polymorphism allows for the constant modification of intermediate filaments structure in response to changes in cellular conditions triggered by extracellular signals (58). These unique physical properties bestow a critical function on intermediate filaments in the dynamic organisation of cytoarchitecture. It is then conceivable that expression of an increased array of intermediate filaments in a given cell may confer on it distinct cytodynamic properties.

A restriction on our findings holds for the possible influence of the endocrine status together with the postmortem period on the immunodetection of GnRH neurones and glial cells. However, previous studies by others have shown that the distribution and the

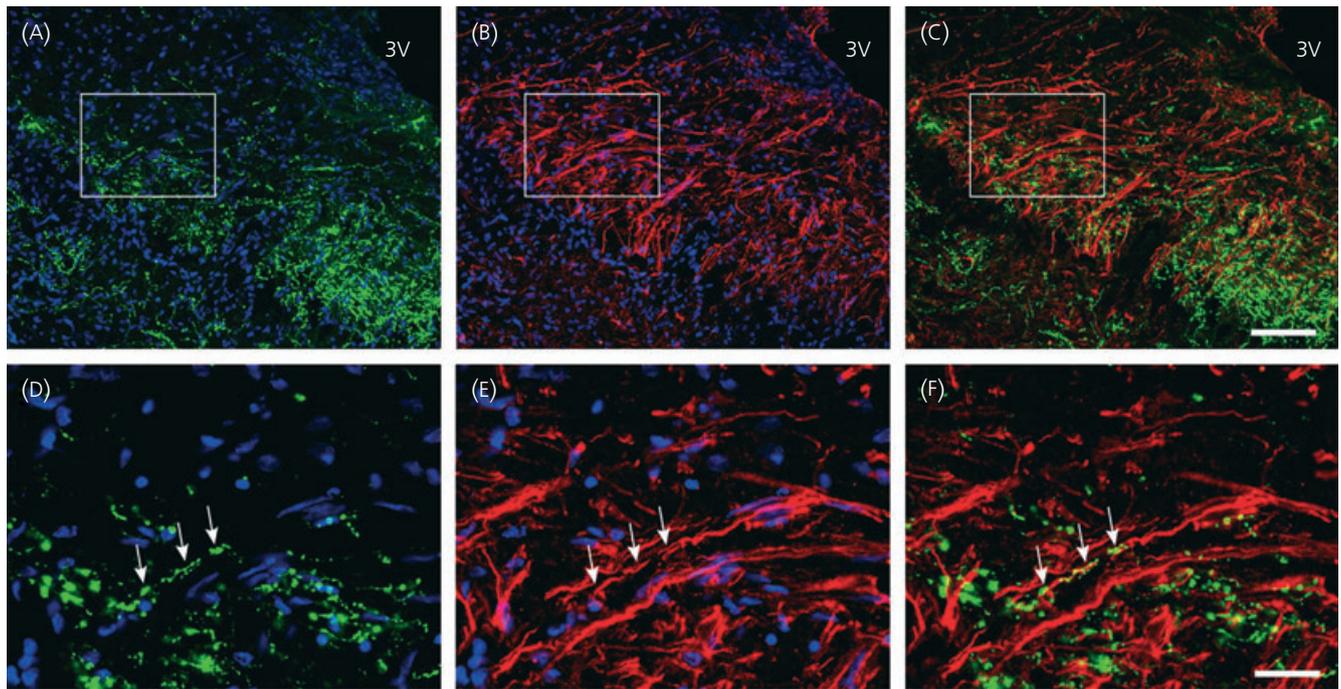


Fig. 5. Anatomical relationship between gonadotrophin-releasing hormone (GnRH)-immunoreactive neurones and nestin-immunoreactive tanycytes in the median eminence of the human hypothalamus. (A–C) Representative micrograph montages of nestin (red) and GnRH green (green) immunofluorescent labelings within the median eminence. (D–E) Higher magnification ($\times 40$) of the distribution of nestin and GnRH (arrow) immunoreactivities in the external region of the median eminence. (C,F) Merged images of nestin and GnRH immunoreactivities. 3V, Third ventricle. Cell nuclei are stained with Hoechst (blue). Scale bars = $120 \mu\text{m}$ (A), $30 \mu\text{m}$ (D).

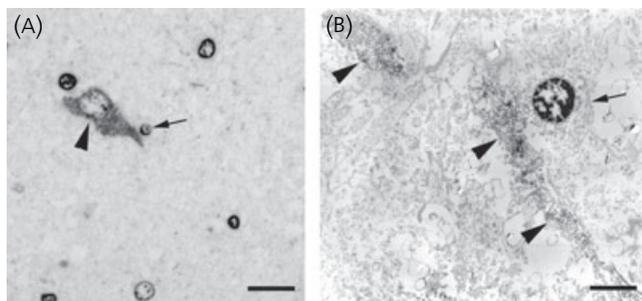


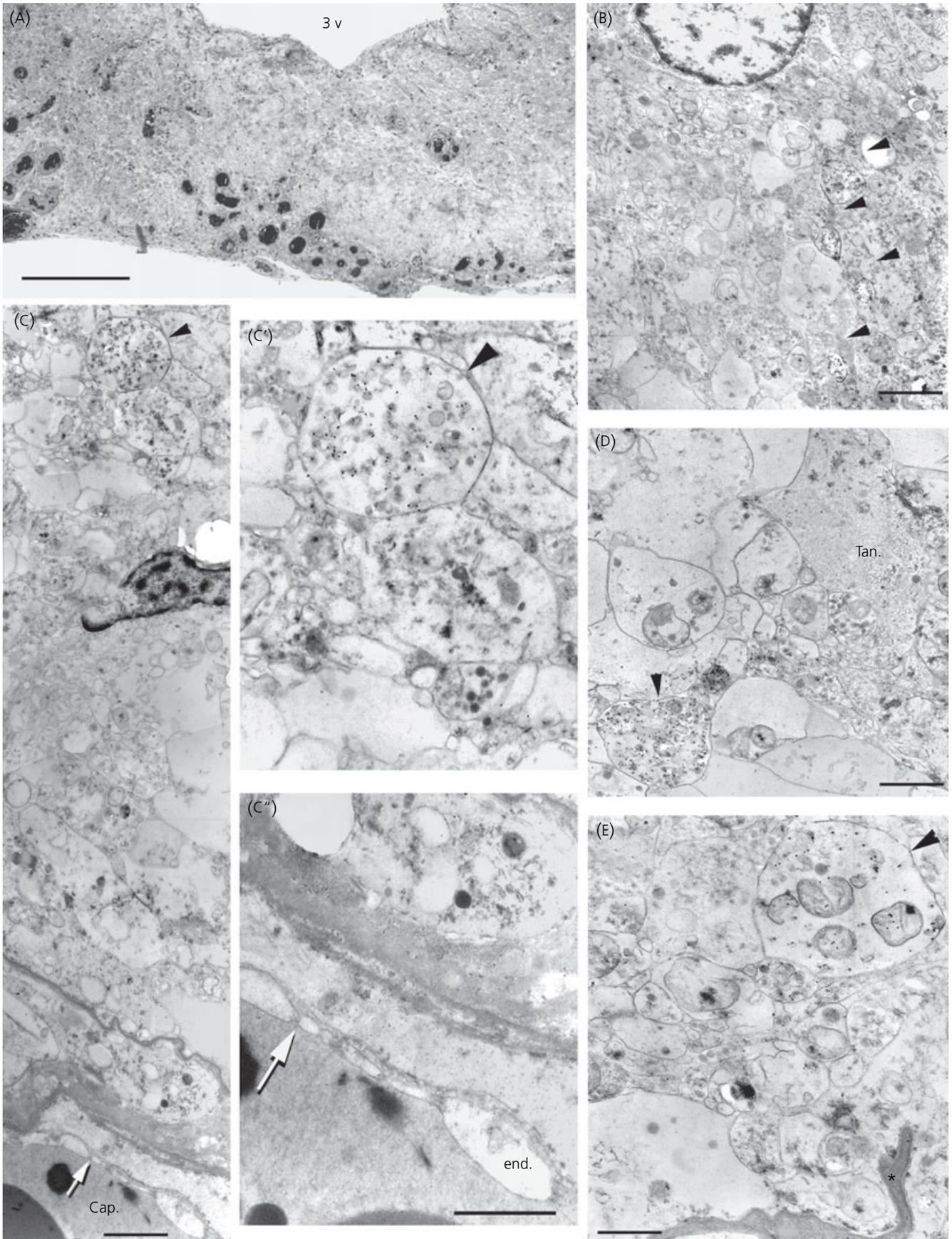
Fig. 6. Representative photomicrographs of a gonadotrophin-releasing hormone (GnRH) neurone from the periventricular zone of the tuberal region of the hypothalamus of a female in a semithin (A) or ultrathin (B) section by light (A) or electron (B) microscopy, respectively. Expression of the GnRH peptide within the neurone is indicated by the presence of osmificated diaminobenzidine (black arrowheads). Astrocyte nuclei are present at the immediate proximity of perikarya and/or dendrites (A,B, arrows). Astroglial processes (B, arrow) are present around dendrites or sections of the perikarya. Scale bars = $10 \mu\text{m}$ (A); $2.5 \mu\text{m}$ (B).

morphology of the GnRH neurones do not vary by the gender, age and postmortem delay ($< 48 \text{ h}$) of the individuals (59). In addition, Rance *et al.* (60) showed that glial cell density, GFAP staining and GnRH neurone morphology were comparable before and after menopause in the human hypothalamus.

Glia-to-neurone signalling and the neuroendocrine control of GnRH secretion in the mammalian brain

There is now evidence that astroglial cells and GnRH neurones communicate via specific signalling pathways. These communication pathways operate by release of growth factors acting via serine-threonine kinase receptors, such as transforming growth factor (TGF)- β_1 (61–65), and growth factors signalling through receptors with tyrosine kinase activity including, among others, the epidermal growth factor (EGF)-related peptides, TGF- α and neuregulins (26, 66–68). Signalling through members of both TGF- β and EGF families may account for part of the plastic remodelling that

Fig. 7. Representative micrographs of the structure and the ultrastructure of the median eminence of a woman by light (A) and electron microscopy (B–E), respectively. (A) Toluidin blue-stained semithin section of the median eminence illustrating the anatomical level at which ultrathin sections were collected to perform the electron microscopic analysis. (B) Representative electron micrograph montage of immunogold-labelled axon terminals (black arrowheads) embedded within an astroglia process. (C) Electron micrograph montage of a gonadotrophin-releasing hormone (GnRH) immunoreactive terminal (black arrowhead) in the external zone of the median eminence in close proximity of a fenestrated (white arrow) capillary (Cap). A high magnification of the nerve terminal (black arrowhead) and of the fenestrated endothelium (end.) are shown in (C') and (C''), respectively. (D) Illustration of a GnRH nerve terminal (arrowhead) surrounded by tanycytic processes (tan.). (E) Representative microphotograph showing an evagination of the basal lamina delineating the pericapillary space (asterisk) towards a GnRH nerve terminal (arrowhead). Scale bars = $250 \mu\text{m}$ (A), $1 \mu\text{m}$ (B,C',C'',D,E), $1.5 \mu\text{m}$ (C).



regulates the direct access of GnRH nerve terminals to the vascular wall and presumably modulates the release of GnRH into the portal vasculature during the reproductive cycle (65). In addition, TGF- α and neuregulin signalling pathways were recently shown to be critical for glial cells to engage neuro–glia interactions able to facilitate GnRH secretion both at the time of puberty and during adulthood (26, 68). Interestingly, in keeping with this concept, some human hypothalamic hamartomas associated with sexual precocity were shown to be rich in astroglial cells containing TG-F α (69). Supporting the notion that astroglial erbB signalling may also contribute physiologically to the process by which the neuroendocrine brain controls the function of GnRH neurones in humans are our recent *in vitro* studies showing that human hypothalamic astrocytes express all the functional erbB receptor signalling machinery (70).

Conclusion

Taken together, the present results show that GnRH neurones morphologically interact with astrocytes and tanocytes in the human brain and thus raise the exiting possibility that glial cells play an important role in the neuroendocrine control of GnRH secretion in humans.

Acknowledgements

This research was supported by the Institut National de la Santé et de la Recherche Médicale (Inserm, France) grant U816, the Université de Lille 2, the imaging Core of IFR114, l'Agence National pour la Recherche (ANR, France) and the Fondation pour la Recherche Médicale (Equipe FRM, France). C.A. was PhD student supported by a fellowship from the Centre Hospitalier Régional Universitaire (CHRU) and the Région Nord Pas de Calais.

Received: 1 December 2006,
revised 15 February 2007, 5 April 2007,
accepted 10 May 2007

References

- Ojeda SR, Terasawa E. Neuroendocrine regulation of puberty. In: Pfaff D, Arnold A, Etgen A, Fahrbach S, Moss R, Rubin R, eds. *Hormone, Brains and Behavior*. New York, NY: Elsevier, 2002: 589–659.
- Plant TM, Barker-Gibb ML. Neurobiological mechanisms of puberty in higher primates. *Hum Reprod Update* 2004; **10**: 67–77.
- Sisk CL, Foster DL. The neural basis of puberty and adolescence. *Nat Neurosci* 2004; **7**: 1040–1047.
- Brann DW, Mahesh VB. Excitatory amino acids: evidence for a role in the control of reproduction and anterior pituitary hormone secretion. *Endocr Rev* 1997; **18**: 678–700.
- Dudas B, Merchenthaler I. Three-dimensional representation of the neurotransmitter systems of the human hypothalamus: inputs of the gonadotrophin hormone-releasing hormone neuronal system. *J Neuroendocrinol* 2006; **18**: 79–95.
- Ojeda SR, Prevot V, Heger S. Regulation of puberty. *Curr Opin Endocrinol Diabetes* 2001; **8**: 154–160.
- Simerly RB. Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. *Annu Rev Neurosci* 2002; **25**: 507–536.
- Terasawa E, Fernandez DL. Neurobiological mechanisms of the onset of puberty in primates. *Endocr Rev* 2001; **22**: 111–151.
- Parent AS, Matagne V, Bourguignon JP. Control of puberty by excitatory amino acid neurotransmitters and its clinical implications. *Endocrine* 2005; **28**: 281–286.
- Herbison AE. Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. *Endocr Rev* 1998; **19**: 302–330.
- Herbison AE, Pape JR. New evidence for estrogen receptors in gonadotropin-releasing hormone neurons. *Front Neuroendocrinol* 2001; **22**: 292–308.
- Dhandapani KM, Mahesh VB, Brann DW. Astrocytes and brain function: implications for reproduction. *Exp Biol Med (Maywood)* 2003; **228**: 253–260.
- Garcia-Segura LM, McCarthy MM. Minireview: role of glia in neuroendocrine function. *Endocrinology* 2004; **145**: 1082–1086.
- Melcangi RC, Martini L, Galbiati M. Growth factors and steroid hormones: a complex interplay in the hypothalamic control of reproductive functions. *Prog Neurobiol* 2002; **67**: 421–449.
- Ojeda SR, Prevot V, Heger S, Lomniczi A, Dziedzic B, Mungenast A. Glia-to-neuron signaling and the neuroendocrine control of female puberty. *Ann Med* 2003; **35**: 244–255.
- Prevot V, De Seranno S, Estrella C. Glial–neuronal–endothelial interactions and the neuroendocrine control of GnRH secretion. In: Hertz L, ed. *Non-Neuronal Cells of the Nervous System: Function and Dysfunction*. Amsterdam: Elsevier, 2004: 199–214.
- Durrant AR, Plant TM. A study of the gonadotropin releasing hormone neuronal network in the median eminence of the rhesus monkey (*Macaca mulatta*) using a post-embedding immunolabelling procedure. *J Neuroendocrinol* 1999; **11**: 813–821.
- King JC, Letourneau RJ. Luteinizing hormone-releasing hormone terminals in the median eminence of rats undergo dramatic changes after gonadectomy, as revealed by electron microscopic image analysis. *Endocrinology* 1994; **134**: 1340–1351.
- Kozłowski GP, Coates PW. Ependymoneuronal specializations between LHRH fibers and cells of the cerebroventricular system. *Cell Tissue Res* 1985; **242**: 301–311.
- Perera AD, Plant TM. Ultrastructural studies of neuronal correlates of the pubertal reaugmentation of hypothalamic gonadotropin-releasing hormone (GnRH) release in the rhesus monkey (*Macaca mulatta*). *J Comp Neurol* 1997; **385**: 71–82.
- Prevot V, Croix D, Bouret S, Dutoit S, Tramu G, Stefano GB, Beauvillain JC. Definitive evidence for the existence of morphological plasticity in the external zone of the median eminence during the rat estrous cycle: implication of neuro–glia–endothelial interactions in gonadotropin-releasing hormone release. *Neuroscience* 1999; **94**: 809–819.
- Romero MT, Silverman AJ, Wise PM, Witkin JW. Ultrastructural changes in gonadotropin-releasing hormone neurons as a function of age and ovariectomy in rats. *Neuroscience* 1994; **58**: 217–225.
- Witkin JW, Ferin M, Popilskis SJ, Silverman AJ. Effects of gonadal steroids on the ultrastructure of GnRH neurons in the rhesus monkey: synaptic input and glial apposition. *Endocrinology* 1991; **129**: 1083–1092.
- Mai JK, Assheuer J, Paxinos G. *Atlas of the Human Brain*. San Diego, CA: Academic Press, 1997.
- De Seranno S, Estrella C, Loyens A, Cornea A, Ojeda SR, Beauvillain JC, Prevot V. Vascular endothelial cells promote acute plasticity in ependymoglia cells of the neuroendocrine brain. *J Neurosci* 2004; **24**: 10353–10363.

- 26 Prevot V, Rio C, Cho GJ, Lomniczi A, Heger S, Neville CM, Rosenthal NA, Ojeda SR, Corfas G. Normal female sexual development requires neuregulin-erbB receptor signaling in hypothalamic astrocytes. *J Neurosci* 2003; **23**: 230–239.
- 27 Romijn HJ, van Uum JF, Breedijk I, Emmering J, Radu I, Pool CW. Double immunolabeling of neuropeptides in the human hypothalamus as analyzed by confocal laser scanning fluorescence microscopy. *J Histochem Cytochem* 1999; **47**: 229–236.
- 28 Beauvillain JC, Tramu G, Mazzuca M. Ultrastructural demonstration of nerve endings containing a substance related to growth hormone-releasing factor in the guinea-pig paraventricular nucleus. *Cell Tissue Res* 1987; **248**: 223–226.
- 29 Beauvillain JC, Tramu G. Immunocytochemical demonstration of LH-RH, somatostatin, and ACTH-like peptide in osmium-postfixed, resin-embedded median eminence. *J Histochem Cytochem* 1980; **28**: 1014–1017.
- 30 Barry J. Characterization and topography of LH-RH neurons in the human brain. *Neurosci Lett* 1976; **3**: 287–291.
- 31 Bugnon C, Bloch B, Fellmann D. Cyto-immunological study of the ontogenesis of the gonadotropic hypothalamo-pituitary axis in the human fetus. *J Steroid Biochem* 1977; **8**: 565–575.
- 32 Dudas B, Mihaly A, Merchenthaler I. Topography and associations of luteinizing hormone-releasing hormone and neuropeptide Y-immunoreactive neuronal systems in the human diencephalon. *J Comp Neurol* 2000; **427**: 593–603.
- 33 King JC, Anthony EL, Fitzgerald DM, Stopa EG. Luteinizing hormone-releasing hormone neurons in human preoptic/hypothalamus: differential intraneuronal localization of immunoreactive forms. *J Clin Endocrinol Metab* 1985; **60**: 88–97.
- 34 Najimi M, Chigr F, Jordan D, Leduque P, Bloch B, Tommasi M, Rebaud P, Kopp N. Anatomical distribution of LHRH-immunoreactive neurons in the human infant hypothalamus and extrahypothalamic regions. *Brain Res* 1990; **516**: 280–291.
- 35 Dahl D, Rueger DC, Bignami A, Weber K, Osborn M. Vimentin, the 57 000 molecular weight protein of fibroblast filaments, is the major cytoskeletal component in immature glia. *Eur J Cell Biol* 1981; **24**: 191–196.
- 36 Pixley SK, VJ. Transition between immature radial glia and mature astrocytes studied with a monoclonal antibody to vimentin. *Brain Res* 1984; **317**: 201–209.
- 37 Voigt T. Development of glial cells in the cerebral wall of ferrets: direct tracing of their transformation from radial glia into astrocytes. *J Comp Neurol* 1989; **289**: 74–88.
- 38 Pixley SK, Kobayashi Y, de Vellis J. A monoclonal antibody against vimentin: characterization. *Brain Res* 1984; **317**: 185–199.
- 39 Eliasson C, Sahlgren C, Berthold CH, Stakeberg J, Celis JE, Betsholtz C, Eriksson JE, Pekny M. Intermediate filament protein partnership in astrocytes. *J Biol Chem* 1999; **274**: 23996–24006.
- 40 Hockfield S, McKay RD. Identification of major cell classes in the developing mammalian nervous system. *J Neurosci* 1985; **5**: 3310–3328.
- 41 Meister B, Hokfelt T, Tsuruo Y, Hemmings H, Ouimet C, Greengard P, Goldstein M. DARPP-32, a dopamine- and cyclic AMP-regulated phosphoprotein in tanycytes of the mediobasal hypothalamus: distribution and relation to dopamine and luteinizing hormone-releasing hormone neurons and other glial elements. *Neuroscience* 1988; **27**: 607–622.
- 42 Prevot V, Dutoit S, Croix D, Tramu G, Beauvillain JC. Semi-quantitative ultrastructural analysis of the localization and neuropeptide content of gonadotropin releasing hormone nerve terminals in the median eminence throughout the estrous cycle of the rat. *Neuroscience* 1998; **84**: 177–191.
- 43 Donahue JE, Stopa EG, Chorsky RL, King JC, Schipper HM, Tobet SA, Blaustein JD, Reichlin S. Cells containing immunoreactive estrogen receptor-alpha in the human basal forebrain. *Brain Res* 2000; **856**: 142–151.
- 44 Kruijver FP, Balesar R, Espila AM, Unmehopa UA, Swaab DF. Estrogen receptor-alpha distribution in the human hypothalamus in relation to sex and endocrine status. *J Comp Neurol* 2002; **454**: 115–139.
- 45 Kruijver FP, Balesar R, Espila AM, Unmehopa UA, Swaab DF. Estrogen-receptor-beta distribution in the human hypothalamus: similarities and differences with ER alpha distribution. *J Comp Neurol* 2003; **466**: 251–277.
- 46 Haydon PGGLIA. Listening and talking to the synapse. *Nat Rev Neurosci* 2001; **2**: 185–193.
- 47 Rodriguez EM, Blazquez JL, Pastor FE, Pelaez B, Pena P, Peruzzo B, Amat P. Hypothalamic tanycytes: a key component of brain-endocrine interaction. *Int Rev Cytol* 2005; **247**: 89–164.
- 48 Prevot V. Glial-neuronal-endothelial interactions are involved in the control of GnRH secretion. *J Neuroendocrinol* 2002; **14**: 247–255.
- 49 Silverman RC, Gibson MJ, Silverman AJ. Relationship of glia to GnRH axonal outgrowth from third ventricular grafts in hpg hosts. *Exp Neurol* 1991; **114**: 259–274.
- 50 Chouaf L, Didier-Bazes M, Aguera M, Tardy M, Sallanon M, Kitahama K, Belin MF. Comparative marker analysis of the ependymocytes of the subcommissural organ in four different mammalian species. *Cell Tissue Res* 1989; **257**: 255–262.
- 51 Xu Y, Tamamaki N, Noda T, Kimura K, Itokazu Y, Matsumoto N, Dezawa M, Ide C. Neurogenesis in the ependymal layer of the adult rat 3rd ventricle. *Exp Neurol* 2005; **192**: 251–264.
- 52 Hatton GI, Perlmutter LS, Salm AK, Tweedle CD. Dynamic neuronal-glial interactions in hypothalamus and pituitary: implications for control of hormone synthesis and release. *Peptides* 1984; **5** (Suppl. 1): 121–138.
- 53 Cashion AB, Smith MJ, Wise PM. The morphometry of astrocytes in the rostral preoptic area exhibits a diurnal rhythm on proestrus: relationship to the luteinizing hormone surge and effects of age. *Endocrinology* 2003; **144**: 274–280.
- 54 Lehman MN, Karsch FJ, Robinson JE, Silverman AJ. Ultrastructure and synaptic organization of luteinizing hormone-releasing hormone (LHRH) neurons in the anestrous ewe. *J Comp Neurol* 1988; **273**: 447–458.
- 55 Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. *Glia* 2005; **50**: 427–434.
- 56 Hatton GI. Dynamic neuronal-glial interactions: an overview 20 years later. *Peptides* 2004; **25**: 403–411.
- 57 Oliet SH, Piet R, Poulain DA, Theodosis DT. Glial modulation of synaptic transmission: insights from the supraoptic nucleus of the hypothalamus. *Glia* 2004; **47**: 258–267.
- 58 Herrmann H, Aebi U. Intermediate filaments and their associates: multi-talented structural elements specifying cytoarchitecture and cytodynamics. *Curr Opin Cell Biol* 2000; **12**: 79–90.
- 59 Dudas B, Merchenthaler I. Close anatomical associations between beta-endorphin and luteinizing hormone-releasing hormone neuronal systems in the human diencephalon. *Neuroscience* 2004; **124**: 221–229.
- 60 Rance NE, McMullen NT, Smialek JE, Price DL, Young WS III. Postmenopausal hypertrophy of neurons expressing the estrogen receptor gene in the human hypothalamus. *J Clin Endocrinol Metab* 1990; **71**: 79–85.
- 61 Bouret S, De Seranno S, Beauvillain JC, Prevot V. Transforming growth factor beta1 may directly influence gonadotropin-releasing hormone gene expression in the rat hypothalamus. *Endocrinology* 2004; **145**: 1794–1801.
- 62 Buchanan CD, Mahesh VB, Brann DW. Estrogen-astrocyte-luteinizing hormone-releasing hormone signaling: a role for transforming growth factor-beta(1). *Biol Reprod* 2000; **62**: 1710–1721.
- 63 Melcangi RC, Galbiati M, Messi E, Piva F, Martini L, Motta M. Type 1 astrocytes influence luteinizing hormone-releasing hormone release from the hypothalamic cell line GT1-1: is transforming growth factor-beta the principle involved? *Endocrinology* 1995; **136**: 679–686.

- 64 Prevot V, Bouret S, Croix D, Takumi T, Jennes L, Mitchell V, Beauvillain JC. Evidence that members of the TGFbeta superfamily play a role in regulation of the GnRH neuroendocrine axis: expression of a type I serine-threonine kinase receptor for TGFbeta and activin in GnRH neurones and hypothalamic areas of the female rat. *J Neuroendocrinol* 2000; **12**: 665–670.
- 65 Prevot V, Cornea A, Mungenast A, Smiley G, Ojeda SR. Activation of erbB-1 signaling in tanycytes of the median eminence stimulates transforming growth factor beta1 release via prostaglandin E2 production and induces cell plasticity. *J Neurosci* 2003; **23**: 10622–10632.
- 66 Ma YJ, Hill DF, Creswick KE, Costa ME, Cornea A, Lioubin MN, Plowman GD, Ojeda SR. Neuregulins signaling via a glial erbB-2-erbB-4 receptor complex contribute to the neuroendocrine control of mammalian sexual development. *J Neurosci* 1999; **19**: 9913–9927.
- 67 Ojeda SR, Urbanski HF, Costa ME, Hill DF, Moholt-Siebert M. Involvement of transforming growth factor alpha in the release of luteinizing hormone-releasing hormone from the developing female hypothalamus. *Proc Natl Acad Sci USA* 1990; **87**: 9698–9702.
- 68 Prevot V, Lomniczi A, Corfas G, Ojeda SR. erbB-1 and erbB-4 receptors act in concert to facilitate female sexual development and mature reproductive function. *Endocrinology* 2005; **146**: 1465–1472.
- 69 Jung H, Carmel P, Schwartz MS, Witkin JW, Bentele KH, Westphal M, Piatt JH, Costa ME, Cornea A, Ma YJ, Ojeda SR. Some hypothalamic hamartomas contain transforming growth factor alpha, a puberty-inducing growth factor, but not luteinizing hormone-releasing hormone neurons. *J Clin Endocrinol Metab* 1999; **84**: 4695–4701.
- 70 Sharif A, Duhem-Tonnelle V, Allet C, Baroncini M, Loyens A, Kerr-Conte J, Blond S, Ojeda SR, Junier MP, Prevot V. Characterization of ligand-activated erbB signalling pathways in human astrocytes. *Soc Neurosci* 2006; **733**: 11/L1.
- 71 Debus E, Weber K, Osborn M. Monoclonal antibodies specific for glial fibrillary acidic (GFA) protein and for each of the neurofilament triplet polypeptides. *Differentiation* 1983; **25**: 193–203.
- 72 Hansen SH, Stagaard M, Mollgard K. Neurofilament-like pattern of reactivity in human foetal PNS and spinal cord following immunostaining with polyclonal anti-glial fibrillary acidic protein antibodies. *J Neurocytol* 1989; **18**: 427–436.
- 73 Osborn M, Debus E, Weber K. Monoclonal antibodies specific for vimentin. *Eur J Cell Biol* 1984; **34**: 137–143.