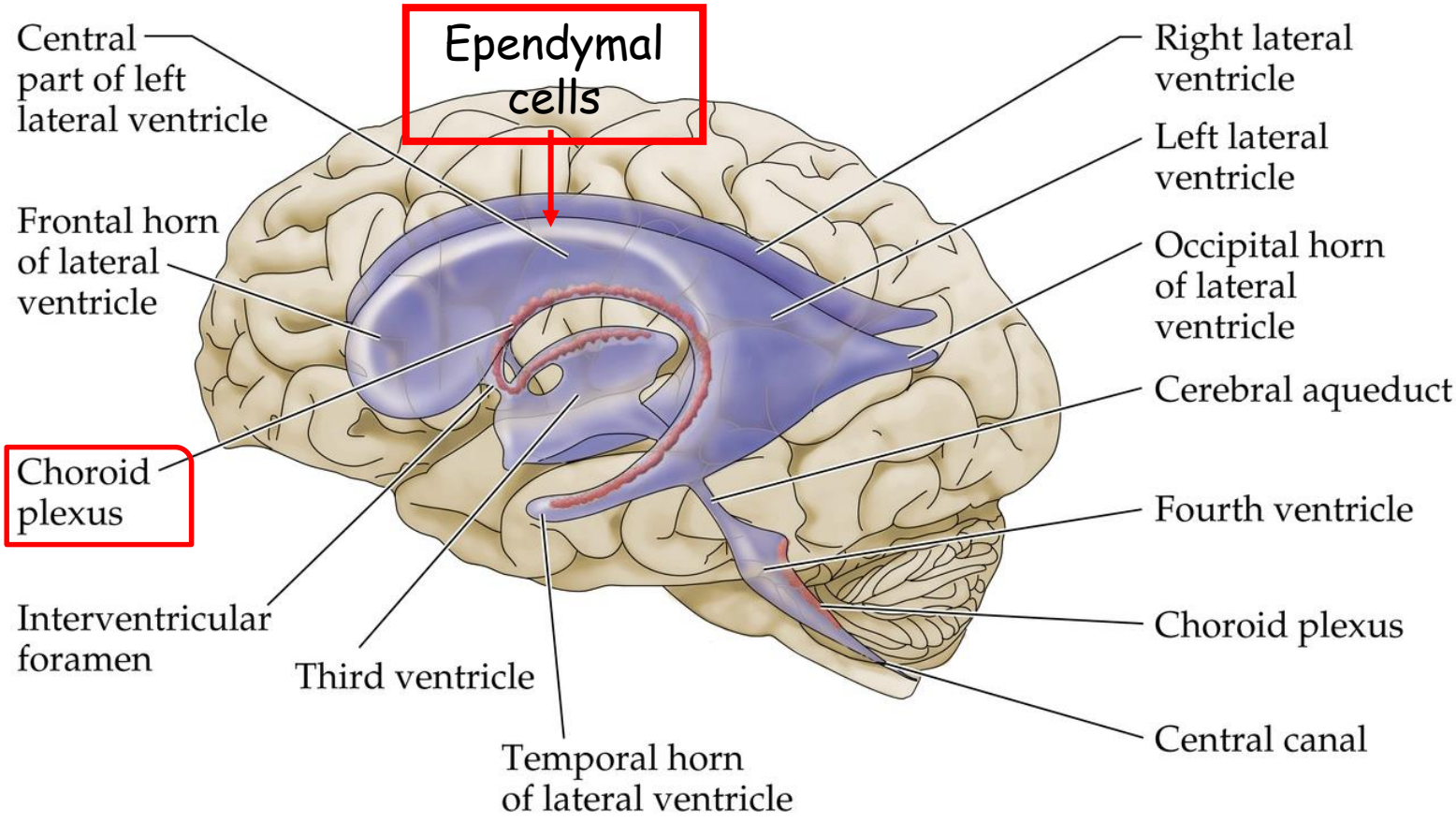
Cells that are mostly neglected in the study of the CNS:

Ependymal cells /Tanycytes – lining of ventricular system

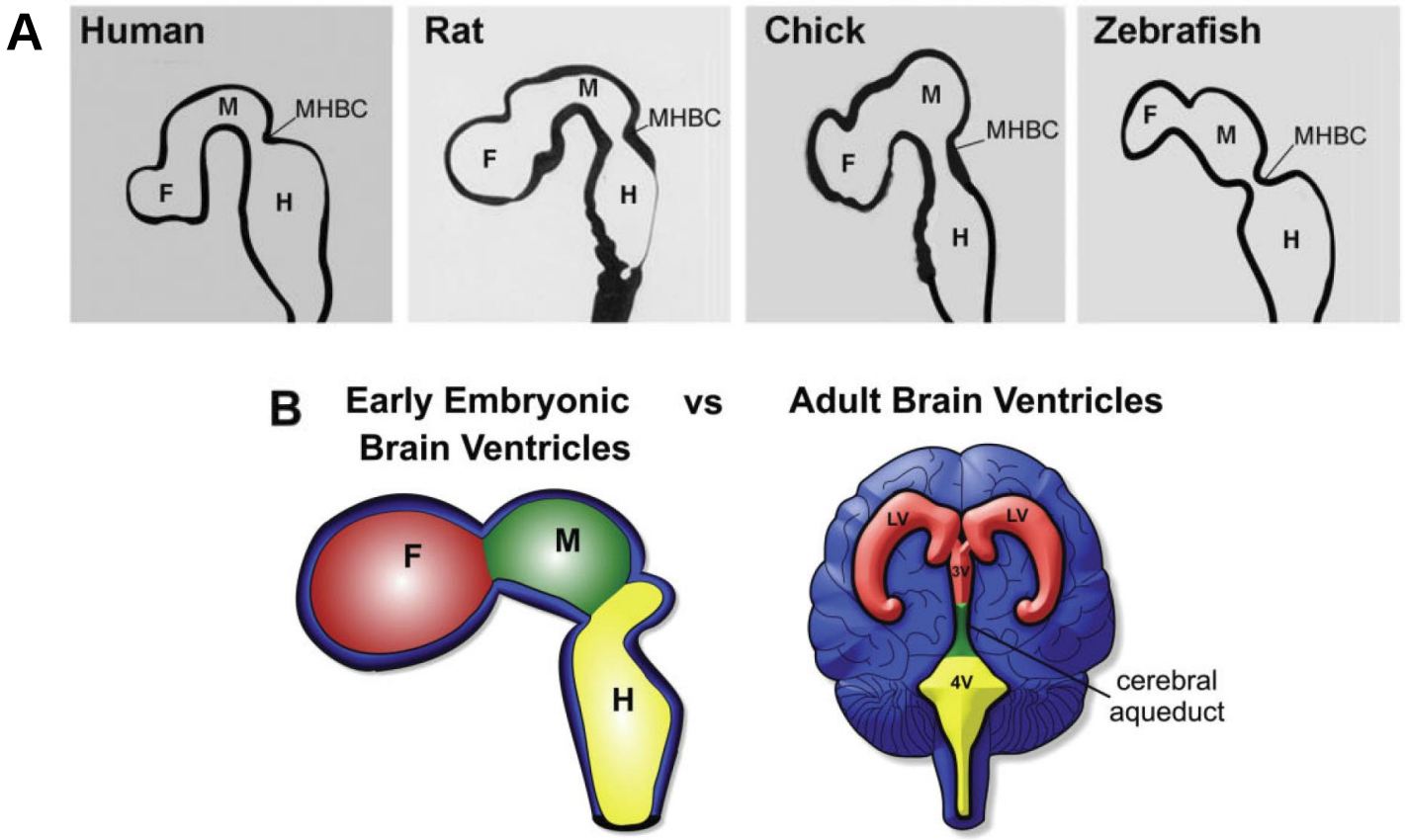
Choroid plexus cells – secretion of cerebrospinal fluid

Endothelial cells - Blood Brain Barrier (BBB)

The ventricular system



Evolutionary conservation of embryonic brain ventricle structure



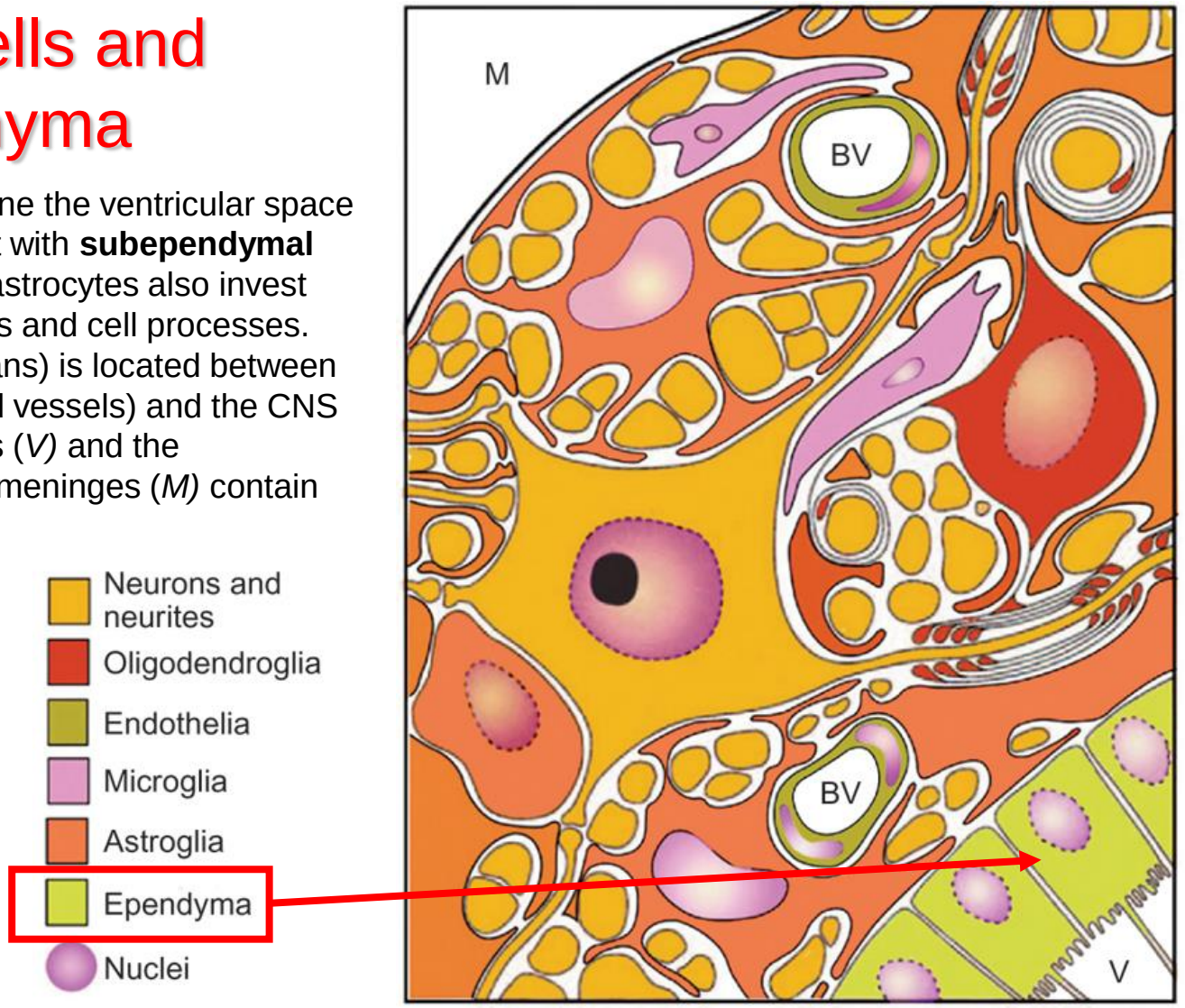
A: Conservation of embryonic brain ventricle structure. Tracings of embryonic brain ventricles at similar corresponding stages in development, all lateral views.

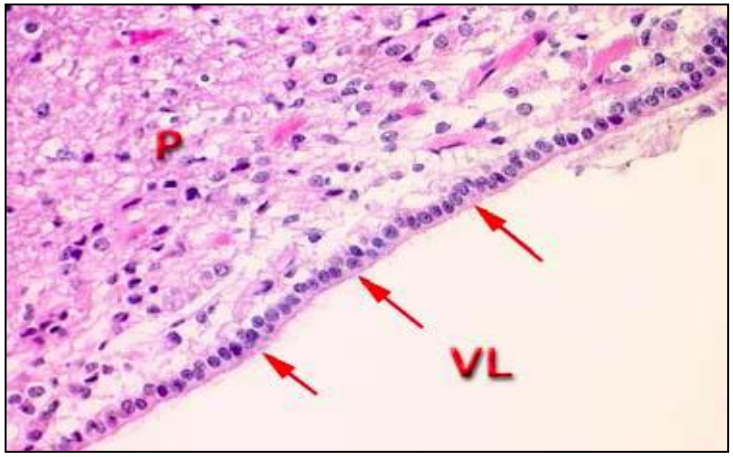
B: Comparison of early embryonic and adult brain ventricles. Colors correspond to the same ventricle regions in the embryo and adult. Not to scale. F, forebrain (telencephalon plus diencephalon); M, midbrain (mesencephalon); H, hindbrain (rhombencephalon); MHBC, midbrain hindbrain boundary constriction

Lowery & Sive, BioEssays 31:446–458, 2009

Relations between ependymal cells and brain parenchyma

Ciliated **ependymal cells** line the ventricular space (*V)* and are in close contact with **subependymal astrocytes**. Note how the astrocytes also invest blood vessels (*BV)*, neurons and cell processes. The pia-astroglia (glia limitans) is located between the exterior (dura and blood vessels) and the CNS parenchyma. The ventricles (*V)* and the subarachnoid space of the meninges (*M)* contain cerebrospinal fluid.





The ventricles of the brain and the central canal of the spinal cord are lined with ependymal cells. These cells are often **ciliated** and form a simple cuboidal or low columnar epithelium. The **lack of tight junctions** between ependymal cells allows a free exchange between cerebrospinal fluid and nervous tissue.

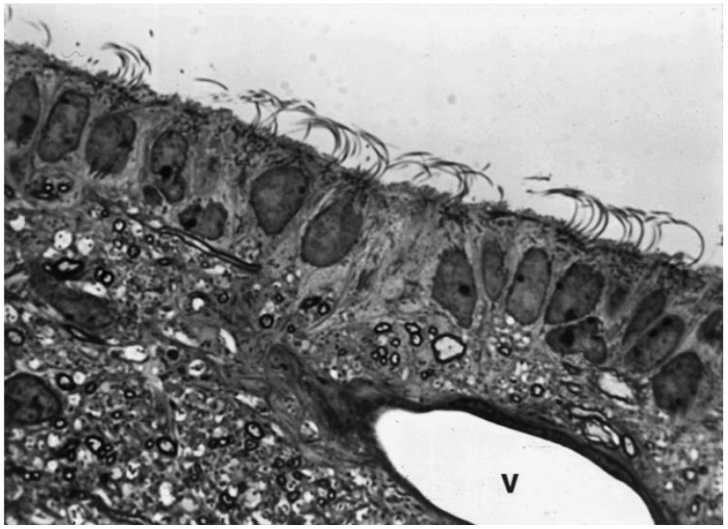
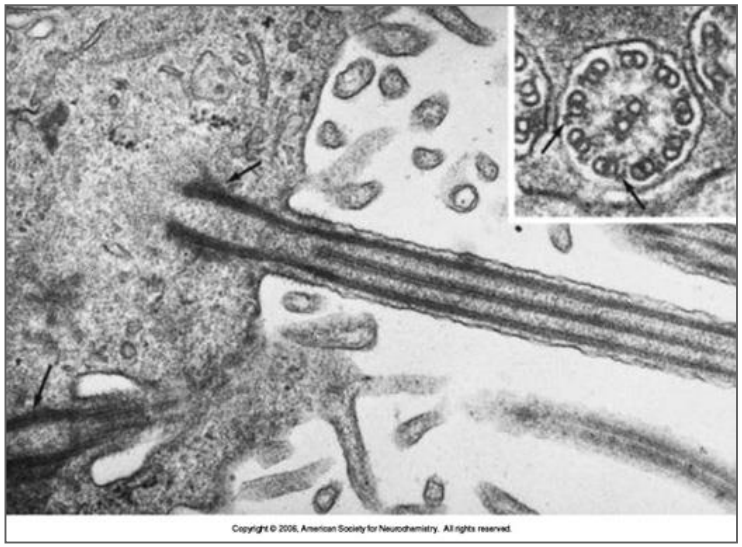
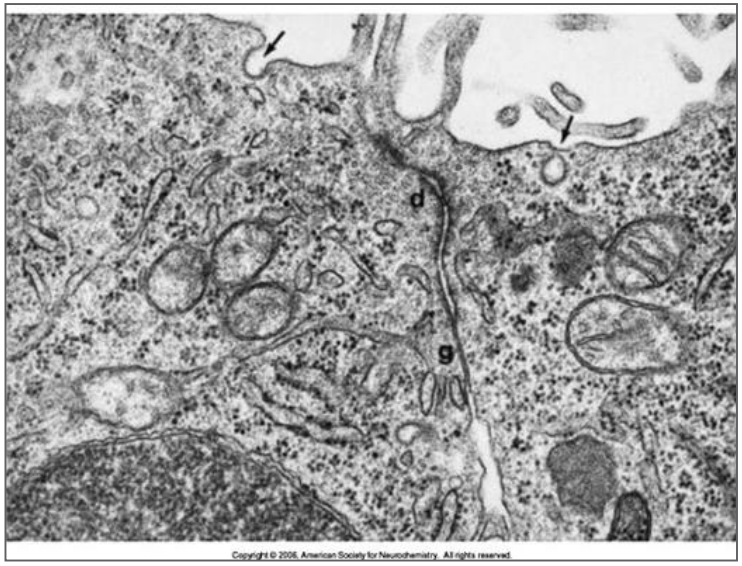


Fig. VIII.1 Ependymal cells. The ependymal cells in this light micrograph are columnar with oval nuclei and contain cilia emanating from basal bodies. Above the nuclei, there are numerous mitochondria. **v** blood vessel. Central canal of the rabbit spinal cord, 1-µm section stained with toluidine blue, ×1,400

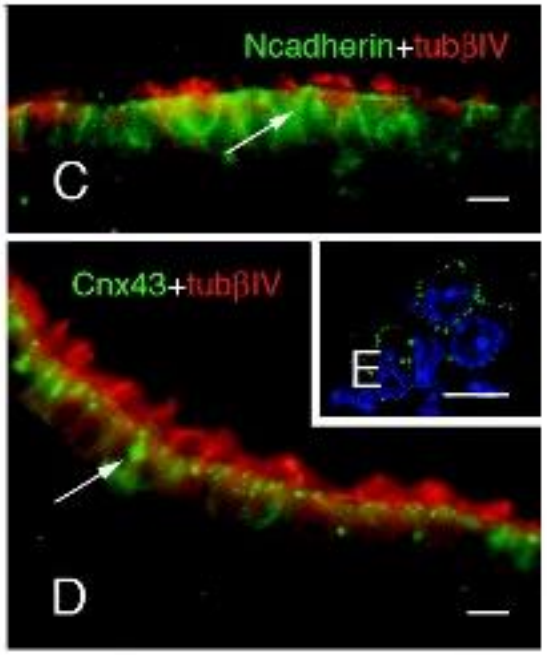
Fig. VIII.3 Ependymal cells. The luminal free surface of the ependyma is shown in this scanning electron micrograph. A tuft of cilia projects from the surface of each ependymal cell, which is otherwise covered with microvilli. Note, however, that circumscribed areas (*arrows*) are denuded of microvilli. Lateral recess of the rat fourth cerebral ventricle, ×5,200 (Courtesy of J.E. Bruni)





Top panel: The surface of an ependymal cell. Surface contains basal bodies (*arrows*) connected to the microtubules of cilia, seen here in longitudinal section. Several microvilli are also present. **Inset:** Ependymal cilia in transverse section possess a central doublet of microtubules surrounded by nine pairs, one of each pair having a characteristic hook-like appendage (*arrows*).

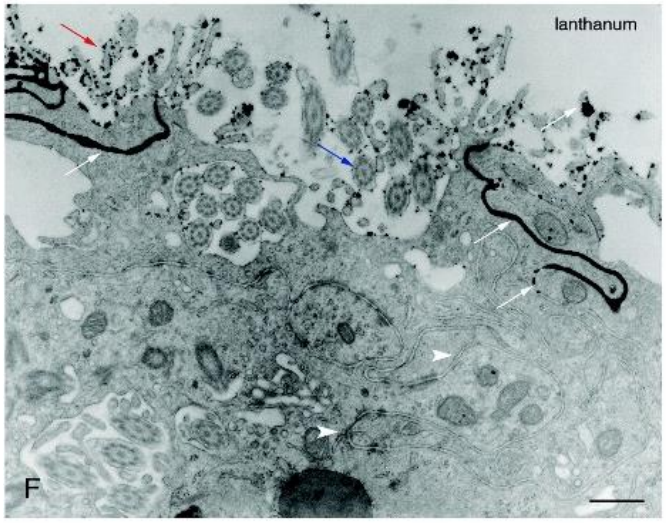
Bottom panel: A typical **desmosome (*d*)** and **gap junction (*g*)** between two ependymal cells. Microvilli and coated pits (*arrows*) are seen along the cell surface.

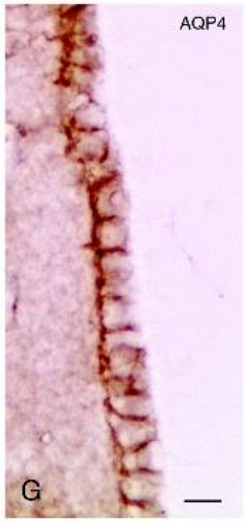


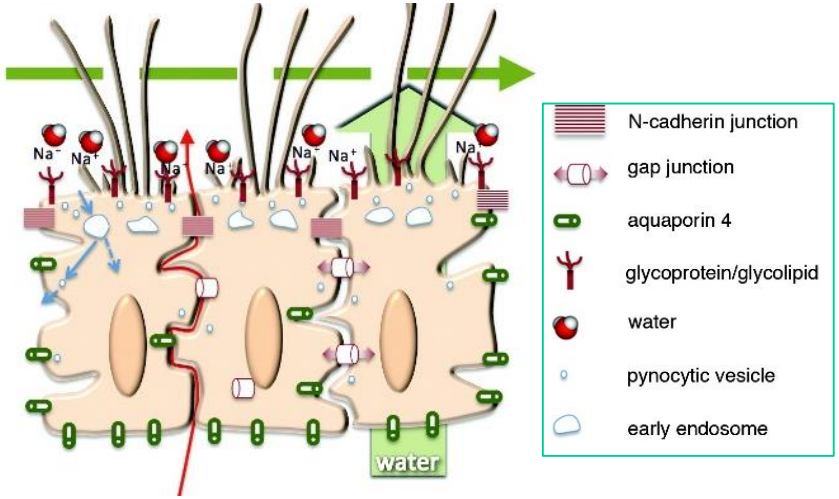
Molecular features of the multiciliated ependyma in the ventricle of the mouse.

Ependymal cells (**C**) express N-cadherin-containing junctions (in green, arrows) in their lateral plasma membrane domains. Tubulin βIV (tubβIV, in red) labels cilia in **C** and **D**. **(D and E).** Multiciliated ependymal cells are joined with connexin43-containing (Cnx43) gap junctions (in green, arrow). Gap junctions in ependymal cells are involved in electrical and metabolic couplings integrating the functioning of the cell layer. Gap junctions play a role in the synchronization of cilia beating and in CSF circulation.

(F) Multiciliated ependymal cells lack tight junctions, as shown with lanthanum nitrate applied to the ventricle and observed under transmission electron microscopy. The tracer (with black electrodensity, white arrows) is passing through the lateral winding extracellular spaces (white arrowheads), proving the absence of functional tight junctions. Motile cilia (blue arrow) and microvilli (yellow arrow) are appreciated in the luminal pole of ependymocytes. **(G)** Aquaporin 4 (AQP4) is present in the laterobasal domain of multiciliated ependyma.







Modified from Jiménez et al.,*Tissue Barriers* **2014,** 2, DOI: 10.4161/tisb.28426

Live imaging of the ependymal cilia in the lateral ventricles of the mouse brain

http://www.jove.com/video/52853/live-imaging-ependymal-cilia-lateral-ventricles-mouse

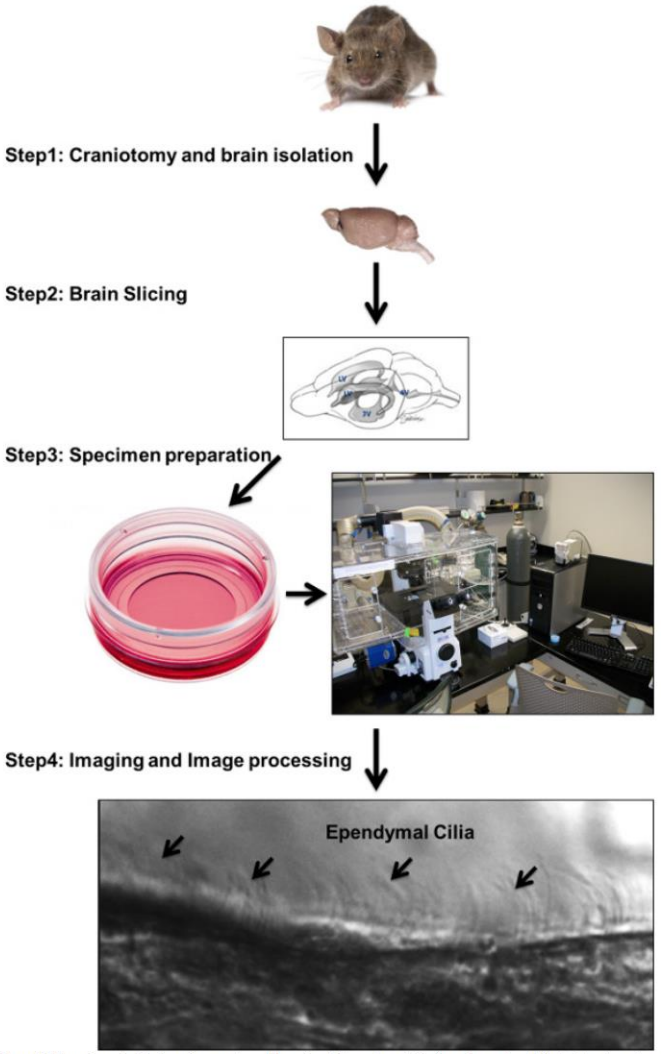


Figure 1: Ependymal cilia imaging protocol flowchart. Ependymal cilia imaging protocol illustrates steps to complete an experiment starting from mouse brainextraction, sectioning and tissue preparation to image acquisition and analysis. An approximate one hour timeline is presented with step-by-step procedure.

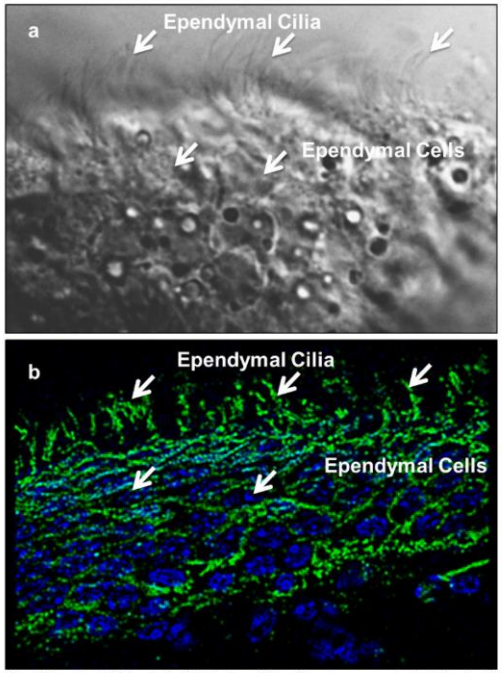


Figure 2: Ependymal cilia localization in the brain ventricles. Shown here are ependymal cells from the lateral ventricle of a mouse brain. (**a**) DIC images of individual ependymal cells (bottom arrows) and cilia (top arrows) are shown. (**b**) An overlay image of a brain section is stained with antibody against a ciliary marker, acetylated a-tubulin, shown in green (top arrows), and counterstained with a nuclear/DNA marker, DAPI, shown in blue (bottom arrows). Please note that panels a and b represent different brain sections.

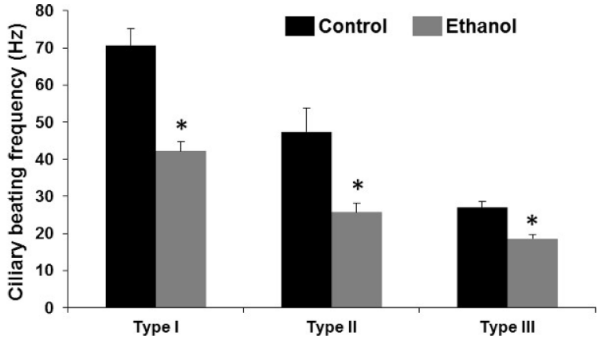


Figure 3: Alcohol and differences in cilia beating frequencies among types of ependymal cells of the mouse brain lateral ventricle. The *ex vivo* brain slice was incubated without (Control) or with (Ethanol) 0.25 % alcohol for 5 min. Compared to control, alcohol treatment significantly decreased cilia beating frequency, as indicated by an asterisk. At least 5-10 independent preparations were used for each ependymal cell type and treatment group.

TANYCYTES

Ependymal cells can specialize into tanycytes, which are rarely ciliated and have long basal processes. Tanycytes form the ventricular lining over the few CNS regions in which the blood-brain barrier is incomplete. They do form **tight junctions** and control the exchange of substances between these regions and surrounding nervous tissue or cerebrospinal fluid.

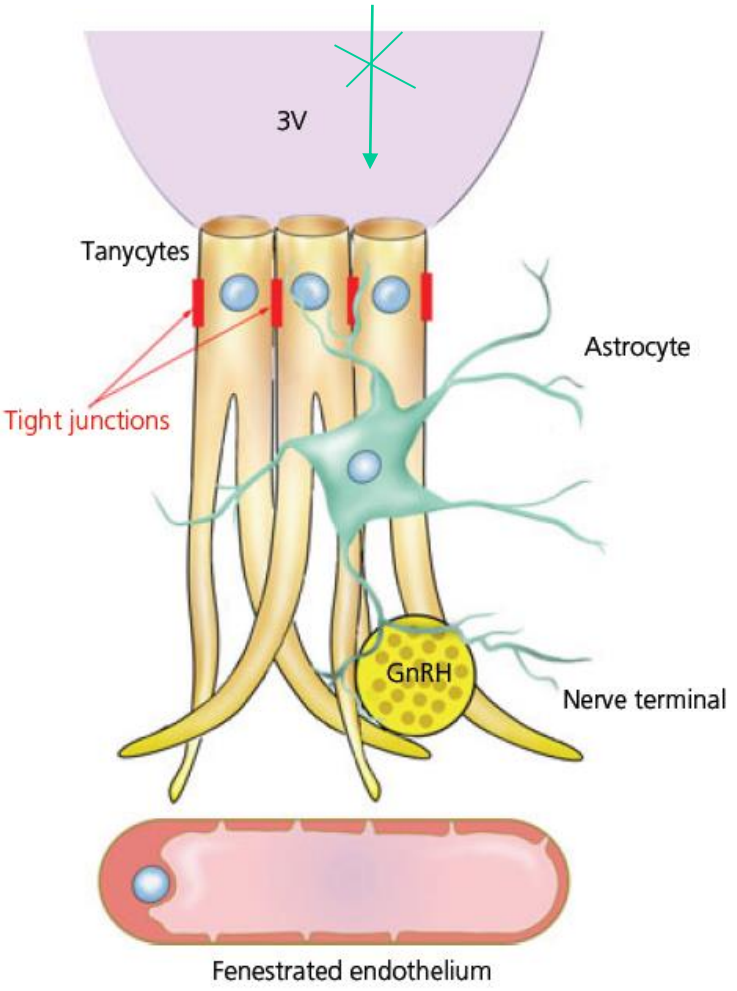
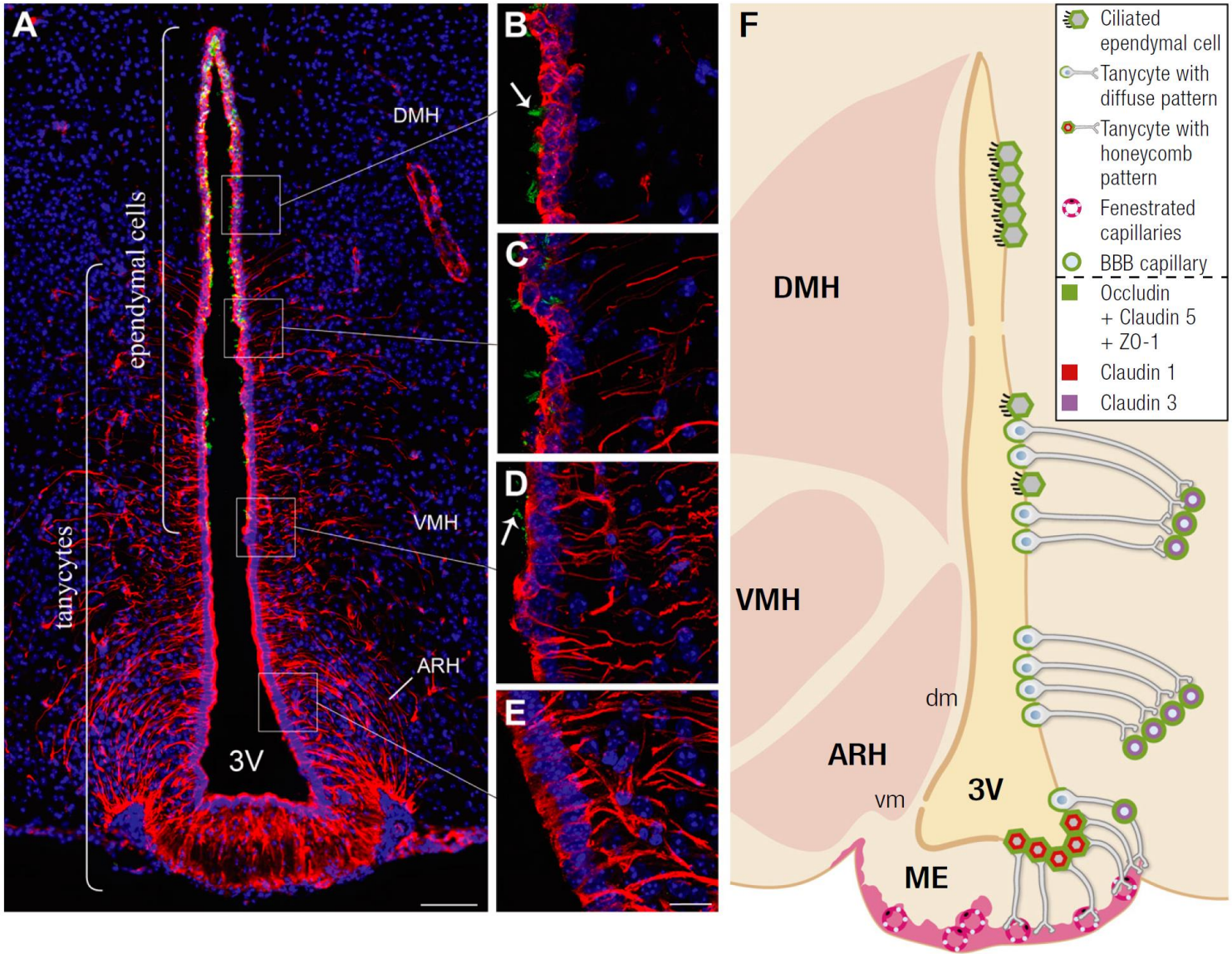


Fig. 1. Schematic representation of the cell types (tanycytes, astrocytes and endothelial cells) and neuronal elements (neuroendocrine terminals) that reside within the median eminence of the hypothalamus. The median eminence of the hypothalamus is the brain structure forming the floor of the third ventricle (3V). The median eminence, which is one of the circumventricular organs of the brain, is capable of conveying information from the brain to the periphery via the release of neurohormones into the circulation and, conversely, sensing information reaching the brain via the bloodstream.

Prevot *et al.* 2010
Journal of Neuroendocrinology **22**, 639–649

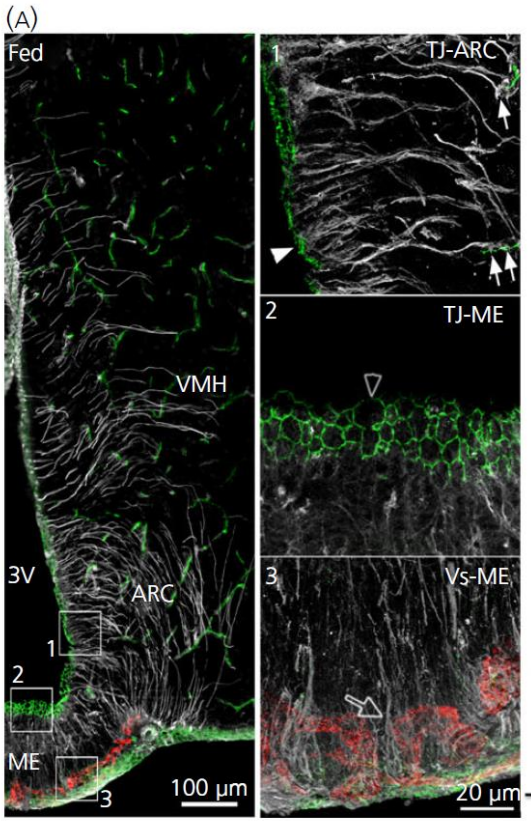
Different tanycyte populations in the hypotalamus / median eminence

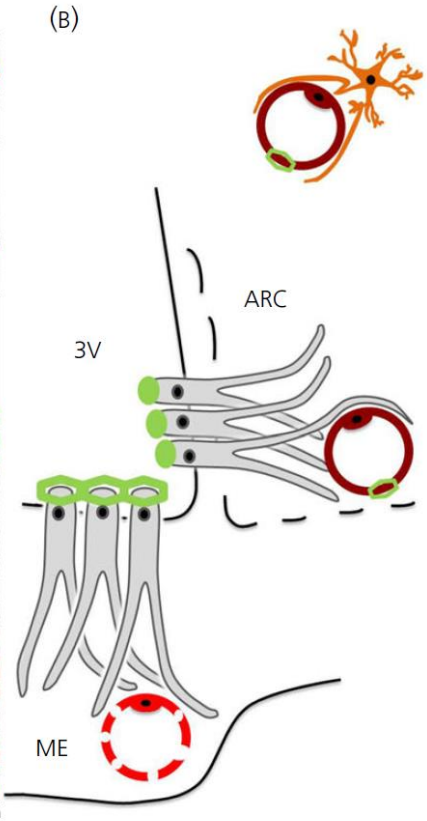
Figure 2. Localization of tanycytes and ependymocytes with beating cilia in the tuberal region of the hypothalamus. (A) Low-magnification photomontage of glu-tubulin (green) and vimentin (red) immunofluorescence. (B–D) High-magnification images showing glu-tubulin immunoreactive cilia (green, arrows) on the ventricular surface at the level of the (B, C) dorsomedial nucleus of the hypothalamus (DMH) and (D) ventromedial nucleus of the hypothalamus (VMH). Note that glu-tubulin immunoreactivity is absent in vimentin-labeled tanycytes of the (E) ARH and (A) ME. Sections are counterstained using Hoechst (blue) to visualize cell nuclei and identify the morphological limits of each hypothalamic structure. Scale bars: (A) 100 μm; (B–E) 20 μm. (F) Representative drawing summarizing the distribution of tight junction proteins in the tuberal region of the hypothalamus (40). 3V, third ventricle; dm, dorsomedial; vm, ventromedial. Adapted with permission from Mullier *et al.* (40).

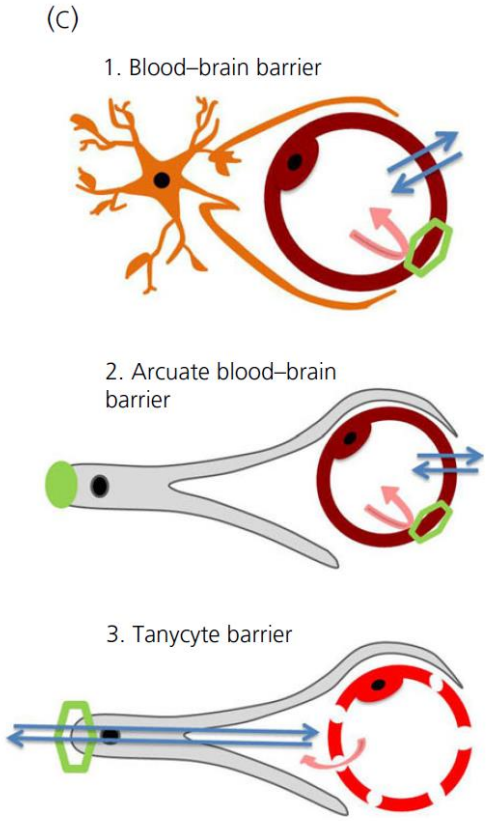


Prevot et al., 2018
doi: 10.1210/er.2017-00235

The organization of the blood-hypothalamus (Arcuate Nucleus, Median Eminence) interface







Blood-Brain barrier

Blood-CSF barrier

Fig. 1. Organisation of the blood–arcuate nucleus (ARC) interface in the mediobasal hypothalamus. (A) Vimentin (white), zonula occludens-1 (ZO-1; green) and MECA-32 (red) immunoreactivity in coronal sections of the hypothalamic tuberal region in fed animals. Tanycytes exhibit a diffuse pattern of tight junction complexes (arrowheads; inset 1) when interacting with ZO-1-positive blood–brain barrier vessels (arrows; inset 1), whereas they display a honeycomb pattern (empty arrowheads; inset 2) when interacting with MECA-32-positive fenestrated vessels (empty arrows; inset 3). (B) Schematic representation of the hypothalamic tuberal region. (C) Schematic representation of different blood–brain interfaces present in the hypothalamic tuberal region including the blood–brain barrier (1), the blood–ARC barrier (2) and the tanycyte barrier (3). Barrier properties are carried by either endothelial cells (1, 2) or tanycytes (3) to maintain brain homeostasis. Paracellular diffusion cannot take place across these barriers, in contrast to fenestrated vessels (pink arrows); consequently, metabolic signals can only enter the brain by specific transcellular transport (blue arrows). Reprinted with permission from Langlet *et al.* (4). 3V, third ventricle; ME, median eminence; TJ, tight junction; VMH, ventromedial hypothalamus; Vs, vessels.

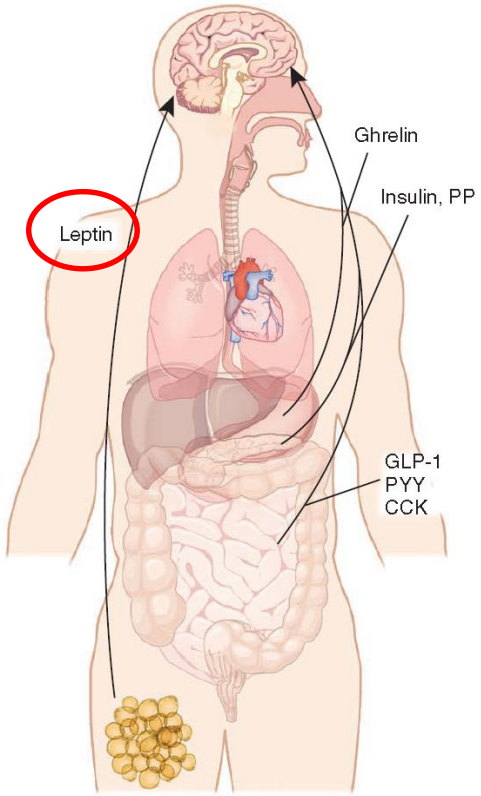
Langlet et al., 2014
doi: 10.1111/jne.12191

Tanycytes: A Gateway to the Metabolic Hypothalamus

F. Langlet*†‡

Journal of Neuroendocrinology, 2014, **26**, 753–760

The central regulation of energy balance relies on the ability of the brain to promptly and efficiently sense variations of metabolic state. To achieve this, circulating hormonal and metabolic signals have to cross the blood–brain interface, where unusual glial cells named tanycytes have been described to play a key role in this process. Tanycytes are specialised polarised ependymoglial cells that line the floor of the third ventricle and send a single process to contact hypothalamic neurones and blood vessels. Although their role in the regulation of energy balance via the modulation of neuronal activity or their chemosensitivity has been already described, recent studies ascribe a new function to tanycytes in the regulation of energy homeostasis as a result of their capacity to regulate the access of metabolic signals to the hypothalamus. This review discusses the peculiar place of tanycytes within the blood–hypothalamus interface, as well as a striking capacity to remodel their own interface to ensure an adaptive metabolic response to energy imbalances.



Central regulation of food intake and energy expenditure. Multiple peripheral factors have been shown to modify food intake and energy expenditure through direct effects on the CNS

Tanycytes

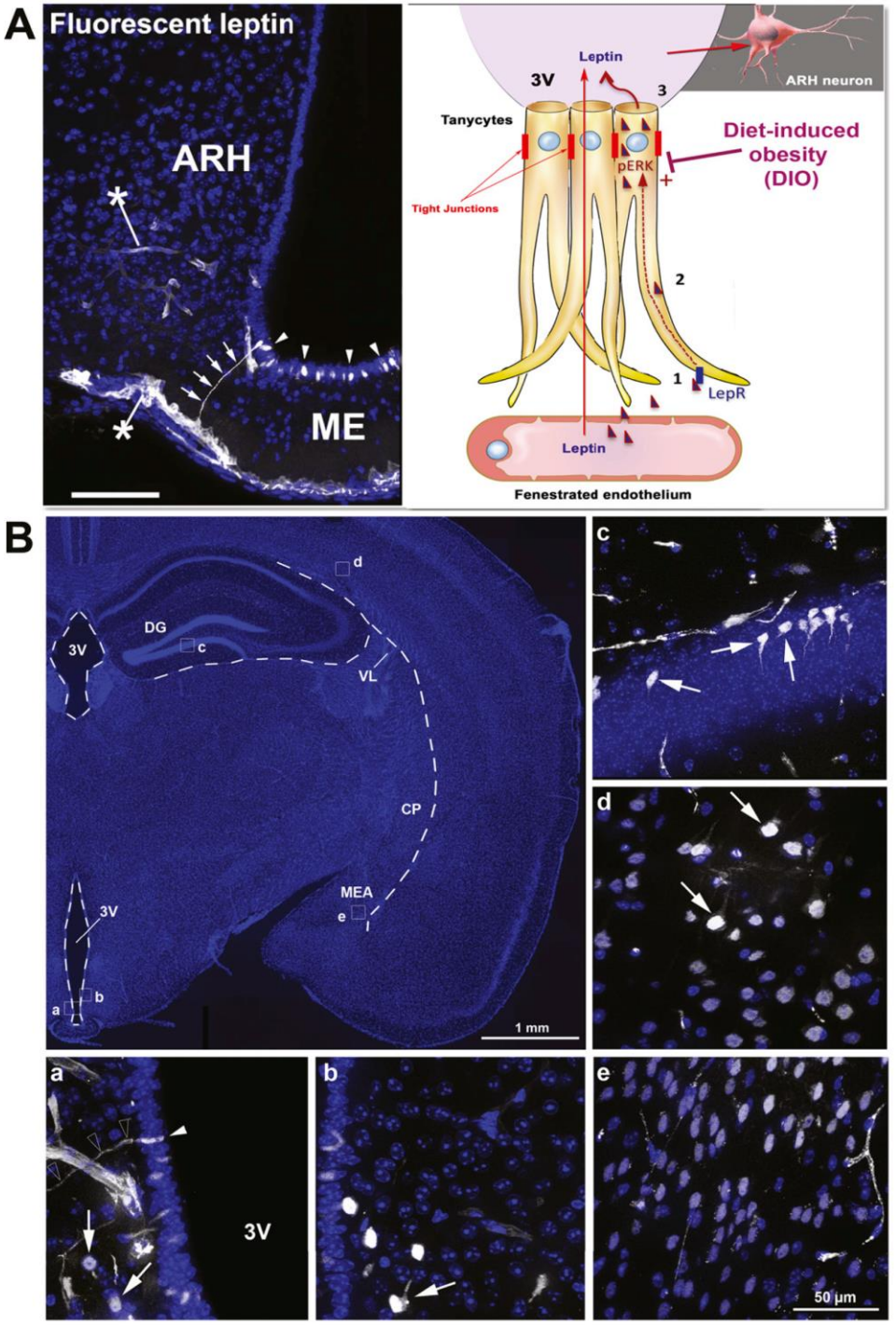
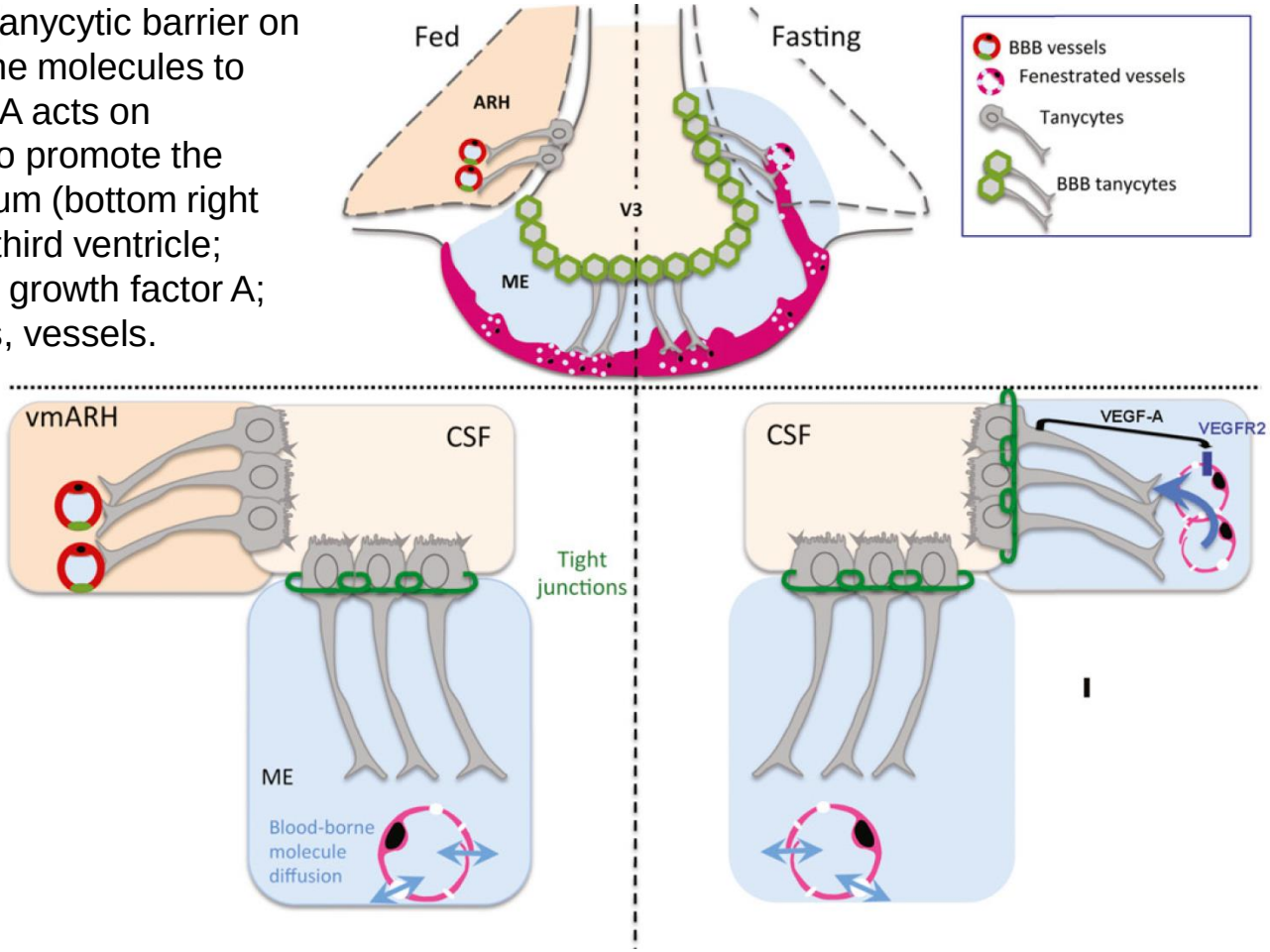


Figure 5. Leptin transported from the periphery into the CSF via the ME reaches target areas both in the hypothalamus and in nonhypothalamic areas bordering the ventricles [adapted with permission from Balland *et al.* (97)]. (A) Left: Representative photomicrograph showing tanycytic processes (arrows) and cell bodies (arrowheads) labeled by fluorescent leptin (25 nmoles per animal; white labeling). Fluorescent bioactive leptin was injected intravenously in wild-type mice. (Asterisks show the BBB vessels of the ARH.) Right: Schematic illustrating the passage of leptin from fenestrated pituitary portal blood vessels to the CSF of the third ventricle (3V) and LepR expressing hypothalamic neurons. (B) Ten minutes later, fluorescent leptin (white labeling) was found not only in downstream hypothalamic neurons (arrows in a and b), but also in other leptin target regions contacting the CSF, such as the (c) hippocampus, (d) cerebral cortex, and (e) medial nucleus of the amygdala (MEA), suggesting that leptin release by tanycytes [arrowheads in (a)] mediates its access to both metabolic and cognitive brain circuits. Similar results were obtained when fluorescent leptin was injected directly into the CSF (data not shown). Dotted lines in the low-magnification view delineate the ventricles. In all panels, cell nuclei are counterstained with Hoechst. Scale bar in (e) indicates the scale for (a) to (e). 3V, third ventricle; CP, caudate-putamen; DG, dentate gyrus; VL, lateral ventricle. Reproduced from Balland *et al.* (97).

Prevot et al., 2018
doi: 10.1210/er.2017-00235

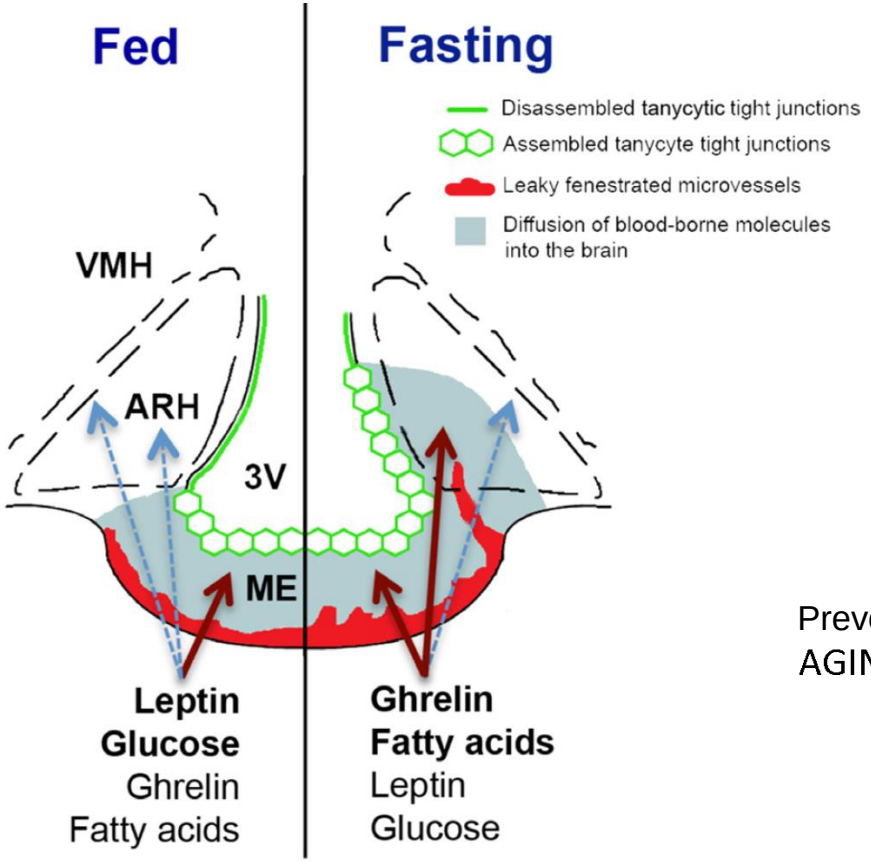
Blood-Arcuate Nucleus interface plasticity

Fasting-induced fenestration of ME microvessel loops reaching the ventromedial ARH (vmARH), and tight junction complex reorganization in ARH tanycytes. Representative drawing summarizing the functional consequences of the structural changes in the tanycytic barrier on the direct access of bloodborne molecules to the vmARH. Tanycytic VEGF-A acts on VEGFR2 in endothelial cells to promote the fenestration of their endothelium (bottom right panel). TJ, tight junction; V3, third ventricle; VEGF-A, vascular endothelial growth factor A; VEGFR, VEGF receptor 2; Vs, vessels.



Prevot et al., 2018
doi: 10.1210/er.2017-00235

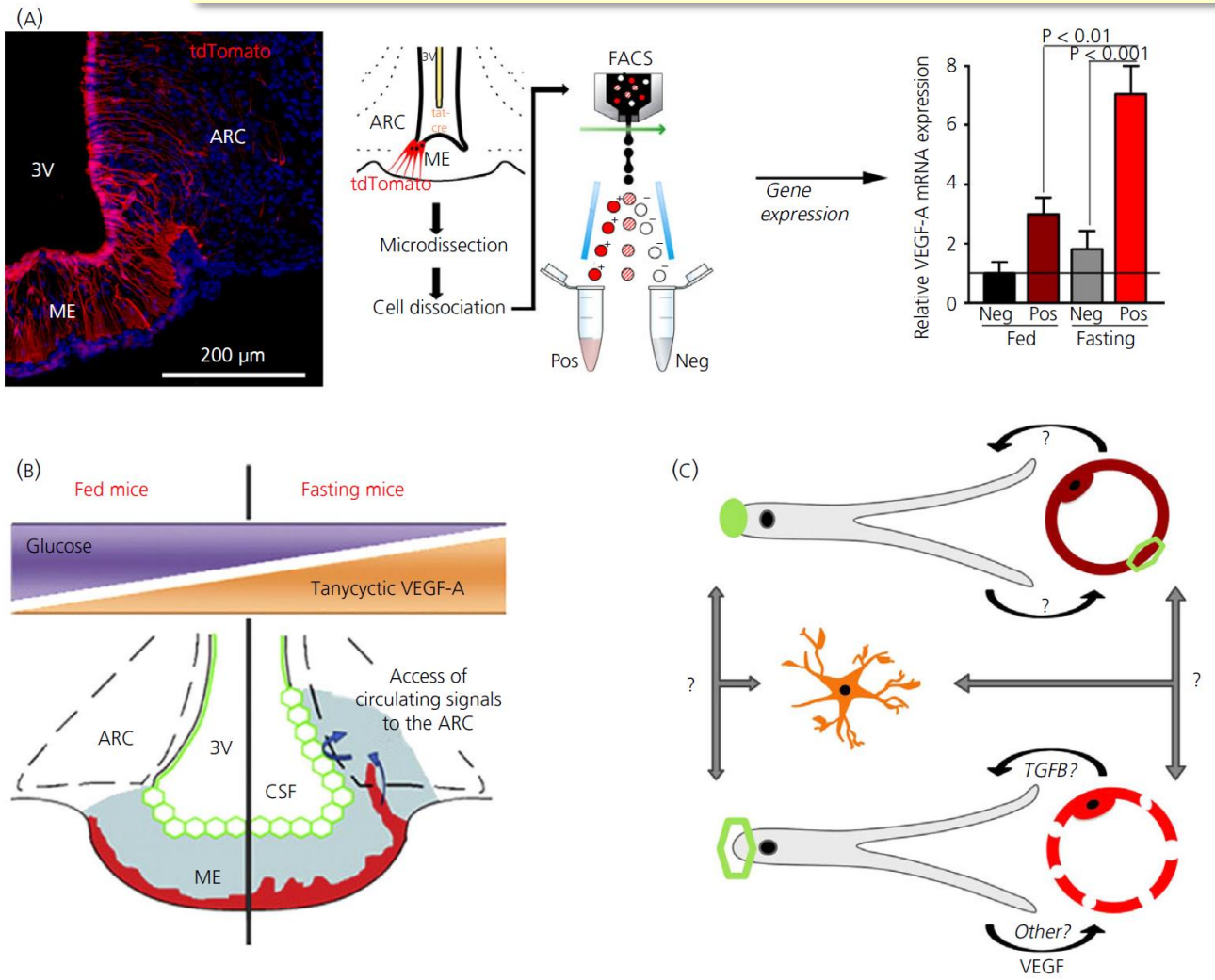
Tanycytes



Prevot et al., 2013
AGING, May 2013, Vol. 5 No.5

Figure 1. Schematic diagram illustrating structural differences between the median eminence and ARH of mice fed *ad libitum* and fasting mice, and their effects on the diffusion of blood-borne signals into the brain. The arcuate nucleus of the hypothalamus (ARH) lies lateral to the third ventricle (3V) and immediately dorsolateral to the median eminence (ME). In mice fed normally (left half of the figure), the fenestrated blood vessels of the ME permit the local diffusion of macromolecules from the circulation, while vessels in the ARH proper exhibit blood-brain barrier properties that block this diffusion (not shown). Hence, circulating metabolic signals whose levels are high in the fed state (e.g., leptin and glucose) require BBB transport to access ARH neurons. Under these conditions, tight junctions (green) between tanycytes line the ventricular wall of the ME, preventing the diffusion of circulating factors into the 3V and CSF. During fasting or energy restriction (right half of the figure), the levels of hormones such as ghrelin rise, along with products of lipolysis (e.g., fatty acids), while leptin and glucose levels fall. Concomitantly, some ME vessels extending into the ARH become fenestrated, while the tight junction barrier along the 3V extends dorsally. These changes allow the freer diffusion of circulating signals that indicate energy restriction to ARH cells, including AgRP/NPY neurons that lie in the ventromedial ARH, while preventing the access of these substances to the rest of the brain through the CSF. The focal plasticity of this dual-faceted blood-hypothalamus barrier thus enhances the orexigenic/anabolic response to energy deficits.

Blood-Arcuate Nucleus interface plasticity



Langlet et al., 2014
doi: 10.1111/jne.12191

Fig. 3. Blood–arcuate nucleus (ARC) interface plasticity is based on cell–cell communication. (A) Isolation of tdTomato-positive tanycytes by fluorescence activated cell sorting following i.c.v. Tat:Cre injection, and real-time polymerase chain reaction analysis of vascular endothelial growth factor (VEGF)-A mRNA in tdTomato-positive (pos; tanycytes) and -negative cells (neg) in fed and fasting mice. Fasting induces the increase of VEGF expression in tanycytes. (B) Schematic representation of blood–ARC interface reorganisation in fed and fasting mice according to glycaemia, and its effects on the diffusion of bloodborne signals into the brain. (C) Alternative hypotheses concerning cell–cell communications implicated in the organisation of the blood–ARC interface. VEGF secreted by tanycytes induces the fenestration of microvessels contacted by them, although other factors (such as transforming growth factor β) and/or cells (such as astrocytes) could influence the blood–ARC interface plasticity. Reprinted with permission from Langlet *et al.* (4). 3V, third ventricle; CSF, cerebrospinal fluid; FACS, fluorescence activated cell sorting; ME, median eminence; Neg, negative; Pos, positive; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

Relationship between GnRH secretory axons and tanycytes

(A) Confrontation in the mediobasal hypothalamus (mouse brain) of neuroendocrine axons secreting GnRH (blue), tanycytes [light gray, labeled for vimentin (VIM)], and the basal lamina [magenta, labeled for laminin (LAM)] of the outer surface of the brain and of hypothalamo-hypophysial capillary vessels. Note in **B1–B2** that ramified GnRH axons travel along tanycytic shafts (asterisks) and terminate (arrows) engulfed by tanycytic endfeet before contact with the basal lamina. The orientation is given in (A) where labeling for VIM and LAM are shown in separate low-power views of 12-mm-thick sections (insets). Scale bar, 10 mm.

(C) Electron micrographs illustrating the dynamic changes occurring in the external zone of the ME that control direct access of GnRH nerve terminals to the pericapillary space during the reproductive cycle in the rat. Left: Electron micrograph of GnRH-immunoreactive terminals (large arrowhead) in the external zone of the ME in close proximity of the fenestrated capillaries (Cap) of the portal vasculature. At most stages of the reproductive cycle, GnRH nerve terminals (labeled with 15-nm gold particles) are entirely embedded in tanycytic endfeet (Tan), which prevent them from contacting the pericapillary space (p.s.) delineated by the parenchymatous basal lamina (arrow). Arrowhead, endothelial basal lamina; short arrow fenestration of the endothelium. Scale bar: 0.5 mm. Right: On proestrus, the time of the occurrence of the preovulatory GnRH/luteinizing hormone surge, a significant fraction of GnRH nerve endings (large arrowhead) directly contact the pericapillary space (p.s.) through filopodial extension of the nerve terminal (arrows). Scale bar: 0.5 mm.

(D) Schematic representation of coordinated glial-endothelial-neuronal interactions regulating the direct access of GnRH neurosecretory terminals to the pericapillary space in the external zone of the ME. In diestrus, high progesterone levels in a context of low circulating estrogens promotes the secretion of Sema7A by ME tanycytes. Sema7A activates integrin b1 (Itgb1) expressed by tanycytes themselves via a paracrine/autocrine action to promote the growth of their endfeet (blue arrows), which engulf GnRH neuroendocrine terminals and thus form a diffusion barrier impeding GnRH release into the pericapillary space and fenestrated capillaries. In parallel, tanycytic Sema7A acts on the Plexin C1 receptor expressed by GnRH neuroendocrine terminals to induce their retraction from the pericapillary space (red arrow). In proestrus, high circulating levels of estrogens promote both nitric oxide (NO) and Sema3A release from the fenestrated endothelial cells of the ME, promoting, respectively, the retraction of tanycytic endfeet from the parenchymatous basal lamina (red arrows) and the neuropilin 1 (NRP1)–mediated outgrowth of GnRH neuroendocrine axons guided by a scaffold of tanycytic processes toward the pericapillary space (blue arrow). Reproduced from Prevot et al. 1998.