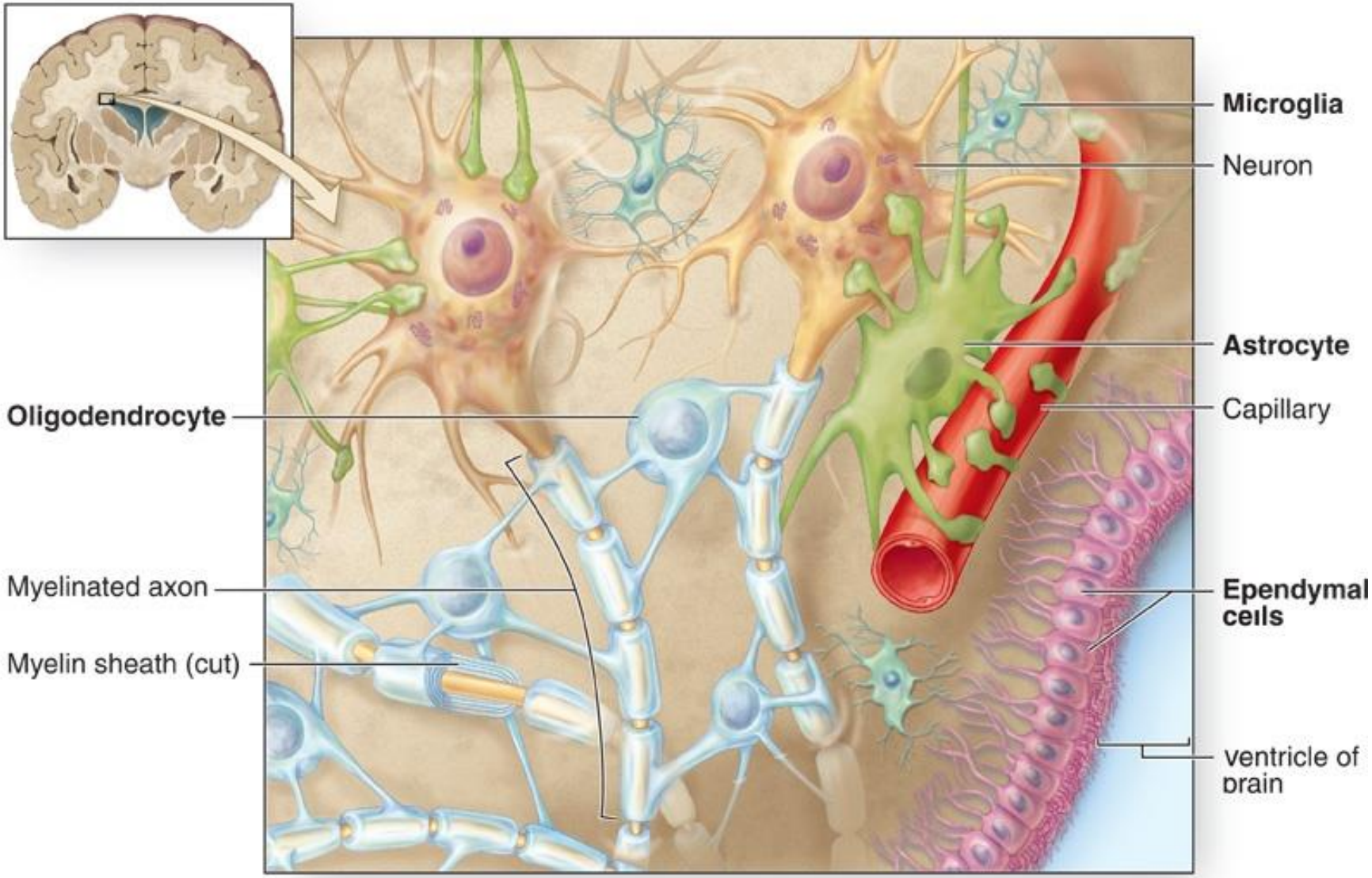
Cellular components of CNS



Cellular components of CNS

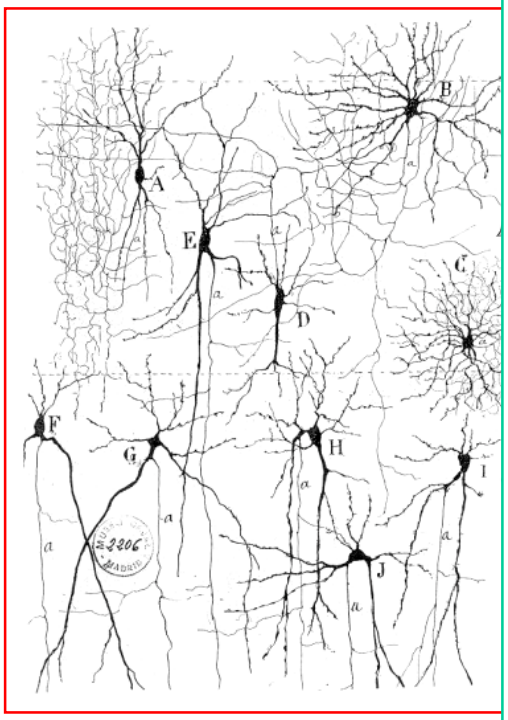
- Neurons
- Glial cells:

Astrocytes (including radial glia), oligodendrocytes, microglia, **ependymal cells**

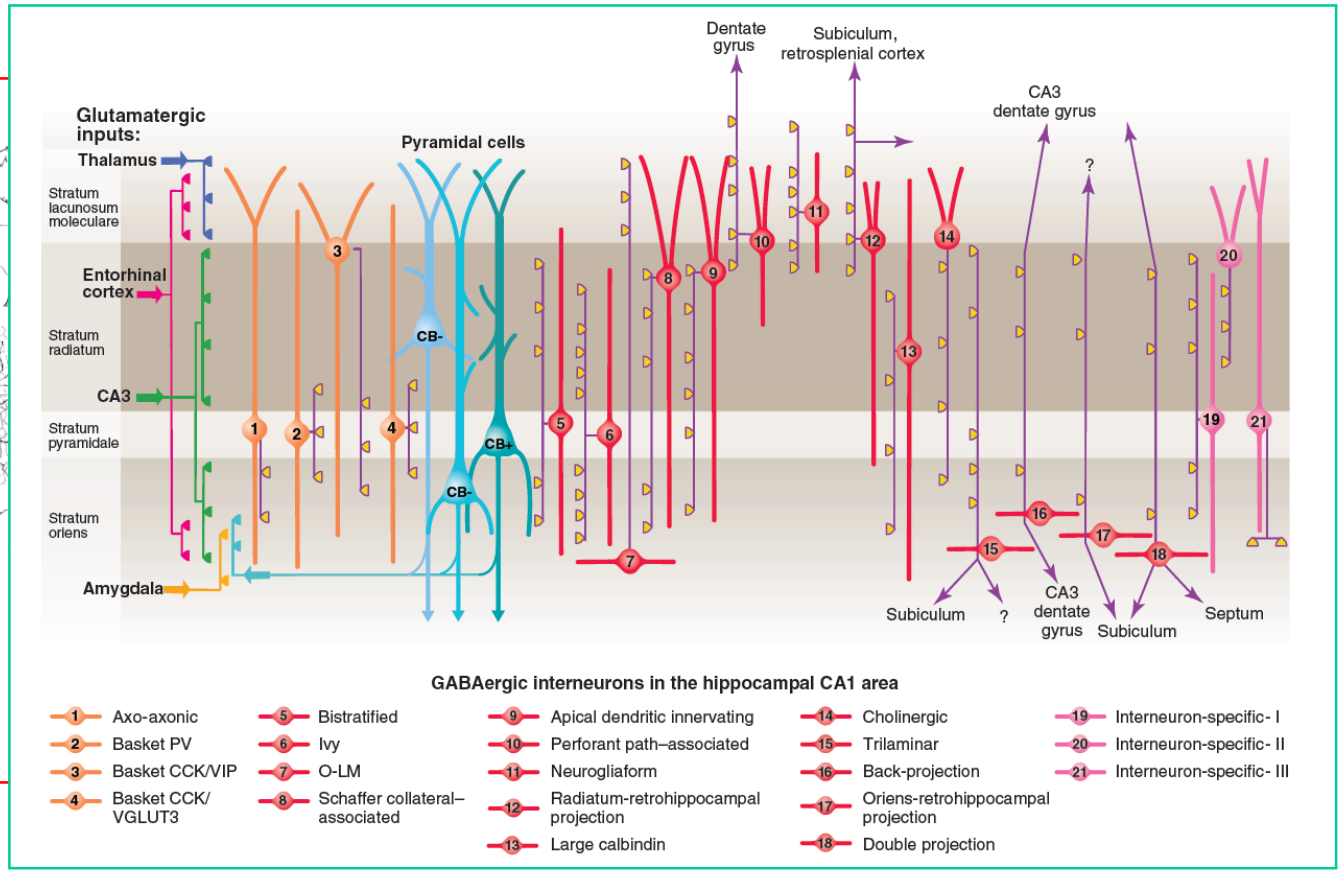
- Epithelial cells of choroid plexus
- Endothelial cells of CNS capillaries

The neuron is the basic functional unit of the nervous system

Neurons come in many different shapes and functions……



(Cajal, 1905)

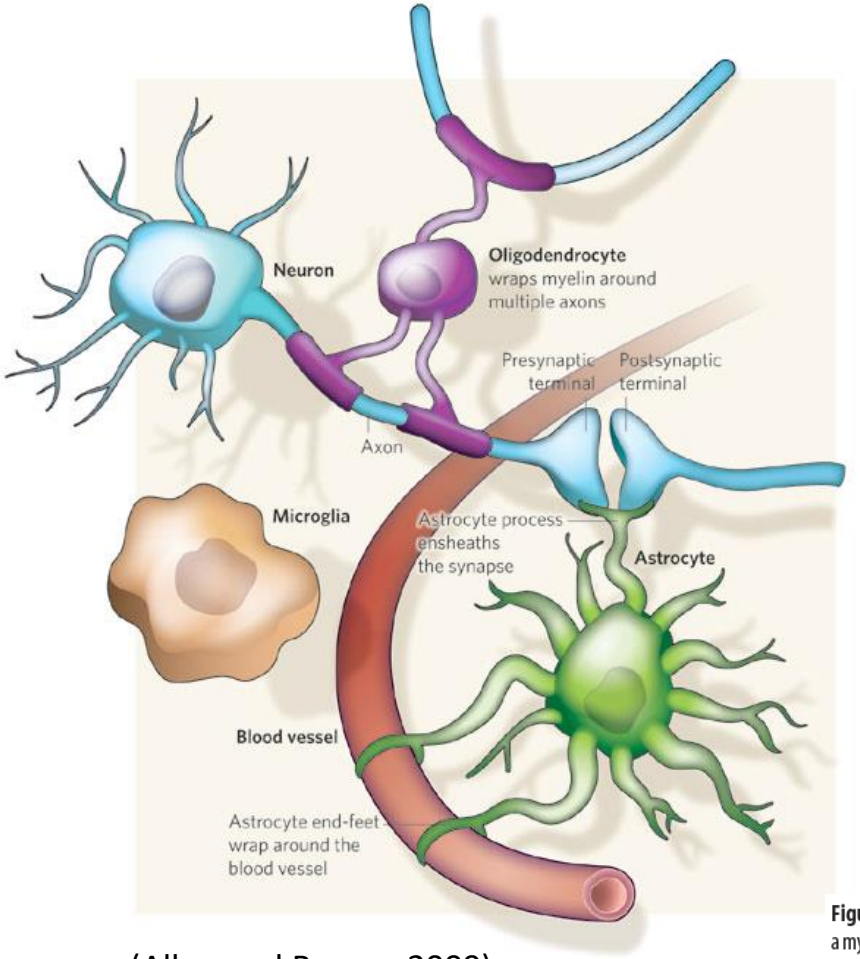


Thomas Klausberger[1,2]* and Peter Somogyi[1]*

SCIENCE VOL 321 4 JULY 2008

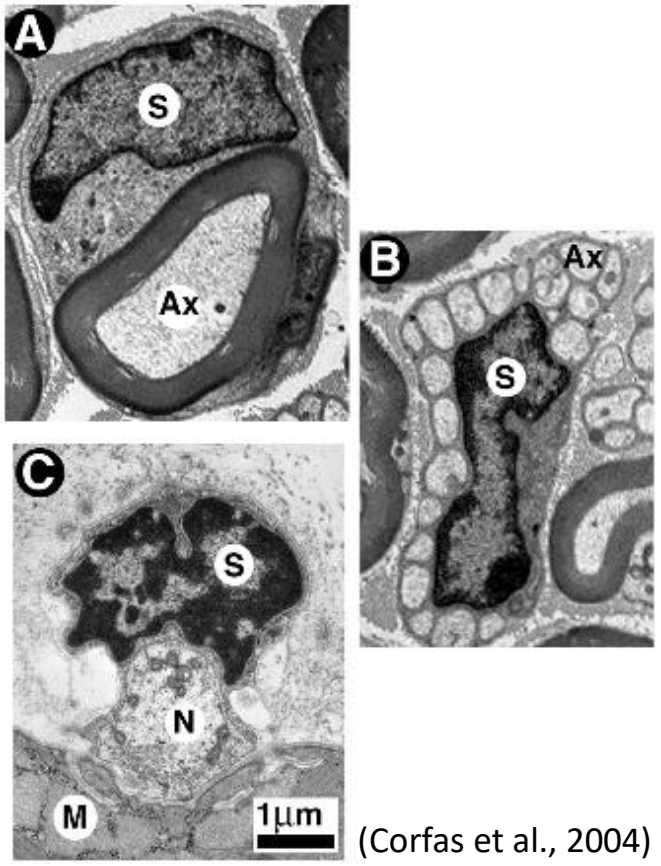
Yet, glia (can) outnumber neurons 10:1 in the human brain

Some examples of CNS glia:



(Allen and Barres, 2009)

Some examples of PNS glia:



(Corfas et al., 2004)

Figure 1. Myelinated, unmyelinated, and perisynaptic Schwann cells as seen with the electron microscope. *A*, Cross section of a myelinated axon of an adult mouse sciatic nerve. The myelin sheath (MS) surrounding the axon (Ax) and the Schwann cell nucleus (S) are clearly visible. *B*, Cross section of a bundle of unmyelinated axons of an adult mouse sciatic nerve. The Schwann cell forms the Remak bundle, a bouquet-like bundle of thin axons, each separated from its neighbor by thin cytoplasmic extensions of the Schwann cell. *C*, Cross section of a frog neuromuscular junction reveals three juxtaposed cellular elements: the perisynaptic Schwann cell, nerve terminal (N), and muscle fiber (M). The perisynaptic Schwann cell body (S indicates nucleus) and its processes cap the nerve terminal, but the processes do not wrap around the nerve terminal region facing acetylcholine receptors on muscle.

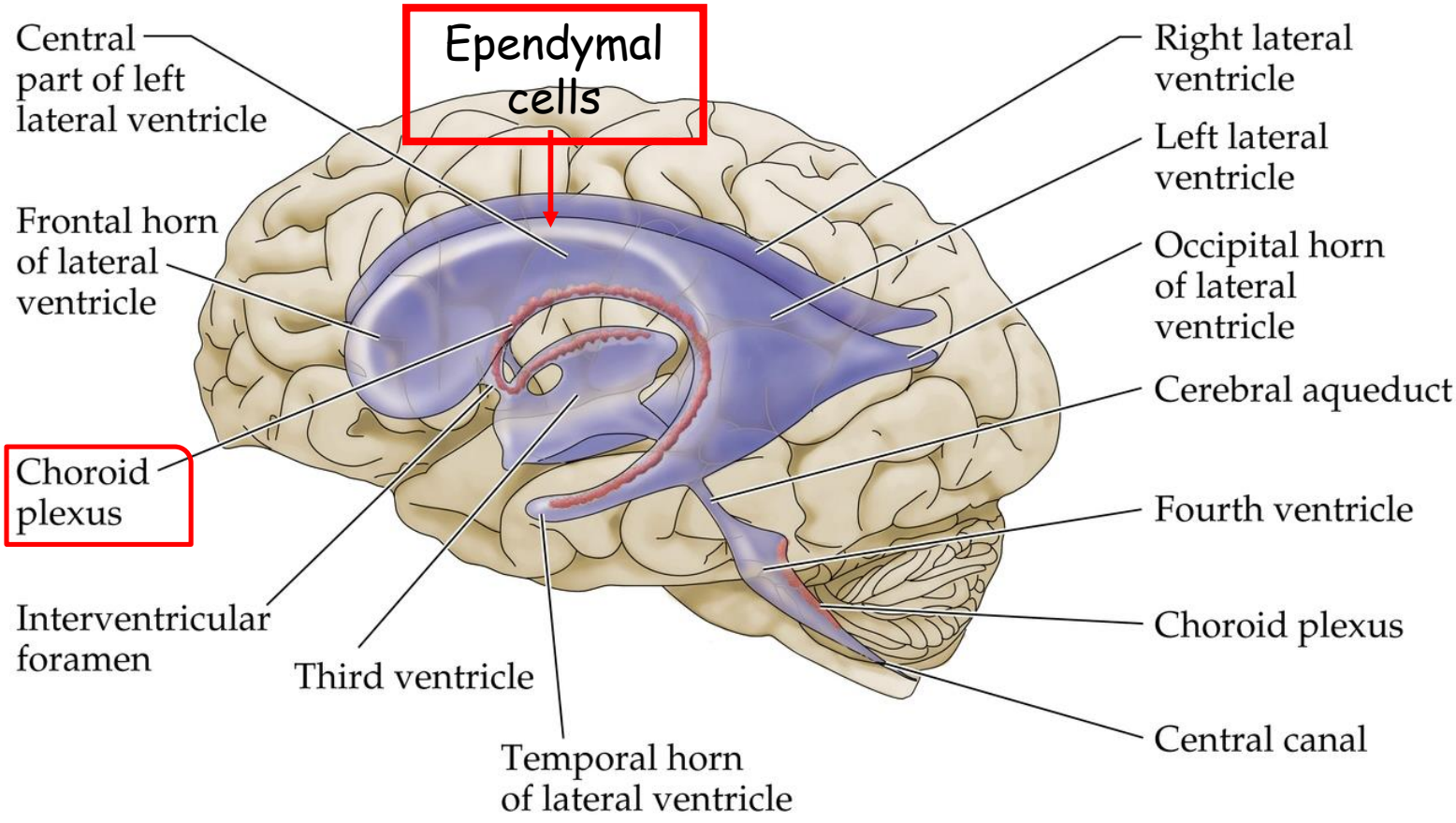
Let’s start with cells that are mostly neglected in the the study of the CNS:

Ependymal cells /Tanycytes – lining of ventricular system

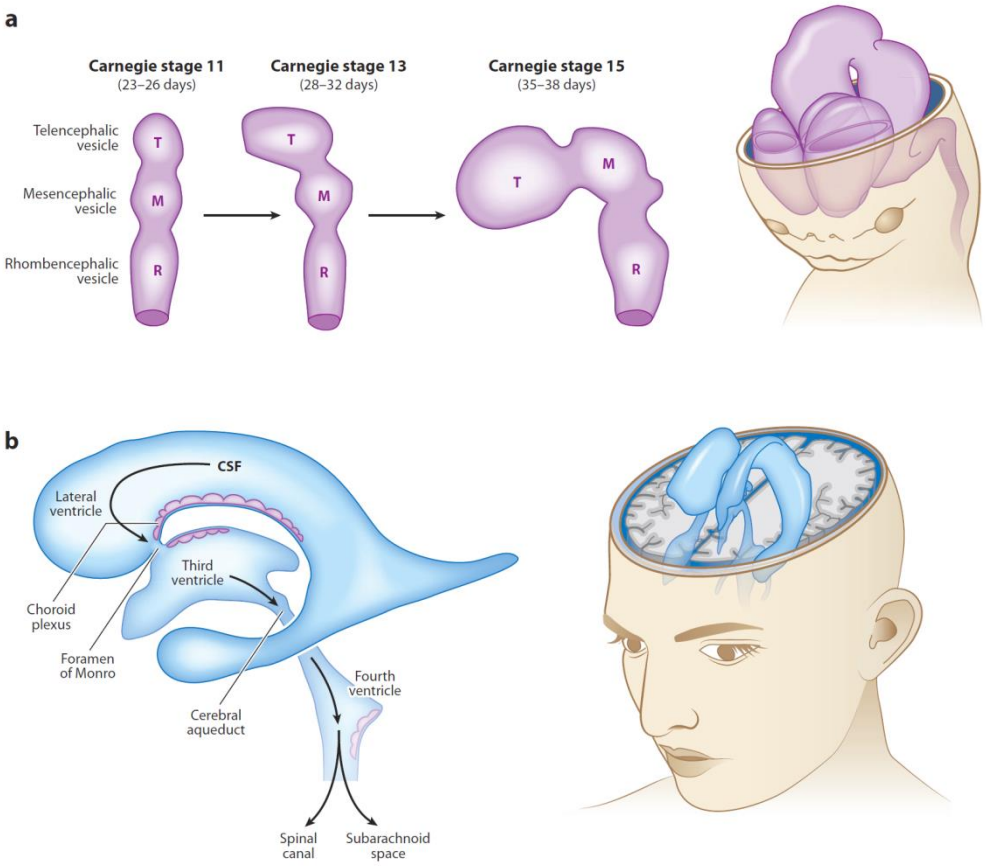
Choroid plexus cells – secretion of cerebrospinal fluid

Endothelial cells - Blood Brain Barrier (BBB)

The ventricular system



Development of the cerebral ventricles



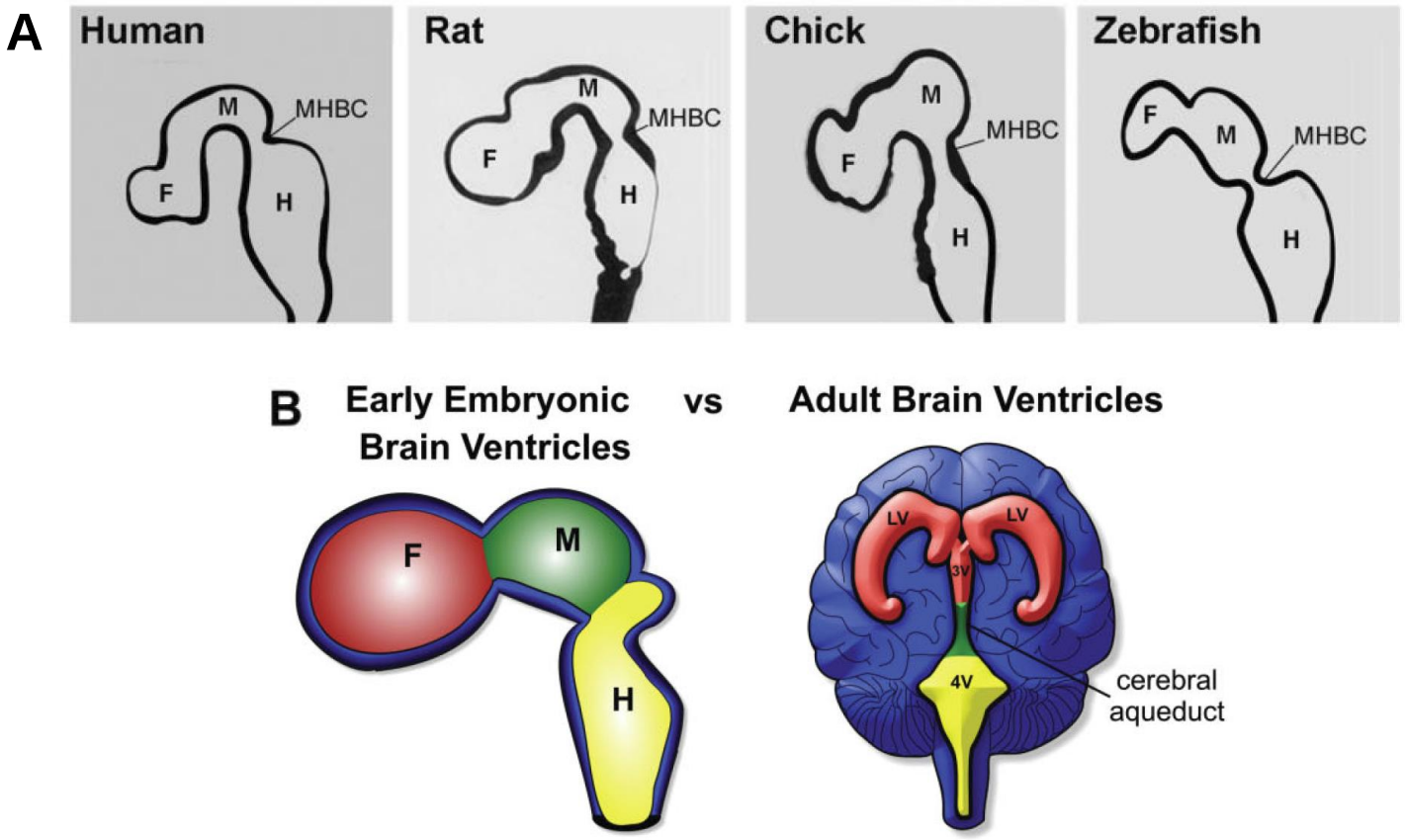
Lehtinen & Walsh. Annu. Rev. Cell Dev. Biol. 2011. 27:653–79

Schematics of the cerebroventricular system during early human brain development and in the mature adult brain

(a) Upon anterior neural tube closure, the three primary brain vesicles [telencephalic (T), mesencephalic (M), and rhombencephalic (R) vesicles] serve as the rudimentary cerebroventricular system for the developing central nervous system (CNS).

(b) In the mature CNS, CSF generated primarily by the choroid plexus tissues located in each ventricle in the brain fills the ventricles, subarachnoid space, and spinal canal. CSF flows from the lateral ventricles via the foramen of Monro/intraventricular foramen into the mesencephalic/third ventricle, and then via the aqueduct of Sylvius/cerebral aqueduct into the hindbrain/fourth ventricle. The CSF then continues through the foramina of Magendie/median apertures and Luschka/lateral apertures into the spinal canal and subarachnoid space, and is finally resorbed into the venous system via arachnoid villi. An adult human circulates approximately 150 ml of CSF within the cerebroventricular system. The CSF is estimated to turn over approximately three to four times per day, so a healthy CNS produces close to 500 ml of CSF daily.

Evolutionary conservation of embryonic brain ventricle structure



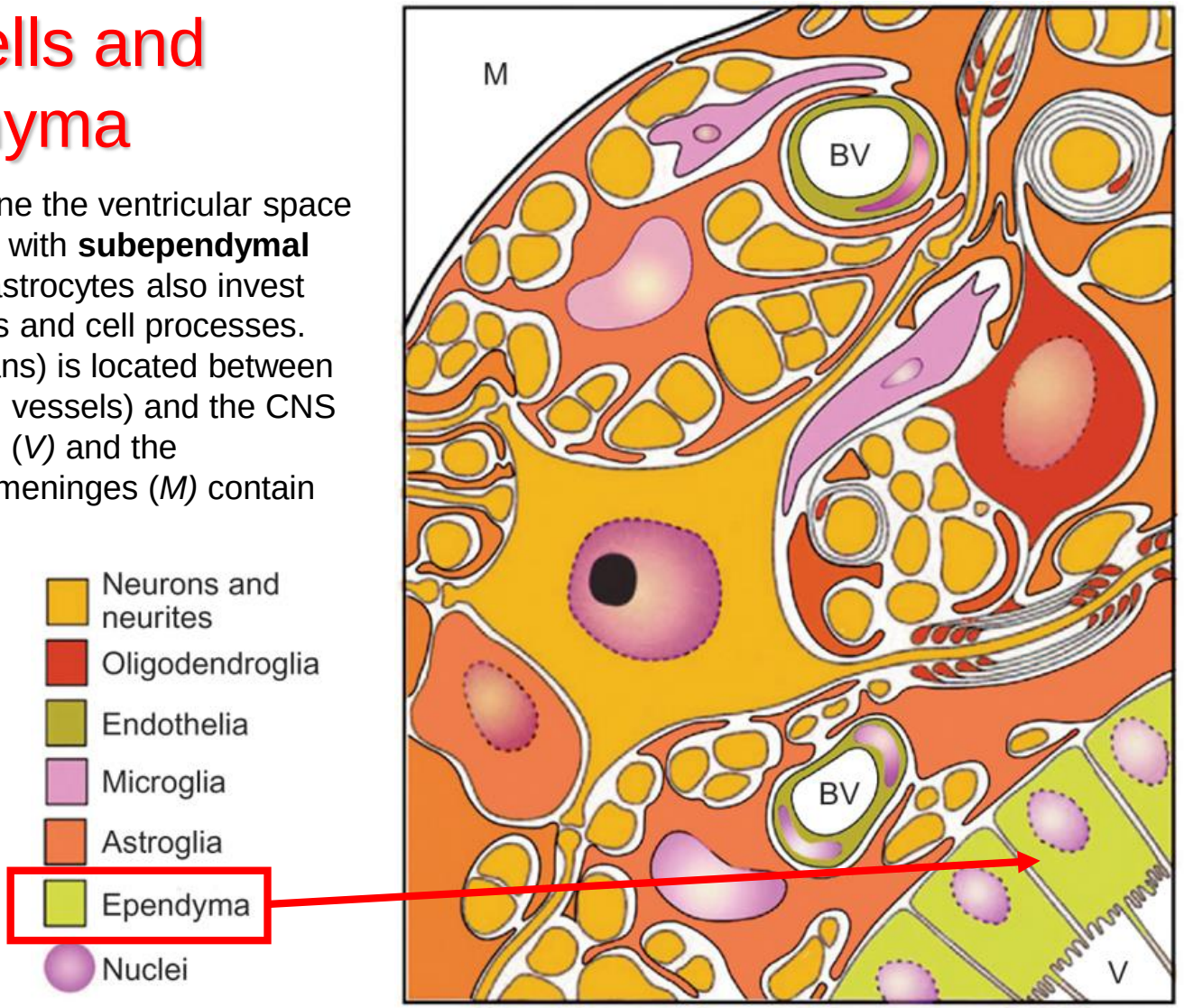
A: Conservation of embryonic brain ventricle structure. Tracings of embryonic brain ventricles at similar corresponding stages in development, all lateral views.

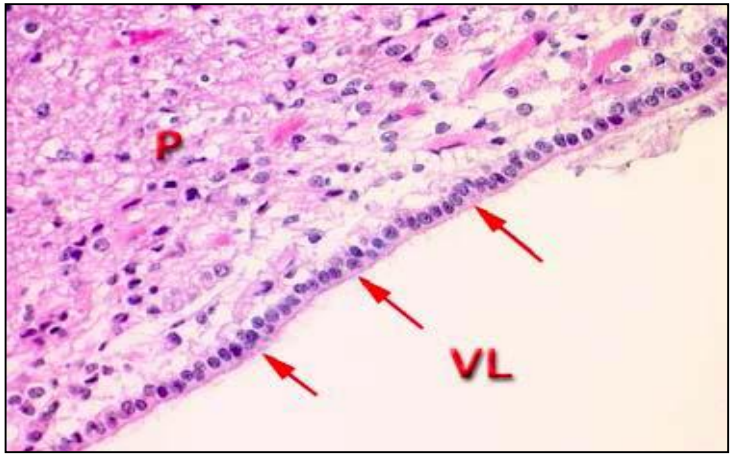
B: Comparison of early embryonic and adult brain ventricles. Colors correspond to the same ventricle regions in the embryo and adult. Not to scale. F, forebrain (telencephalon plus diencephalon); M, midbrain (mesencephalon); H, hindbrain (rhombencephalon); MHBC, midbrain hindbrain boundary constriction

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.

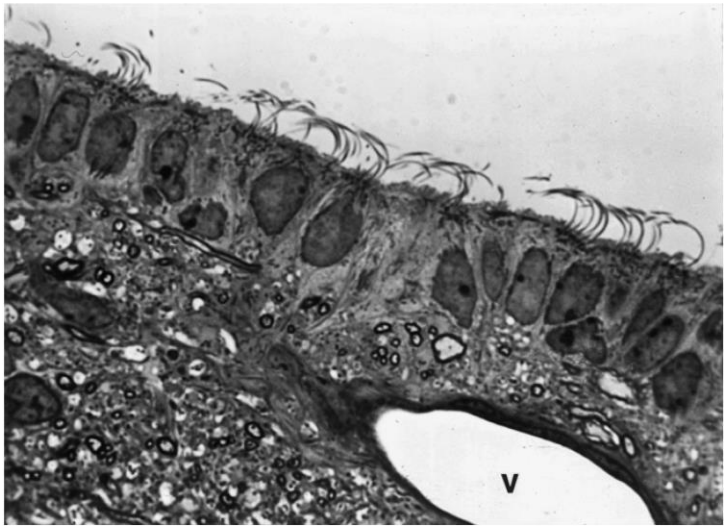
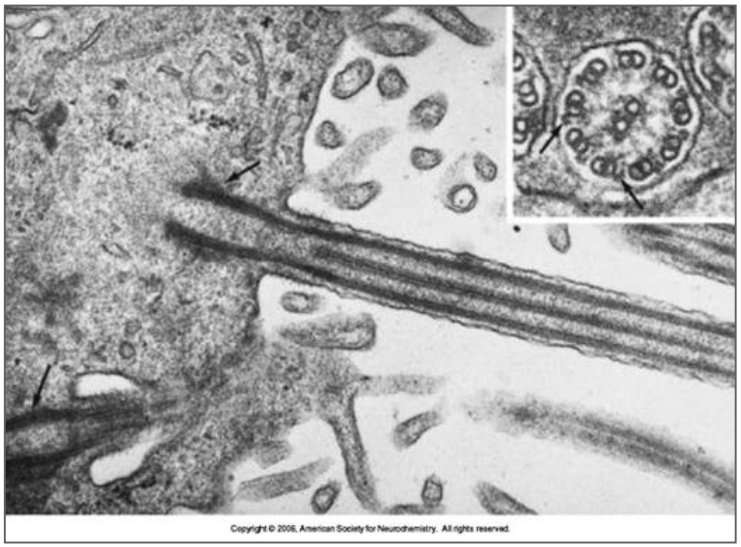
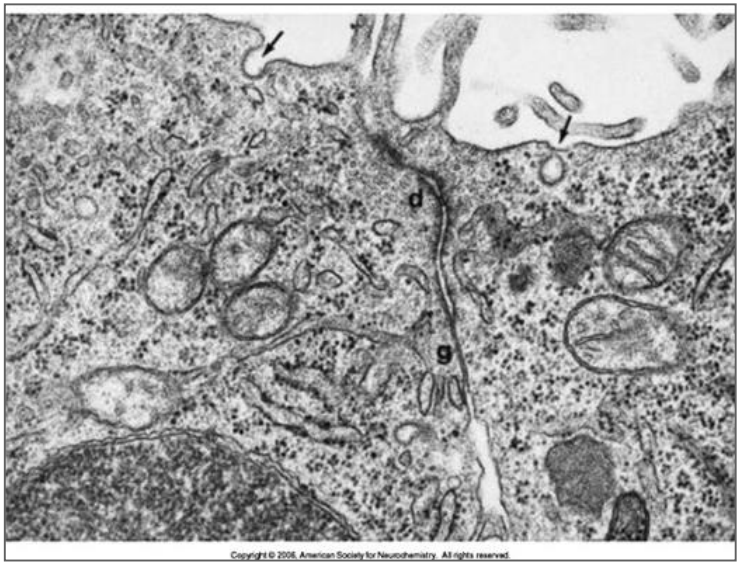


Fig. VIII.1 Ependymal cells. The ependymal cells in this light micrograph are columnar with oval nuclei and contain cilia emanating from basal bodies. Above the nuclei, there are numerous mitochondria. **v** blood vessel. Central canal of the rabbit spinal cord, 1-µm section stained with toluidine blue,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

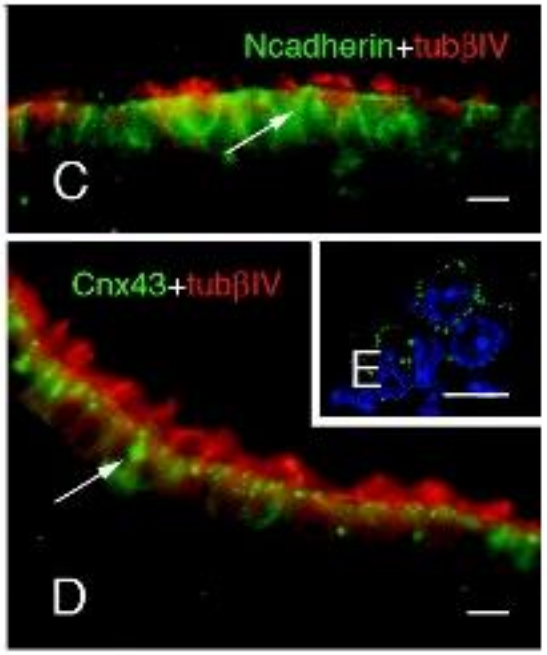
Ependymal cells





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*).

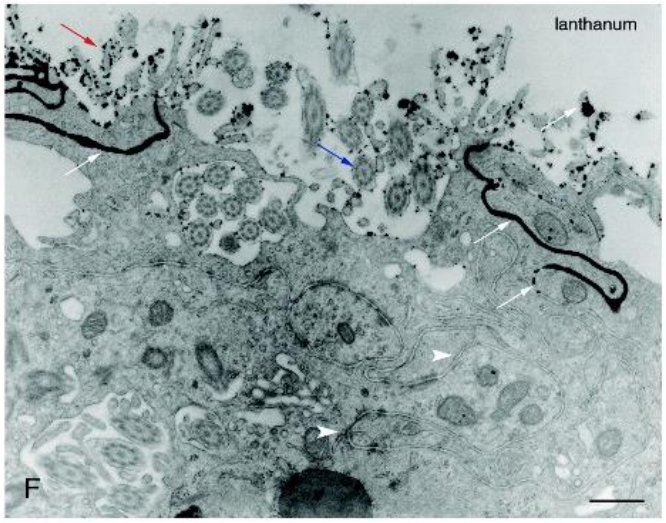
Bottom panel: A typical **desmosome (*d*)** and **gap junction (*g*)** between two ependymal cells. Microvilli and coated pits (*arrows*) are seen along the cell surface.

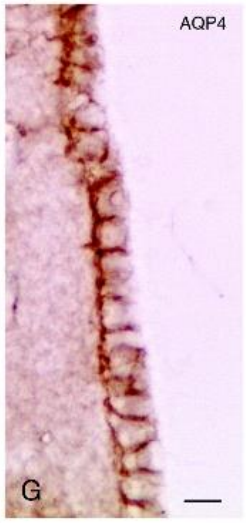


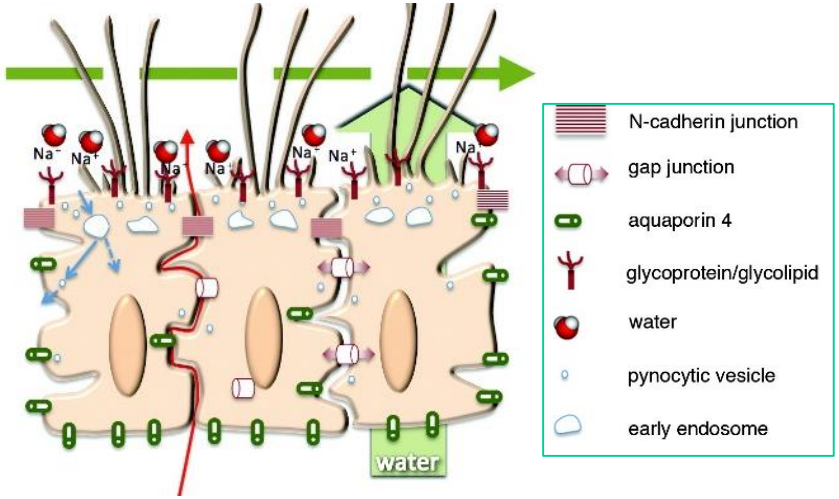
Molecular features of the multiciliated ependyma in the ventricle of the mouse.

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

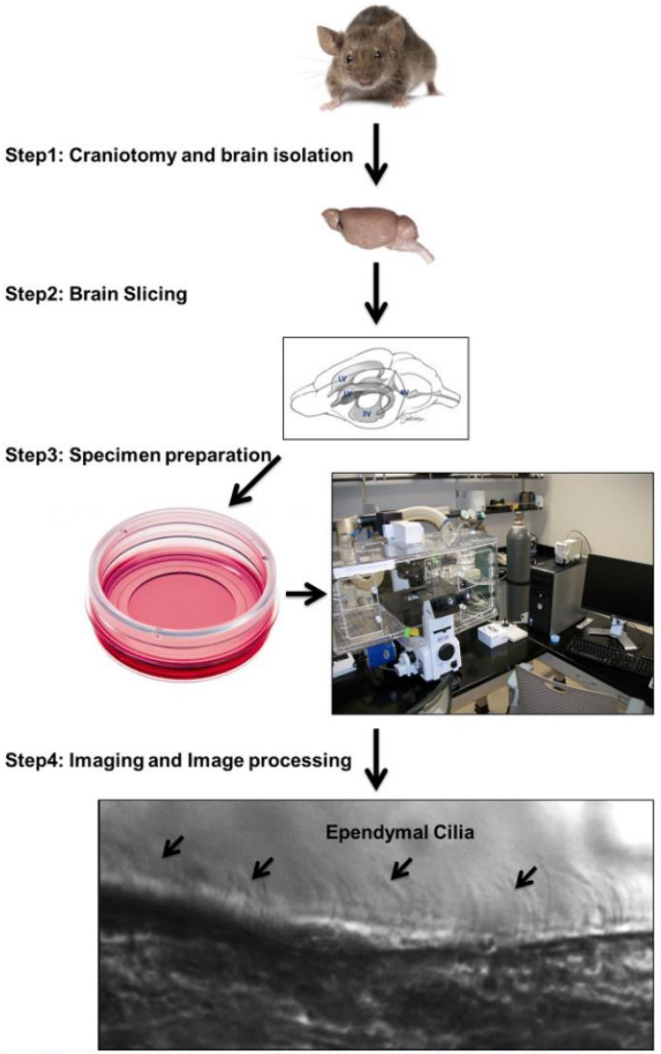


Figure 1: Ependymal cilia imaging protocol flowchart. Ependymal cilia imaging protocol illustrates steps to complete an experiment starting from mouse brainextraction, sectioning and tissue preparation to image acquisition and analysis. An approximate one hour timeline is presented with step-by-step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

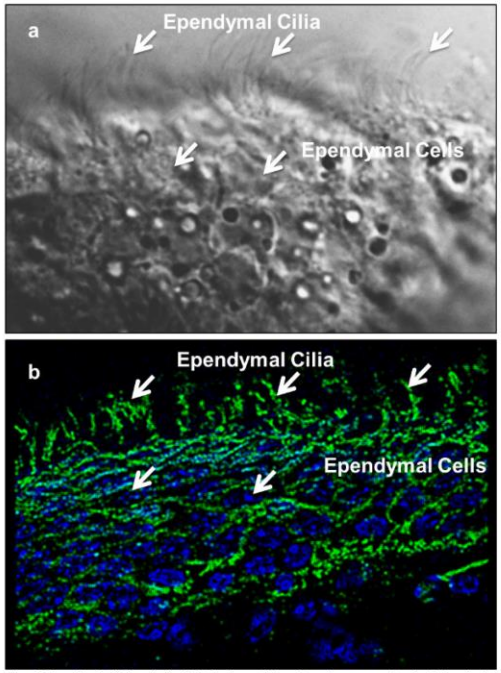


Figure 2: Ependymal cilia localization in the brain ventricles. Shown here are ependymal cells from the lateral ventricle of a mouse brain. (**a**) DIC images of individual ependymal cells (bottom arrows) and cilia (top arrows) are shown. (**b**) An overlay image of a brain section is stained with antibody 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.

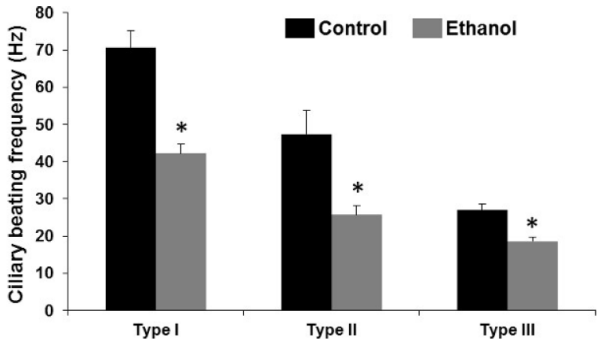


Figure 3: Alcohol and differences in cilia beating frequencies among types of ependymal cells of the mouse brain lateral ventricle. The *ex vivo* brain slice was incubated without (Control) or with (Ethanol) 0.25 % alcohol for 5 min. Compared to control, alcohol treatment significantly decreased cilia 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.

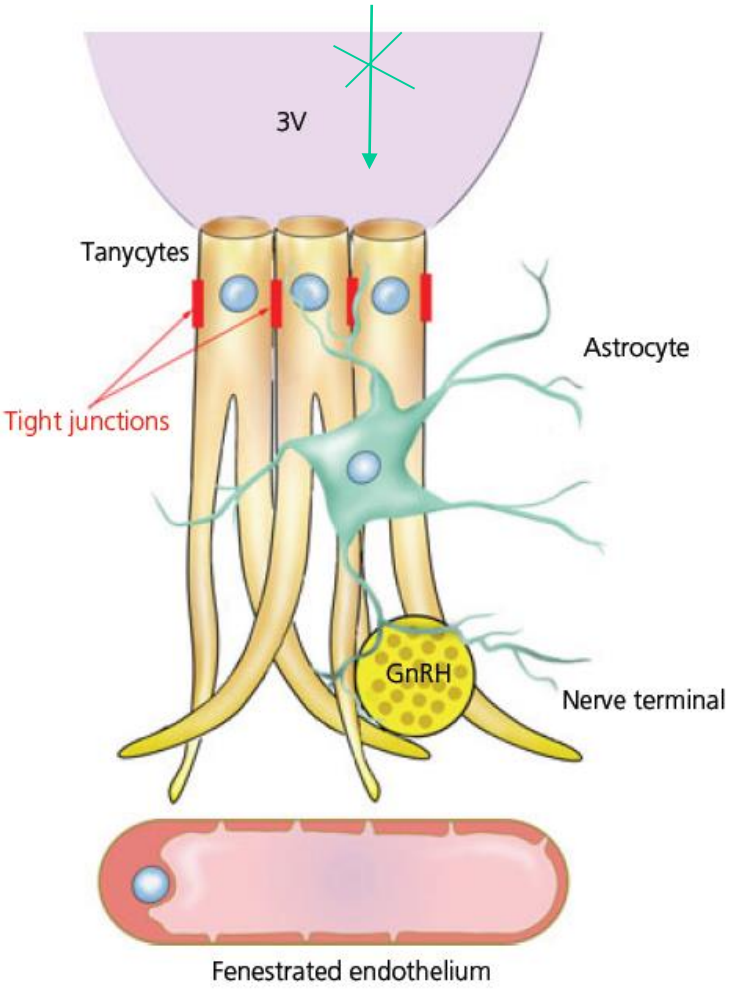


Fig. 1. Schematic representation of the cell types (tanycytes, astrocytes and endothelial cells) and neuronal elements (neuroendocrine terminals) that reside within the median eminence of the hypothalamus. The median eminence of the hypothalamus is the brain structure forming the floor of the third ventricle (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

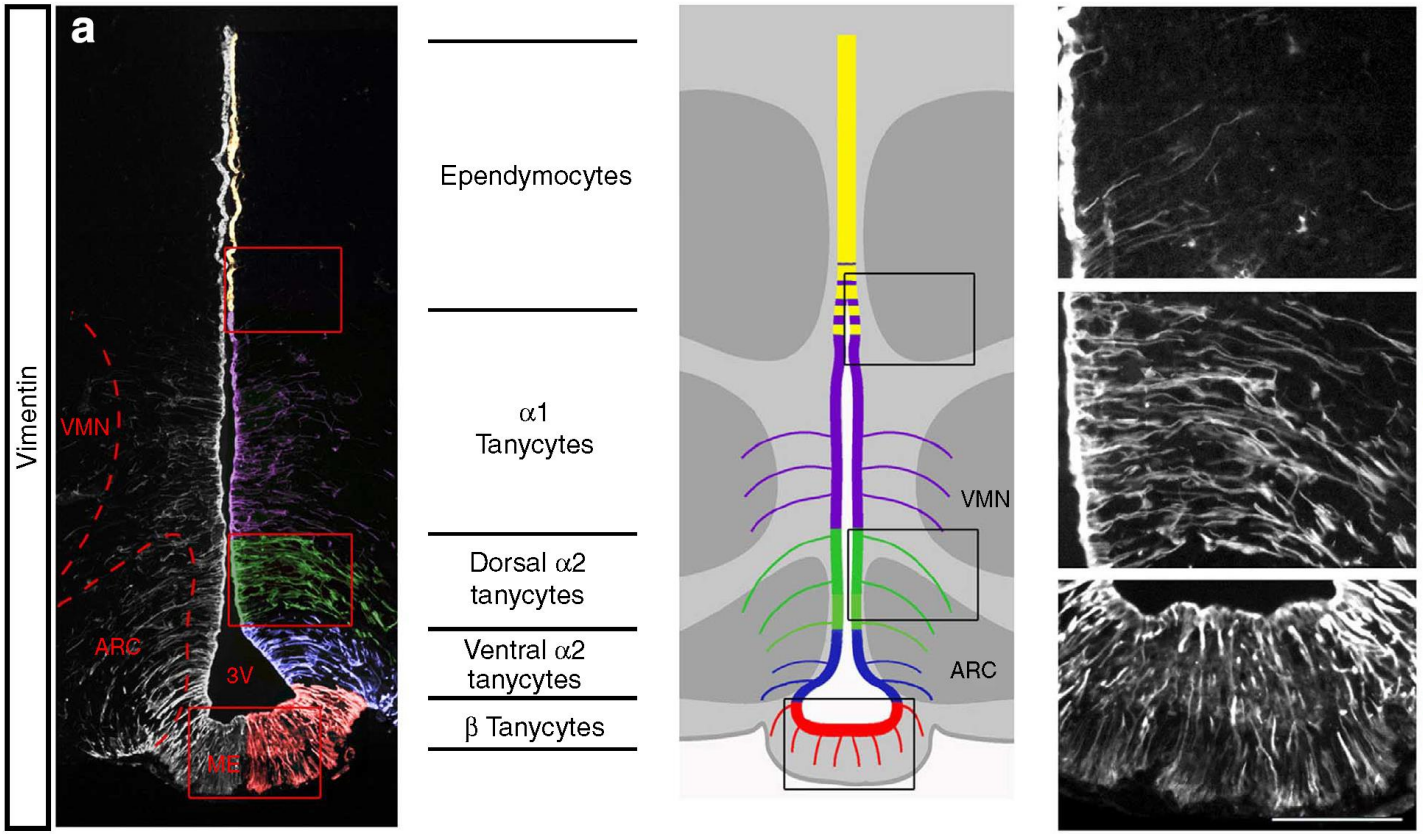


Figure 1 | *GLAST::CreER*^{T2} marks α-tanycytes subpopulations in the adult hypothalamus. (**a**) Left hand panel: coronal section (c.s.) through third ventricle (3V), immunolabelled with Vimentin. The Vimentin^{+ive} process distinguishes tanycytes (false-coloured in right-hand side of image) from ependymocytes. Central panel cartoon shows position and process projection of tanycyte subtypes: purple, α1; green, dα2; blue, vα2; red, β. Note β-tanycytes divide into medial β2 and lateral β1 subsets[13,14]. Right-hand (rh) panel shows high-power magnifications of boxed regions. Ventrally, all ventricular cells appear to have a Vimentin^{+ive} process; medially, many ventricular cells have a Vimentin^{+ive} process; dorsally, the ependymocyte/α1 boundary is indistinct, with intermingling of tanycytes and ependymocytes. (**b–d**): Confocal images, showing c.s. through central hypothalamus, counter-

Robins et al., 2013

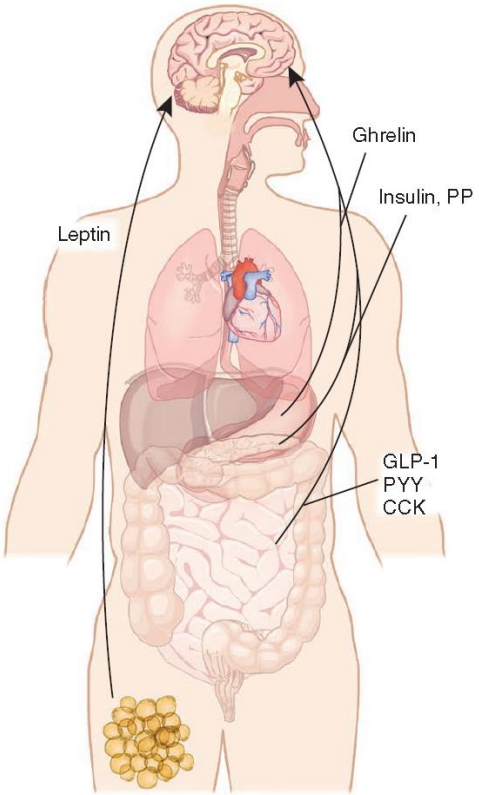
NATURE COMMUNICATIONS | 4:2049 | DOI: 10.1038

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

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

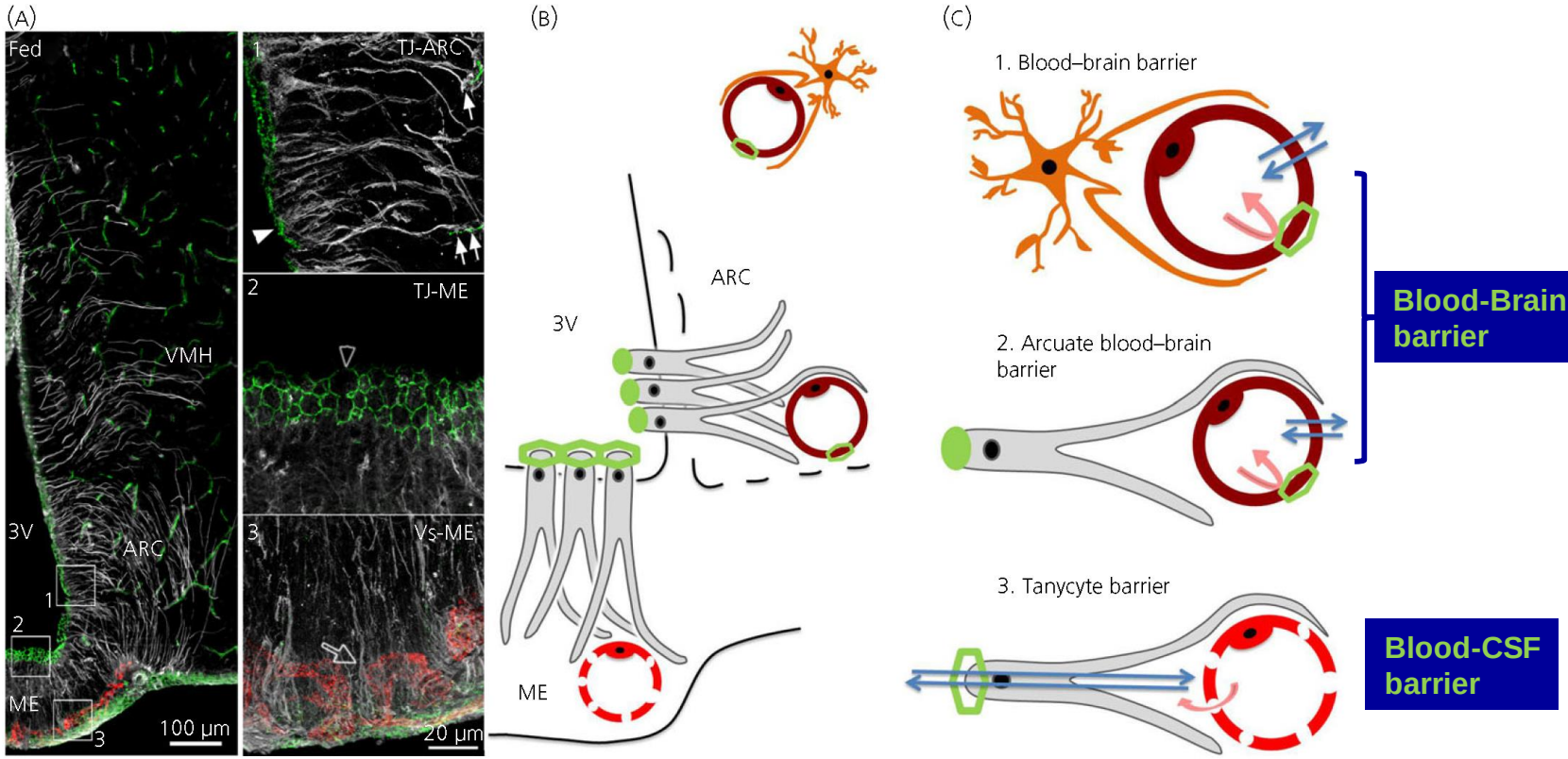


Fig. 1. Organisation of the blood–arcuate nucleus (ARC) interface in the mediobasal hypothalamus. (A) Vimentin (white), zonula occludens-1 (ZO-1; green) and MECA-32 (red) immunoreactivity in coronal sections of the hypothalamic tuberal region in fed animals. Tanycytes exhibit a diffuse pattern of tight 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

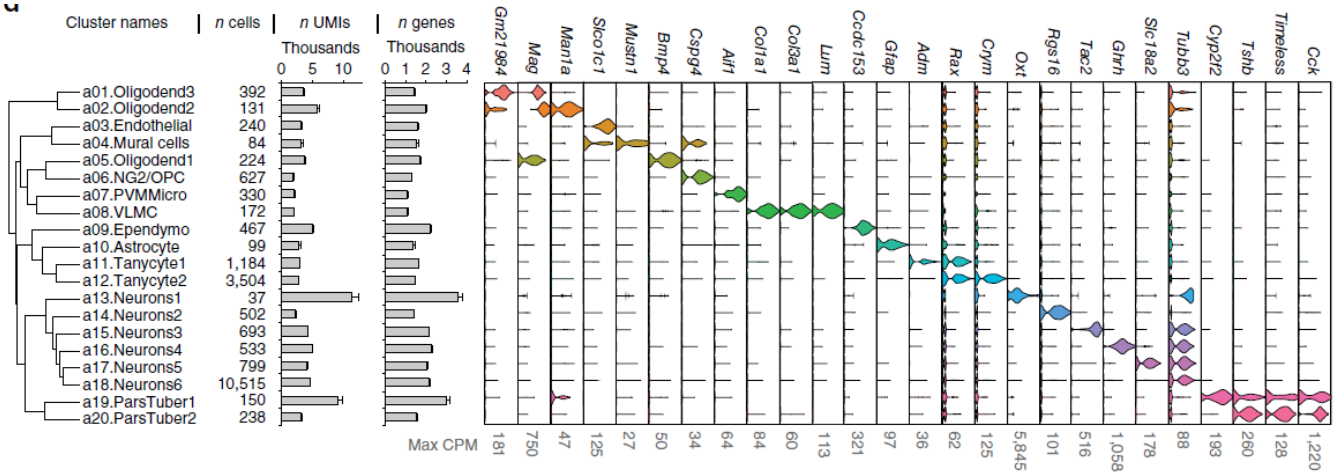
- **How can we define the many different cell types present in the hypothalamus/median eminence?**
- **Can an 'expression profile' approach help in this task?**

A molecular census of arcuate hypothalamus and median eminence cell types

John N Campbell[1], Evan Z Macosko[2–4], Henning Fenselau[1], Tune H Pers[5,6], Anna Lyubetskaya[1], Danielle Tenen[1], Melissa Goldman[2,3], Anne M J Verstegen[1], Jon M Resch[1], Steven A McCarroll[2–4,7,8], Evan D Rosen[1,8], Bradford B Lowell[1,7] & Linus T Tsai[1]

The hypothalamic arcuate–median eminence complex (Arc-ME) controls energy balance, fertility and growth through molecularly distinct cell types, many of which remain unknown. To catalog cell types in an unbiased way, we profiled gene expression in 20,921 individual cells in and around the adult mouse Arc-ME using Drop-seq. We identify 50 transcriptionally distinct Arc-ME cell populations, including a rare tanycyte population at the Arc-ME diffusion barrier, a new leptin-sensing neuron population, multiple agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) subtypes, and an orexigenic somatostatin neuron population. We extended Drop-seq to detect dynamic expression changes across relevant physiological perturbations, revealing cell type–specific responses to energy status, including distinct responses in AgRP and POMC neuron subtypes. Finally, integrating our data with human genome-wide association study data implicates two previously unknown neuron populations in the genetic control of obesity. This resource will accelerate biological discovery by providing insights into molecular and cell type diversity from which function can be inferred.

The technique: **Drop-seq** single-cell expression profiling



Campbell et al. 2017, Nature Neuroscience, doi:10.1038/nn.4495

Using expression patterns of cell type–specific marker genes, a single identity was assignedto to each cluster: neurons (*Tubb3*+), ependymocytes (*Ccdc153*+), tanycytes (*Rax*+), oligodendrocyte lineage cells (*Mag*+), oligodendrocyte precursor cells (also known as NG2 cells), *Cspg4*+), macrophages (*Aif1*+), endothelial cells (*Slco1c1*+), mural cells (*Mustn1*+) and astrocytes (*Gfap*+

Cell

New!

Resource

The technique:
Drop-seq single-cell expression profiling

Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

Evan Z. Macosko,[1,2,3,*] Anindita Basu,[4,5] Rahul Satija,[4,6,7] James Nemesh,[1,2,3] Karthik Shekhar,[4] Melissa Goldman,[1,2] Itay Tirosh,[4] Allison R. Bialas,[8] Nolan Kamitaki,[1,2,3] Emily M. Martersteck,[9] John J. Trombetta,[4] David A. Weitz,[5,10] Joshua R. Sanes,[9] Alex K. Shalek,[4,11,12] Aviv Regev,[4,13,14] and Steven A. McCarroll[1,2,3,*]

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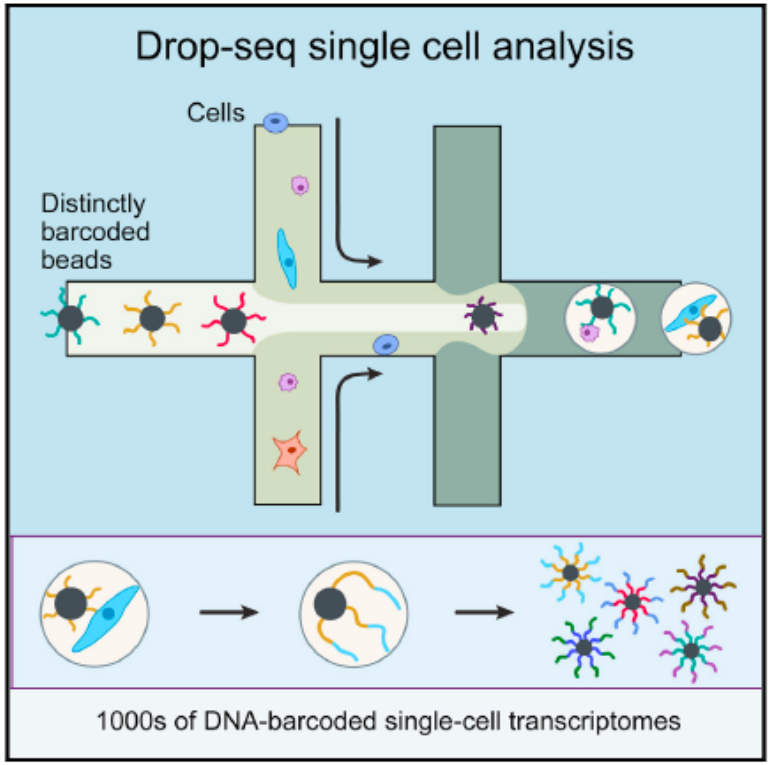
http://dx.doi.org/10.1016/j.cell.2015.05.002

Macosko et al, 2015 Cell
http://dx.doi.org/10.1016/j.cell.2015.05.002

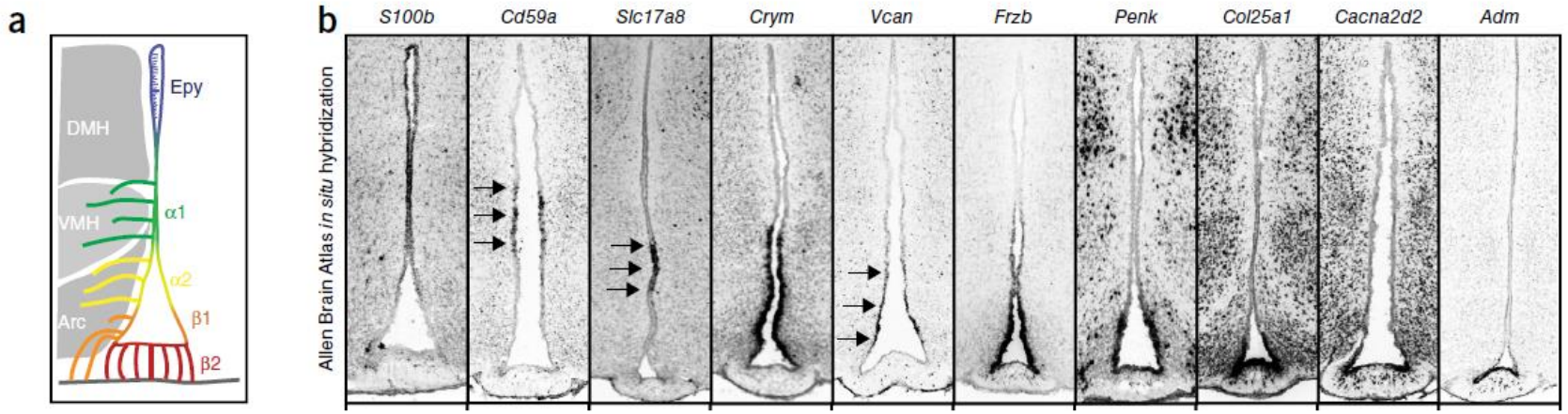


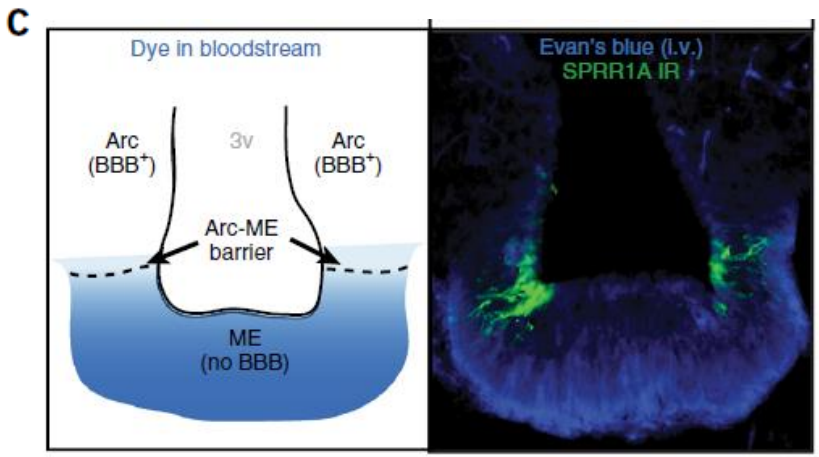
SUMMARY

Cells, the basic units of biological structure and function, vary broadly in type and state. Single-cell genomics can characterize cell identity and function, but limitations of ease and scale have prevented its broad application. Here we describe Drop-seq, a strategy for quickly profiling thousands of individual cells by separating them into nanoliter-sized aqueous droplets, associating a different barcode with each cell's RNAs, and sequencing them all together. Drop-seq analyzes mRNA transcripts from thousands of individual cells simultaneously while remembering transcripts' cell of origin. We analyzed transcriptomes from 44,808 mouse retinal cells and identified 39 transcriptionally distinct cell populations, creating a molecular atlas of gene expression for known retinal cell classes and novel candidate cell subtypes. Drop-seq will accelerate biological discovery by enabling routine transcriptional profiling at single-cell resolution.

Highlights

- Drop-seq enables highly parallel analysis of individual cells by RNA-seq
- Drop-seq encapsulates cells in nanoliter droplets together with DNA-barcoded beads





Subclustering analysis of Drop-seq data revealed eight clusters of ependymal cells (either ependymocytes or tanycytes). Previous studies in rodents have described four tanycyte subtypes (α1, α2, β1 and β2) occupying distinct regions along the third ventricle (**a**). To trace the anatomical origin of the ependymal cell clusters, the markers of each cluster were cross-referenced with *in situ* hybridization data (**b**) from the Allen Mouse Brain Atlas (http://mouse.brain-map.org/). Drop-seq data nearly double the number of ependymal cell subtypes thought to exist and provide insight into each's possible functions.

The gene *Sprr1a* was found only at the border between Arc and ME (c), where tanycytes are thought to form a diffusion barrier. Small proline-rich (SPRR) proteins, including SPRR1A, are crucial constituents of the cornified envelope, the diffusion barrier in the skin.

Figure 2 Ependymal cell types. (**a**) Illustration of known subtypes of hypothalamic ependymal (Epy) cells, their approximate anatomical locations and the orientations of their processes. Ependymocytes have cilia in the ventricle and tanycytes have basal processes in the brain parenchyma and median eminence. DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus. (**b**) Marker gene expression shown by *in situ* hybridization of coronal brain sections (Allen Mouse Brain Atlas; top) and ependymal cell feature plot (bottom) derived from tSNE plot (**Fig. 1b**).

(**c**) Bottom left, schematic of an experiment to define the diffusion barrier between Arc and ME; 3v, third ventricle. Bottom right, confocal micrograph comparing SPRR1A immunoreactivity (IR) to the location of the Arc-ME diffusion barrier, visualized by extravasation of intravascular (i.v.) Evan's blue; micrograph is representative of 2 mice.

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