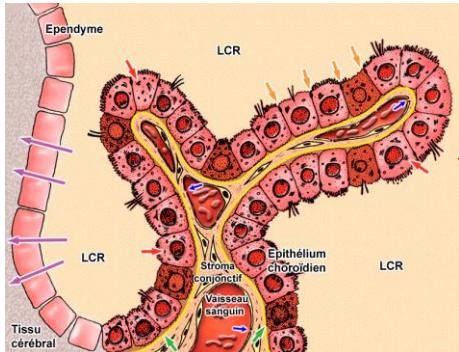
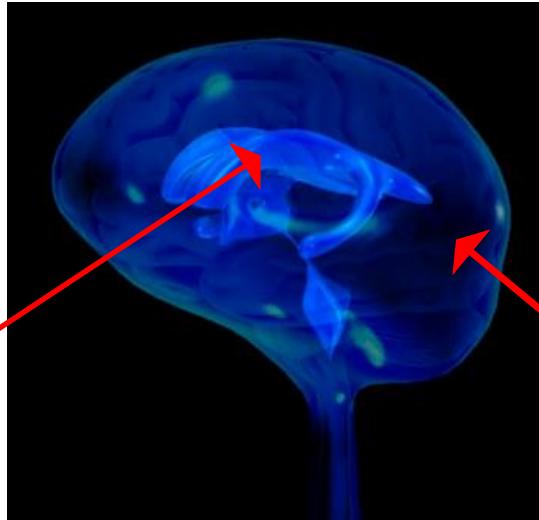


Two main interfaces between the blood and the central nervous system



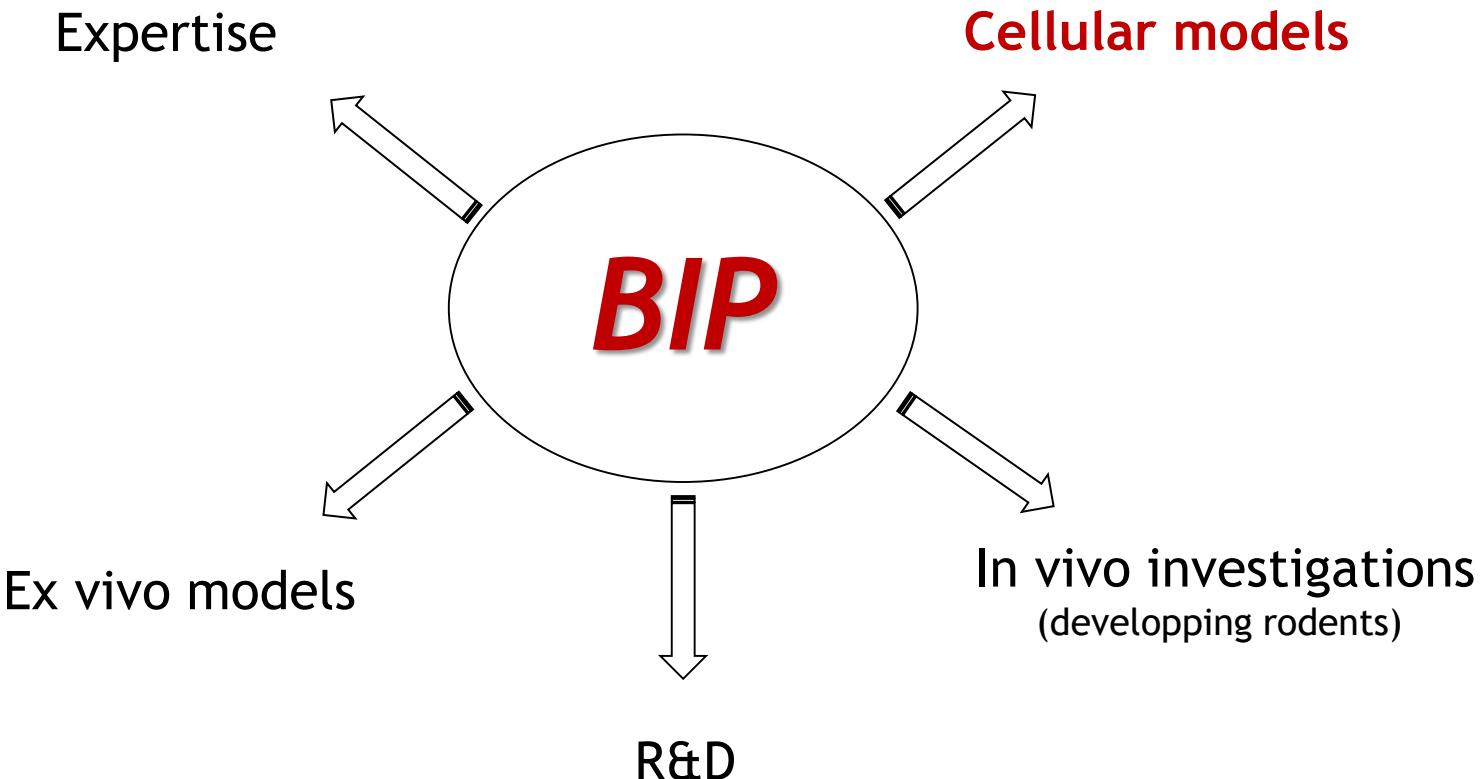
Blood-cerebrospinal fluid barrier
(choroid plexuses)

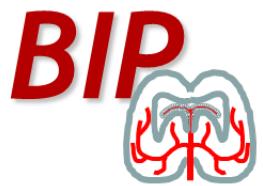


Blood-brain parenchyma barrier
(microvessels)

- ✓ *Protection and cerebral homeostasis/ Neuroprotective therapeutic strategies*
- ✓ *Cerebral drug delivery*
- ✓ *Neuroimmune Interactions/neuroinflammatory diseases*

*to develop and propose original tools to study
blood-brain interfaces functions*





Blood-brain interfaces exploratory platform



*Neurocampus Louis Jouvet, CRNL
Hôpital du Vinatier, Bron*



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Nathalie Strazielle, PharmD, PhD

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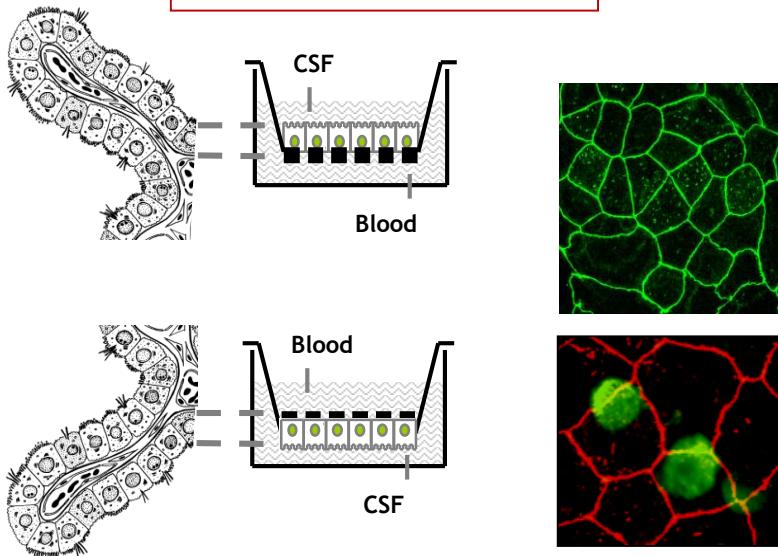


Cellular models of blood-brain interfaces

Primary cultures to obtain an optimal differentiation

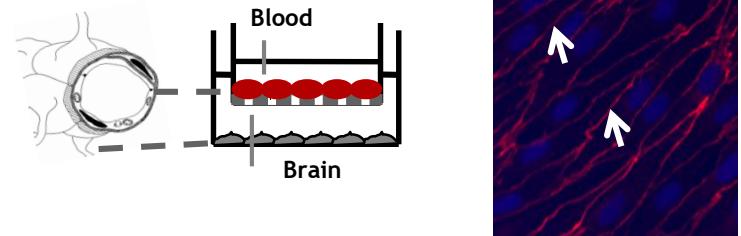
choroid plexus epithelial cells

➤ Blood/CSF barrier
(rat, mouse)



brain capillary endothelial cells

➤ Blood-brain barrier
(rat)

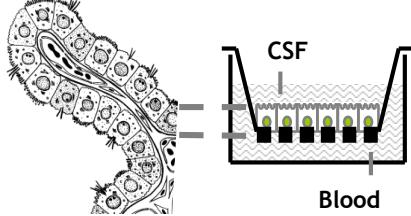


Analytical expertise

- Immunocytology
- Spectrophotometry
- Immunofluorescence
- Protein extraction
- UV-Fluorescence HPLC
- Radiodetection
- RNA isolation
- Elisa

Cellular models of blood-brain interfaces

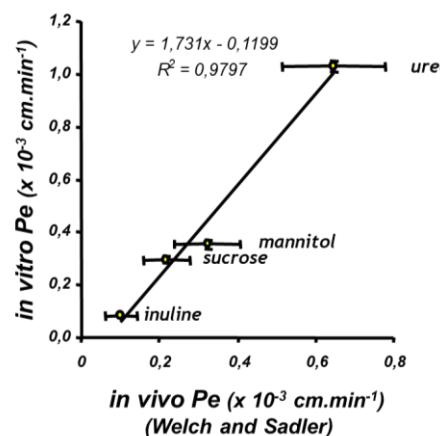
Blood/CSF barrier



Pharmacotoxicological applications

- ✓ Drug delivery
- ✓ Transport

- *In vitro / in vivo correlation of epithelial permeability towards passive diffusion markers*



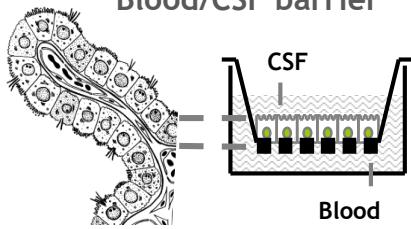
- *Epithelial permeability coefficient (cPe) of antiretroviral nucleoside analogs*

Nucleoside analog	Concentration	Basolateral to apical	Apical to basolateral
AZT	5 µM	0.335 ± 0.030	0.914 ± 0.154 **
	1 mM	0.604 ± 0.020 **	0.644 ± 0.039 *
d4T	5 µM	0.109 ± 0.008	0.174 ± 0.018 **
	1 mM	0.136 ± 0.002 **	0.165 ± 0.012 \$
ddl	5 µM	0.018 ± 0.015	0.106 ± 0.035 **
	1 mM	0.027 ± 0.013	0.058 ± 0.010 **, \$\$
3TC	5 µM	0.024 ± 0.014	0.127 ± 0.025 **
	1 mM	0.035 ± 0.006	0.055 ± 0.004 *, \$\$
ddC	5 µM	-0.009 ± 0.050	0.009 ± 0.028
	1 mM	0.007 ± 0.012	0.035 ± 0.009

- AIDS, 2003, 17:1473-1485
- Eur J Med Chem, 2015, 98:237e249

Cellular models of blood-brain interfaces

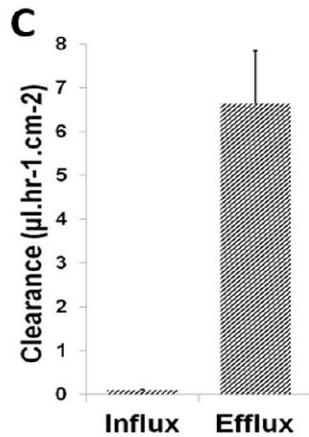
Blood/CSF barrier



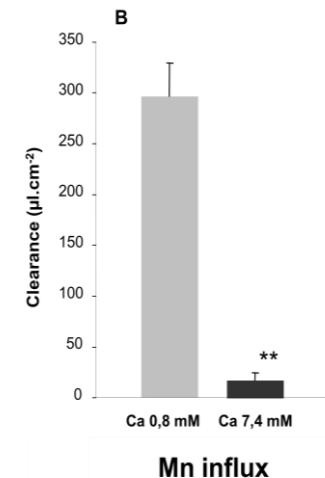
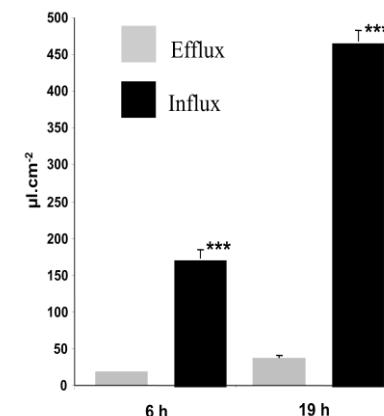
Pharmacotoxicological applications

- ✓ Drug delivery
- ✓ Transport

- IgG transport



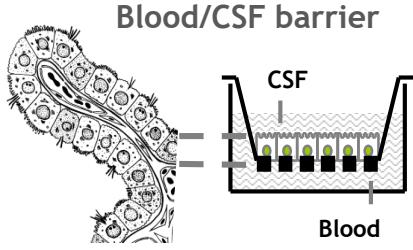
- Manganese transport



- Mol Pharmaceutics, 2013, 10:1473-1491
- J Neurochem, 2011, 117:747-756

Cellular models of blood-brain interfaces

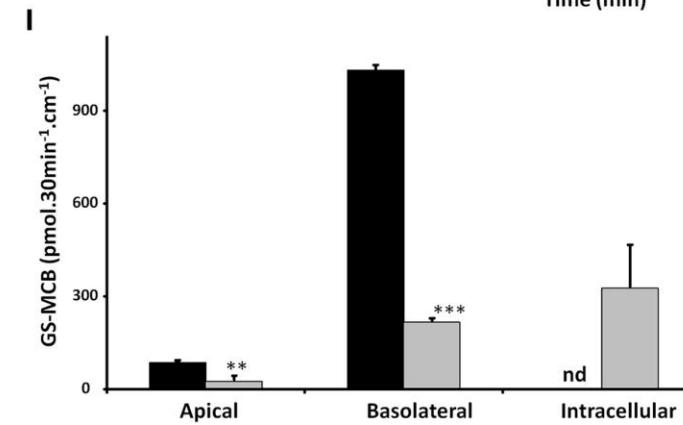
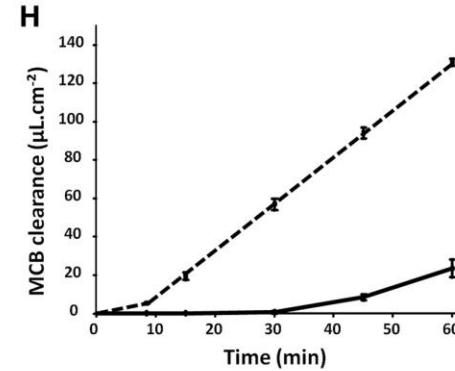
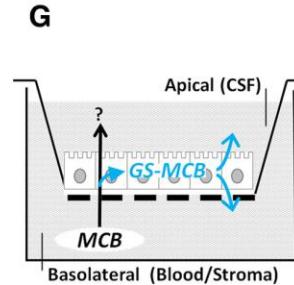
Blood/CSF barrier



Pharmacotoxicological applications

- ✓ Metabolic barrier towards drugs and toxicants

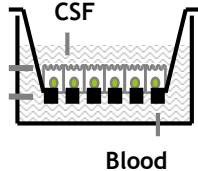
Metabolism en passage of the toxic compound MCB and polarized elimination of its glutathione-conjugated metabolite



- J Neurosci, 2018, 38:3466 -79
- J Cereb Blood Flow Metab, 2006, 26:1165-75
- J Neurosci, 1999, 19:6275-89

Cellular models of blood-brain interfaces

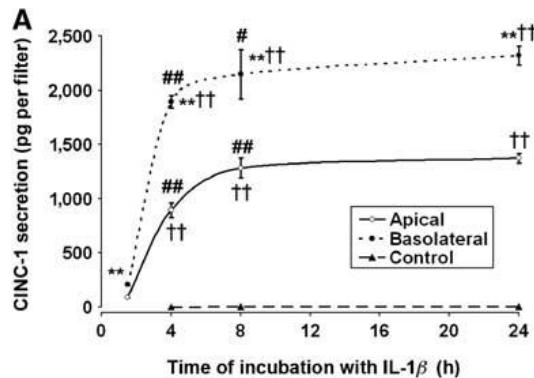
Blood/CSF barrier



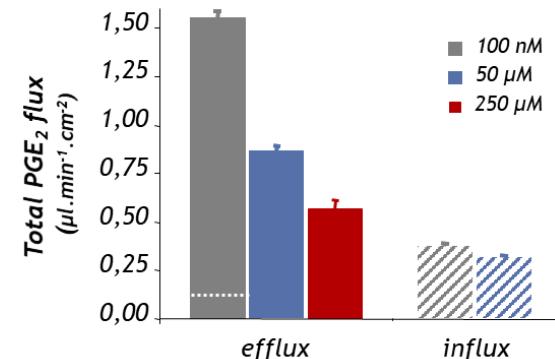
Physiopathological applications

- ✓ Secretion and transport of inflammatory mediators

- Polarity of Cinc-1 secretion induced by the cytokine IL-1 β



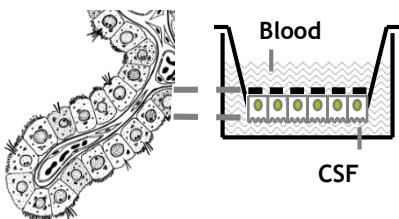
- PGE2 transcellular flux



- In preparation
- J Cereb Blood Flow Metabol 2012, 32:93-104
- J Cereb Blood Flow Metabol 2009, 29:1503-16
- J Infectious Dis 2006, 194:341-9
- J Neurochem 2005, 94:1580-1593

Cellular models of blood-brain interfaces

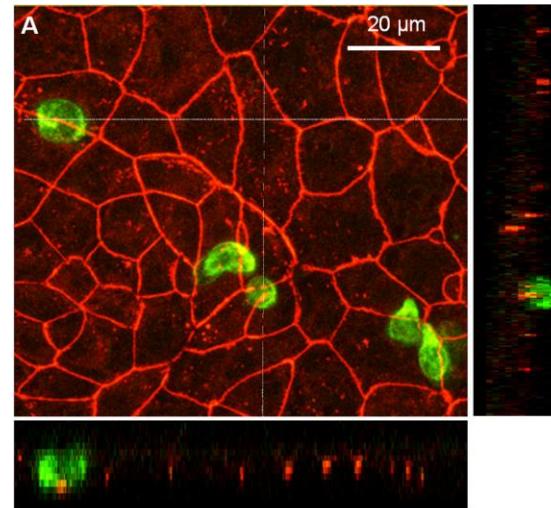
Blood/CSf barrier



Physiopathological applications

- ✓ Cellular infiltration
- ✓ Neuroinfection

Transepithelial traffic of immune cells

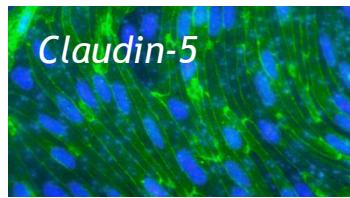
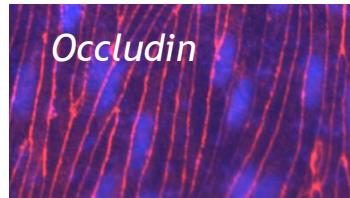
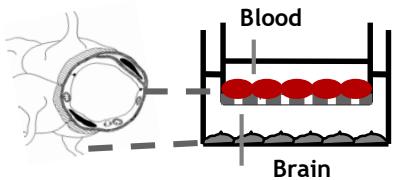


- *Acta Neuropathol Commun.* 2020 Jan 23;8(1):4
- *PLoS ONE* 11(3): e0150945



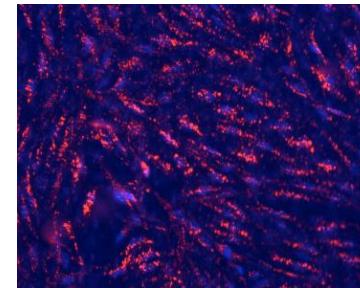
Cellular models of blood-brain interfaces

Blood-brain barrier

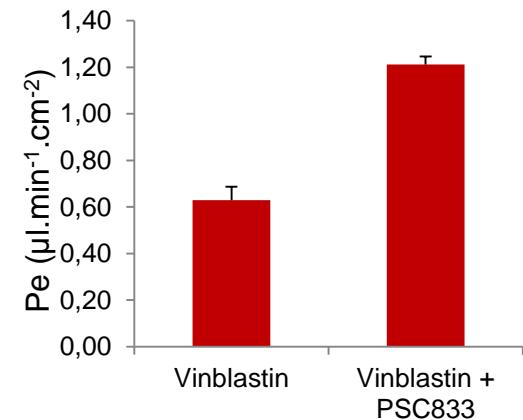
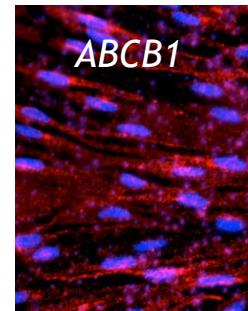


Exemples of molecular transport

- receptor-mediated



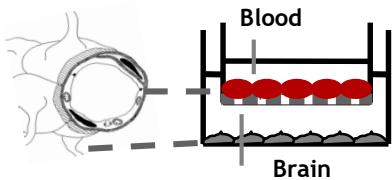
- mediated by SLC/ABC transporters





Cellular models of blood-brain interfaces

Blood-brain barrier

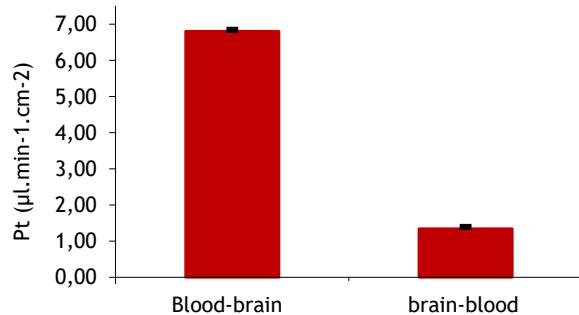


- ✓ Drug delivery
- ✓ Transport
- ✓ Neuroprotection

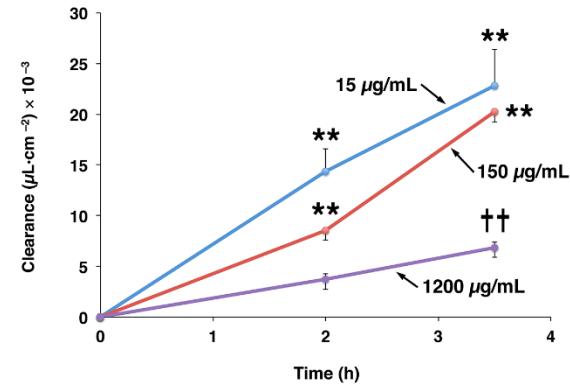
Applications

- ✓ Neuroinflammation
- ✓ Neuroinfection

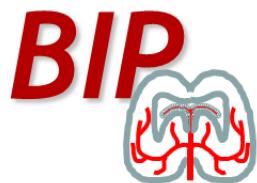
- *Polarized transport of an inflammatory modulator*



- *Saturability of the blood-to-brain transport of a pharmacological polypeptide*



- J Neurotrauma. 2020 doi: 10.1089/neu.2020.7229,
- One article in preparation
- One patent deposited by a partner



Blood-brain interfaces exploratory platform



Instrumentation available on the facility

- *Analytic*

- **HPLC** (equipped with 2 detectors Dual UV and fluorescence, automatic refrigerated sample holder, Shimadzu)
- **Liquid Scintillation** (^{14}C , ^3H) (Tricarb 4910 TR, Packard)
- **Thermostated fluorescence plate reader** (Spark, Tecan)

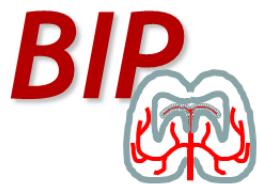
- *Histology and cellular imaging*

- **Cryostat** (NX50, Microm)
- **Fluorescence Macroscopy** (Axiozoom V16, Zeiss)
- **Access to epifluorescence** (Axioimager M.2, Zeiss (FLUID) and confocal (LSM800, Zeiss, CRNL) microscopes
- **Access to light sheet microscope** (Zeiss Spim, IFR Lyon-Est)

- *Molecular analyses through qRT-PCR*

- **Lightcyclers LC 2.0 et LC 480 Roche instrument**





Blood-brain interfaces exploratory platform



Partnerships

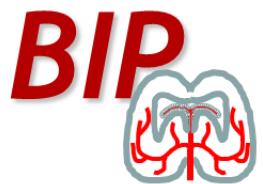
Public laboratories : *Brown University, Göttingen Medicin University, Institut Cochin, Observatoire Français des nocardioses, Université de Lyon, Tokyo University*

Private companies : *Axoltis, Gaoma, Merck-Serono, Neurogen, Novartis, Servier, SAAT Université Grenoble-Alpes, Vect-Horus*

- ✓ **Fee-for-services** :
 - ADR Rhône-Alpes Auvergne INSERM
 - Brain-i
- ✓ **Research contracts** : (quotes, Brain-i)
- ✓ **Collaborations** : Development of innovative blood-brain interfaces-oriented tools and approaches

Institutional fundings EC-FP7, ANR-IHU





Blood-brain interfaces exploratory platform



➤ **in vivo Investigations** (developing rodents)

Permeability constants Blood/CSF and Blood/Brain

$$K_{in\ csf} = C_t / AUC_{0 \rightarrow t}$$

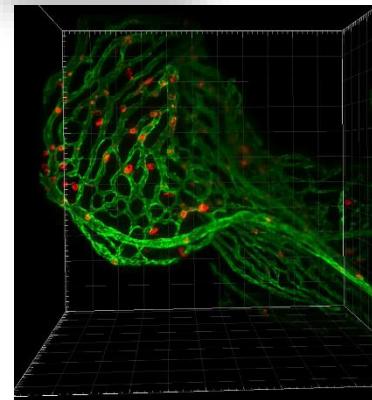
$$K_{w\ csf} = K_{in\ csf} \times V_{csf}$$

$$K_{in\ cx,\ pons,\ cb}$$



Cell tracking at the blood-CSF barrier

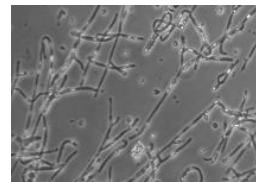
- *Acta Neuropathol Commun* 2020, 8:4
- *J Neurosci.* 2018, 38:3466-3479
- *Fluids Barriers CNS* 2015, 12:8.



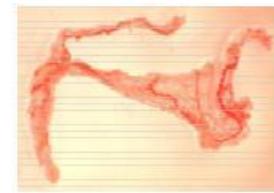
➤ Ex vivo models of blood-brain interfaces

**Isolated cerebral microvessels and choroid plexuses (live tissue),
from healthy and diseased animals**

- Molecular studies
(transcriptomic, proteomic)



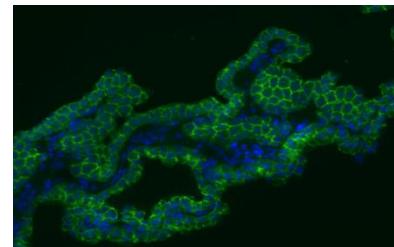
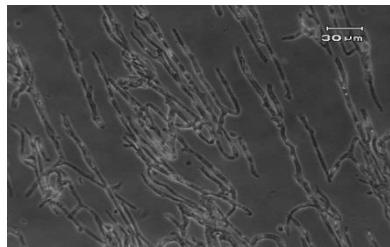
- Functional studies
(uptake, metabolism, secretion)



Sci Rep 2019, 9:5998; Am J Physiol Cell Physiol 2018, 315:C445-C456; J Neurosci. 2018, 38:3466-3479; Front Neurosci 2015, 9:123; Fluids Barriers CNS 2013, 10:25; PLoS One 2013, ;8:e65629; Histochem Cell Biol; 2012,138:861-79; J Neurochem 2011, 117:747-56; ...

➤ Primate tissue bank (*Partnership : Cynbiose*)

Macaca fascicularis (mRNA, proteins, paraffin sections, cryopreserved tissue, validated through quality control



Analytical tools

- Immuno-cytology
- Immuno-fluorescence
- Protein extraction
- UV/fluorescence-HPLC
- Radiodetection
- RNA isolation
- Elisa

Selected publications BIP : Developed Méthodologies, exemple of applications, expert reviews.

Strazielle N, Ghersi-Egea JF. Demonstration of a coupled metabolism-efflux process at the choroid plexus as a mechanism of brain protection toward xenobiotics. *J Neurosci*. 1999 Aug 1;19(15):6275-89

Strazielle N, Ghersi-Egea JF, Ghiso J, Dehouck MP, Frangione B, Patlak C, Fenstermacher J, Gorevic P. In vitro evidence that beta-amyloid peptide 1-40 diffuses across the blood-brain barrier and affects its permeability. *J Neuropathol Exp Neurol*. 2000 Jan;59(1):29-38

Strazielle N, Belin MF, Ghersi-Egea JF. Choroid plexus controls brain availability of anti-HIV nucleoside analogs via pharmacologically inhibitable organic anion transporters. *AIDS*. 2003 Jul 4;17(10):1473-85.

Strazielle N, Khuth ST, Murat A, Chalon A, Giraudon P, Belin MF, Ghersi-Egea JF. Pro-inflammatory cytokines modulate matrix metalloproteinase secretion and organic anion transport at the blood-cerebrospinal fluid barrier. *J Neuropathol Exp Neurol*. 2003 Dec;62(12):1254-64

Khuth ST, Strazielle N, Giraudon P, Belin MF, Ghersi-Egea JF. Impairment of blood-cerebrospinal fluid barrier properties by retrovirus-activated T lymphocytes: reduction in cerebrospinal fluid-to-blood efflux of prostaglandin E2. *J Neurochem*. 2005 Sep;94(6):1580-93. Epub 2005 Jul 18.

Ghersi-Egea JF, Strazielle N, Murat A, Jouvet A, Buénerd A, Belin MF. Brain protection at the blood-cerebrospinal fluid interface involves a glutathione-dependent metabolic barrier mechanism. *J Cereb Blood Flow Metab*. 2006 Sep;26(9):1165-75. Epub 2006 Jan 4.

Batisson M, Strazielle N, Hejmadi M, Thomas D, Ghersi-Egea JF, Etienne J, Vandenesch F, Lina G. Toxic shock syndrome toxin-1 challenges the neuroprotective functions of the choroidal epithelium and induces neurotoxicity. *J Infect Dis*. 2006 Aug 1;194(3):341-9. Epub 2006 Jun 30.

Gazzin S, Strazielle N, Schmitt C, Fevre-Montange M, Ostrow JD, Tiribelli C, Ghersi-Egea JF. Differential expression of the multidrug resistance-related proteins ABCb1 and ABCc1 between blood-brain interfaces. *J Comp Neurol*. 2008 Oct 10;510(5):497-507.

Szmydynger-Chodobska J, Strazielle N, Zink BJ, Ghersi-Egea JF, Chodobski A. The role of the choroid plexus in neutrophil invasion after traumatic brain injury. *J Cereb Blood Flow Metab*. 2009 Sep;29(9):1503-16.

Ginguéné C, Champier J, Maallem S, Strazielle N, Jouvet A, Fèvre-Montange M, Ghersi-Egea JF. P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) localize in the microvessels forming the blood-tumor barrier in ependymomas. *Brain Pathol*. 2010 Sep;20(5):926-35

Schmitt C, Strazielle N, Richaud P, Bouron A, Ghersi-Egea JF. Active transport at the blood-CSF barrier contributes to manganese influx into the brain. *J Neurochem*. 2011 May;117(4):747-56.

Gazzin S, Berengeno AL, Strazielle N, Fazzari F, Raseni A, Ostrow JD, Wennberg R, Ghersi-Egea JF, Tiribelli C. Modulation of Mrp1 (ABCc1) and Pgp (ABCb1) by bilirubin at the blood-CSF and blood-brain barriers in the Gunn rat. *PLoS One*. 2011 Jan 31;6(1):e16165.

Szmydynger-Chodobska J, Strazielle N, Gandy JR, Keefe TH, Zink BJ, Ghersi-Egea JF, Chodobski A. Posttraumatic invasion of monocytes across the blood-cerebrospinal fluid barrier. *J Cereb Blood Flow Metab*. 2012 Jan;32(1):93-104.

Selected publications BIP : Developed Méthodologies, exemple of applications, expert reviews.

- Kratzer I, Vasiljevic A, Rey C, Fevre-Montange M, Saunders N, Strazielle N, Ghersi-Egea JF. Complexity and developmental changes in the expression pattern of claudins at the blood-CSF barrier. *Histochem Cell Biol*. 2012 Dec;138(6):861-79.
- Schmitt C, Strazielle N, Ghersi-Egea JF. Brain leukocyte infiltration initiated by peripheral inflammation or experimental autoimmune encephalomyelitis occurs through pathways connected to the CSF-filled compartments of the forebrain and midbrain. *J Neuroinflammation*. 2012 Aug 7;9:187.
- Strazielle N, Ghersi-Egea JF. Physiology of blood-brain interfaces in relation to brain disposition of small compounds and macromolecules. *Mol Pharm*. 2013 May 6;10(5):1473-91.
- Kratzer I, Liddelow SA, Saunders NR, Dziegielewska KM, Strazielle N, Ghersi-Egea JF. Developmental changes in the transcriptome of the rat choroid plexus in relation to neuroprotection. *Fluids Barriers CNS*. 2013 Aug 1;10(1):25.
- Badaut J and Ghersi-Egea JF. The Choroid Plexus and Cerebrospinal Fluid system: roles in neurodegenerative diseases. In Neman J and Chen TC, *The Choroid Plexus and Cerebrospinal Fluid: Emerging Roles in CNS Development, Maintenance, and Disease Progression*, Elsevier, Academic press, San Diego, 2014.
- Ghersi-Egea JF, Babikian A, Blondel S, Strazielle N. Changes in the cerebrospinal fluid circulatory system of the developing rat: quantitative volumetric analysis and effect on blood-CSF permeability interpretation. *Fluids Barriers CNS*. 2015 Mar 10;12:8.
- Cecioni S, Aouadi K, Guiard J, Parrot S, Strazielle N, Blondel S, Ghersi-Egea JF, Chapelle C, Denoroy L, Praly JP. Novel routes to either racemic or enantiopure α -amino-(4-hydroxy-pyrrolidin-3-yl)acetic acid derivatives and biological evaluation of a new promising pharmacological scaffold. *Eur J Med Chem*. 2015 Jun 15;98:237-49.
- Saunders NR, Dziegielewska KM, Møllgård K, Habgood MD, Wakefield MJ, Lindsay H, Stratzielle N, Ghersi-Egea J-F and Liddelow SA. Influx mechanisms in the embryonic and adult rat choroid plexus: a transcriptome study. *Front. Neurosci*. 2015 9:123.
- Moretti R, Pansiot J, Bettati D, Strazielle N, Ghersi-Egea JF, Damante G, Fleiss B, Titomanlio L, Gressens P. Blood-brain barrier dysfunction in disorders of the developing brain. *Front Neurosci*. 2015 Feb 17;9:40.
- Strazielle N, Ghersi-Egea JF. Efflux transporters in blood-brain interfaces of the developing brain. *Front Neurosci*. 2015 Feb 5;9:21.
- Ghersi-Egea JF and Damkier H. Blood-brain interfaces organization in relation to inorganic anion transport, CSF secretion and circulation. In *Brain Edema: From Molecular Mechanisms to Clinical Practice*. J Badaut and N Plesnila eds. Oxford: Academic Press, 2017, pp. 29-48.
- Strazielle N, Creidy R, Malcus C, Boucraut J, Ghersi-Egea JF. T-Lymphocytes Traffic into the Brain across the Blood-CSF Barrier: Evidence Using a Reconstituted Choroid Plexus Epithelium. *PLoS One*. 2016 Mar 4;11(3):e0150945.
- Virgone-Carlotta A, Dufour E, Bacot S, Ahmadi M, Cornou M, Moni L, Garcia J, Chierici S, Garin D, Marti-Batlle D, Perret P, Ghersi-Egea JF, Moulin Sallanon M, Fagret D, Ghezzi C. New diketopiperazines as vectors for peptide protection and brain delivery: Synthesis and biological evaluation. *J Labelled Comp Radiopharm*. 2016 Oct;59(12):517-530.

Selected publications BIP : Developed Méthodologies, exemple of applications, expert reviews.

Strazielle N, Ghersi-Egea JF. Potential Pathways for CNS Drug Delivery Across the Blood-Cerebrospinal Fluid Barrier. *Curr Pharm Des.* 2016;22(35):5463-5476.

Ghersi-Egea JF, Strazielle N, Catala M, Silva-Vargas V, Doetsch F, Engelhardt B. Molecular anatomy and functions of the choroidal blood-cerebrospinal fluid barrier in health and disease. *Acta Neuropathol.* 2018 Mar;135(3):337-361.

Kratzer I, Strazielle N, Saudrais E, Mönkkönen K, Maleval C, Blondel S, Ghersi-Egea JF. Glutathione conjugation at the blood-CSF barrier efficiently prevents exposure of the developing brain fluid environment to blood-borne reactive electrophilic substances. *J Neurosci.* 2018 38(14):3466 -3479.

Ghersi-Egea JF, Saudrais E, and Strazielle N. Barriers to drug distribution into the perinatal and postnatal brain. *Pharm Res.* 2018 Mar 7;35(4):84.

Saudrais E, Strazielle N, Ghersi-Egea JF. Choroid plexus glutathione peroxidases are instrumental in protecting the brain fluid environment from hydroperoxides during postnatal development. *Am J Physiol Cell Physiol.* 2018 Oct 1;315(4):C445-C456.

Koehn LM, Dziegielewska KM, Møllgård K, Saudrais E, Strazielle N, Ghersi-Egea JF, Saunders NR, Habgood MD. Developmental differences in the expression of ABC transporters at rat brain barrier interfaces following chronic exposure to diallyl sulfide. *Sci Rep.* 2019 Apr 12;9(1):5998.

Mottahedin A, Blondel S, Ek J, Leverin AL, Svedin P, Hagberg H, Mallard C, Ghersi-Egea JF, Strazielle N. N-acetylcysteine inhibits bacterial lipopeptide-mediated neutrophil transmigration through the choroid plexus in the developing brain. *Acta Neuropathol Commun.* 2020 Jan 23;8(1):4.

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