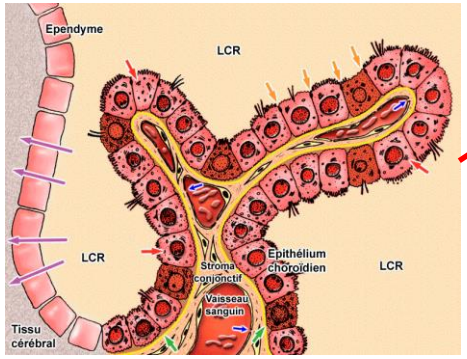
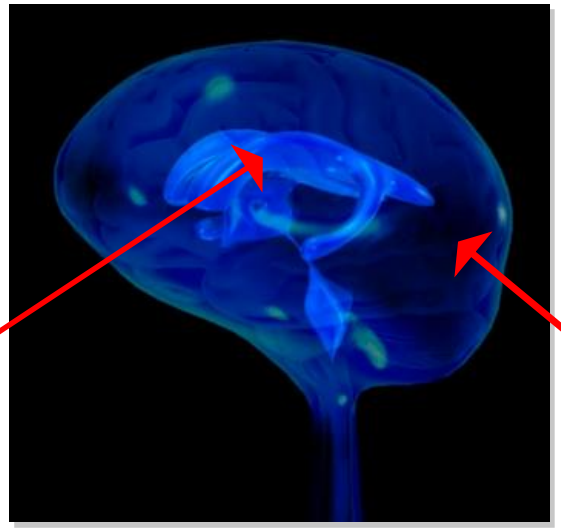
Blood-brain interfaces exploratory platform

Two main interfaces between the blood and the central nervous system





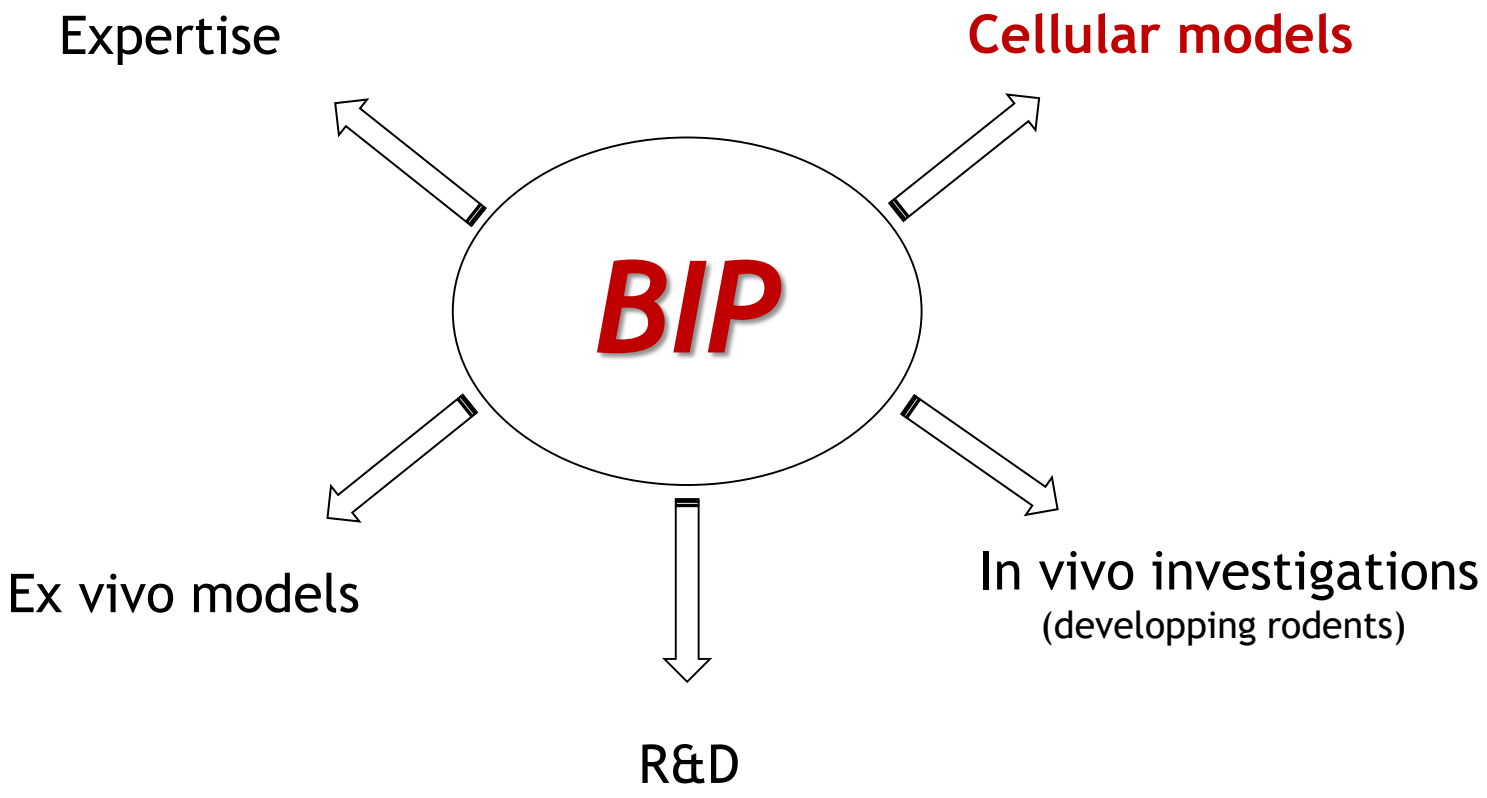
Blood-cerebrospinal fluid barrier (choroid plexuses)

Blood-brain parenchyma barrier (microvessels)

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

Blood-brain interfaces exploratory platform

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



Blood-brain interfaces exploratory platform

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Nathalie Strazielle, PharmD, PhD

ns.brain.i@gmail.com

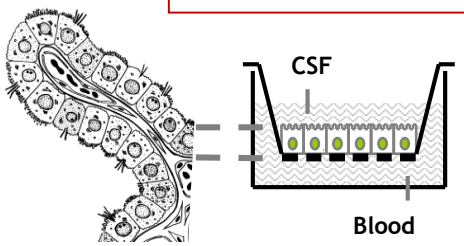
Blood-brain interfaces exploratory platform

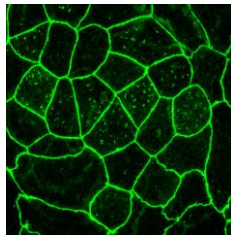
Cellular models of blood-brain interfaces

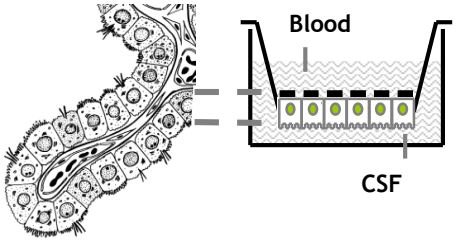
Primary cultures to obtain an optimal differentiation

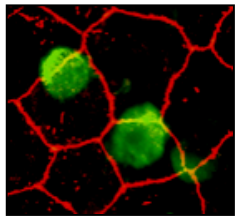
choroid plexus epithelial cells

➢ Blood/CSF barrier
rat, mouse



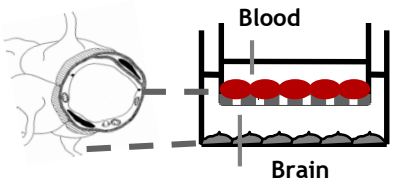


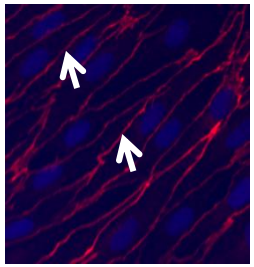




brain capillary endothelial cells

➢ Blood-brain barrier
rat, monkey in partnership with CYNBIOSE.



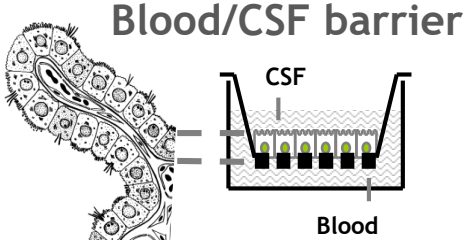


Analytical expertise

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

Blood-brain interfaces exploratory platform

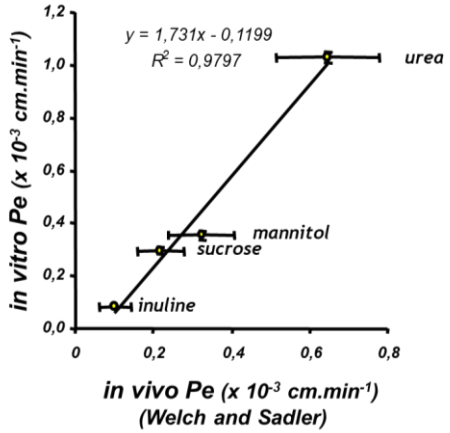
Cellular models of blood-brain interfaces



Pharmacotoxicological applications

- ✓ Drug delivery
- ✓ Transport

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



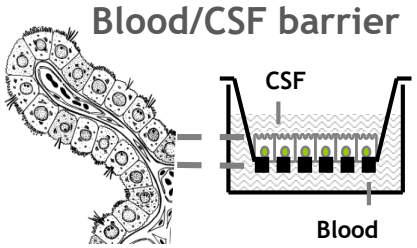
- *Epithelial permeability coefficient (cPe) of antiretroviral nucleoside analogs*

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	$
ddI	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*

Blood-brain interfaces exploratory platform

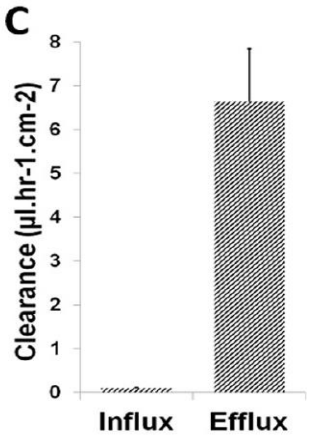
Cellular models of blood-brain interfaces



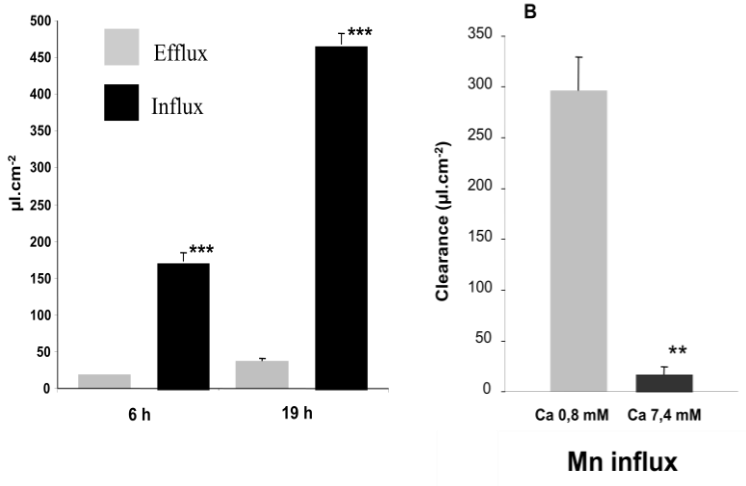
Pharmacotoxicological applications

- ✓ Drug delivery
- ✓ Transport

- ***IgG transport***



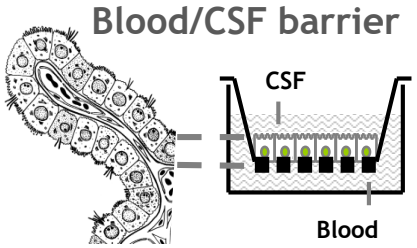
- ***Manganese transport***



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

Blood-brain interfaces exploratory platform

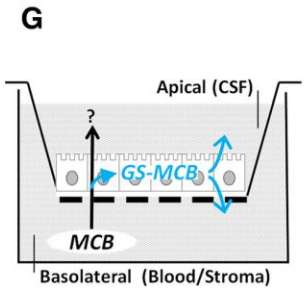
Cellular models of blood-brain interfaces

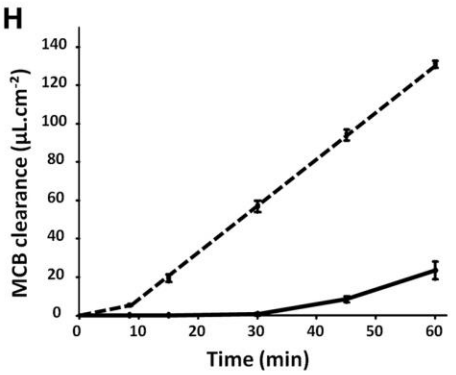


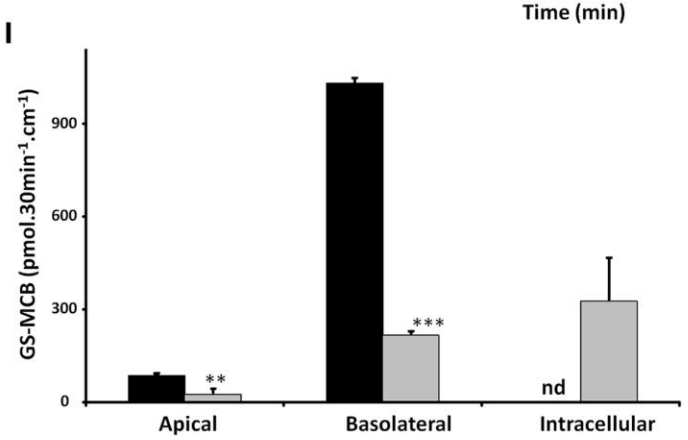
Pharmacotoxicological applications

- ✓ Metabolic barrier towards drugs and toxicants

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



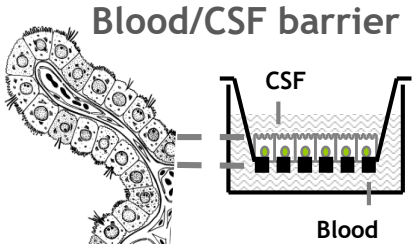




- *Article in preparation*
- *J Neurosci, 2018, 38:3466 -79*
- *J Cereb Blood Flow Metab, 2006, 26:1165-75*
- *J Neurosci, 1999, 19:6275-89*

Blood-brain interfaces exploratory platform

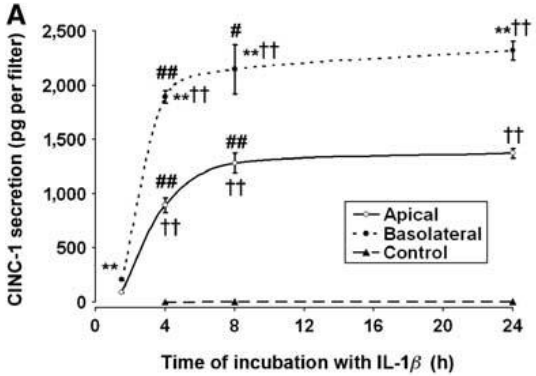
Cellular models of blood-brain interfaces



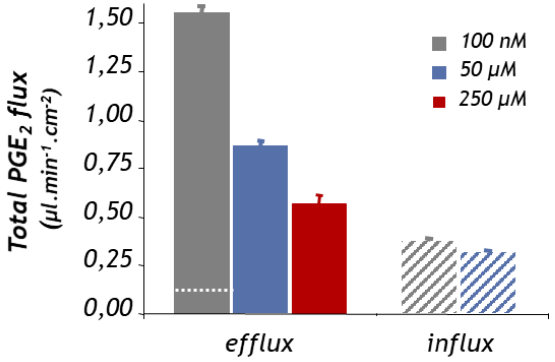
Physiopathological applications

- ✓ Secretion and transport of inflammatory mediators

- **Polarity of Cinc-1 secretion induced by the cytokine Il-1**



- **PGE2 transcellular flux**

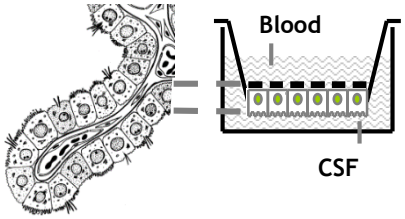


- *Article in preparation*
- *J Cereb Blood Flow Metabol 2012, 32:93-104*
- *J Cereb Blood Flow Metabol 2009, 29:1503-16*
- *J Infectious Dis 2006, 194:341-9*
- *J Neurochem 2005, 94:1580-1593*

Blood-brain interfaces exploratory platform

Cellular models of blood-brain interfaces

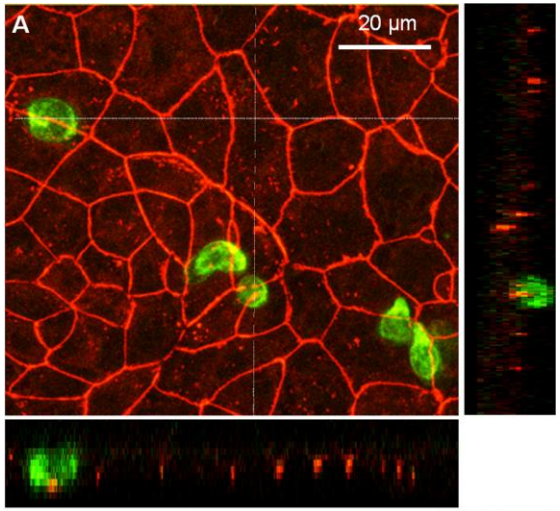
Blood/CSF barrier



Physiopathological applications

- ✓ Cellular infiltration
- ✓ Neuroinfection

Transepithélial traffic of immune cells

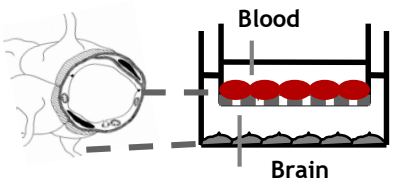


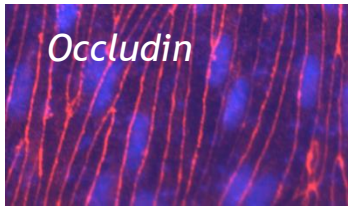
- *Fluids Barriers CNS. 2024 Aug 16;21(1):66.*
- *Acta Neuropathol Commun. 2020 Jan 23;8(1):4*
- *PLoS ONE 11(3): e0150945*

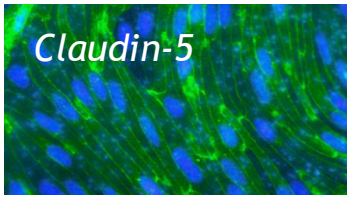
Blood-brain interfaces exploratory platform

Cellular models of blood-brain interfaces

Blood-brain barrier (rat, monkey)

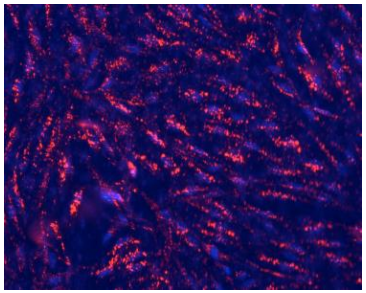




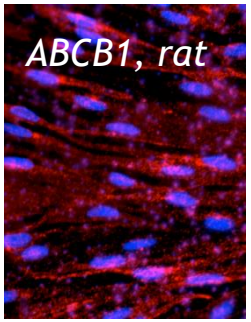


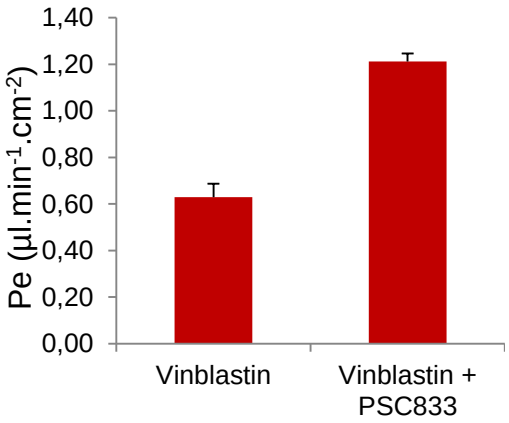
Exemples of molecular transport

- *receptor-mediated*



- *mediated by SLC/ABC transporters*

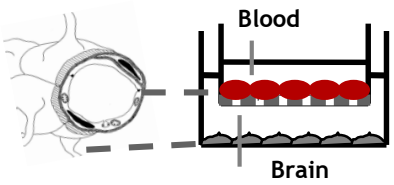




Blood-brain interfaces exploratory platform

Cellular models of blood-brain interfaces

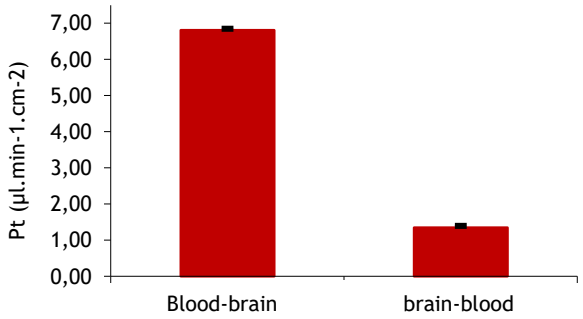
Blood-brain barrier



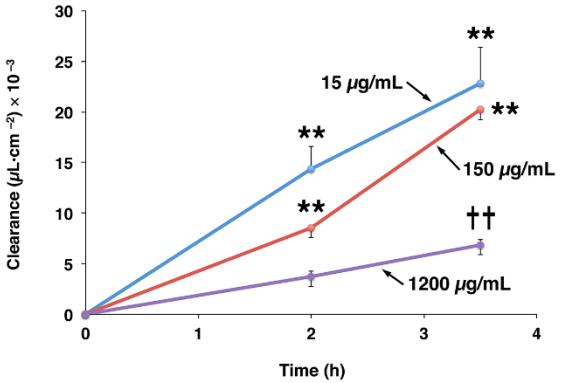
Applications

- ✓ Drug delivery
- ✓ Transport
- ✓ Neuroprotection
- ✓ Neuroinflammation
- ✓ Neuroinfection

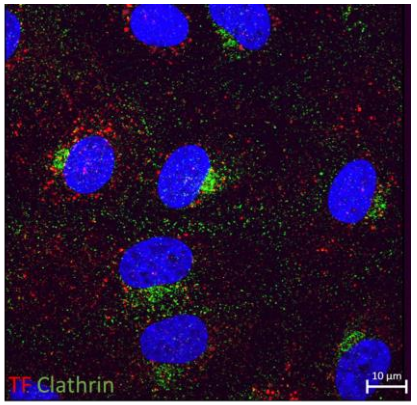
- *Polarized transport of an inflammatory modulator*



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



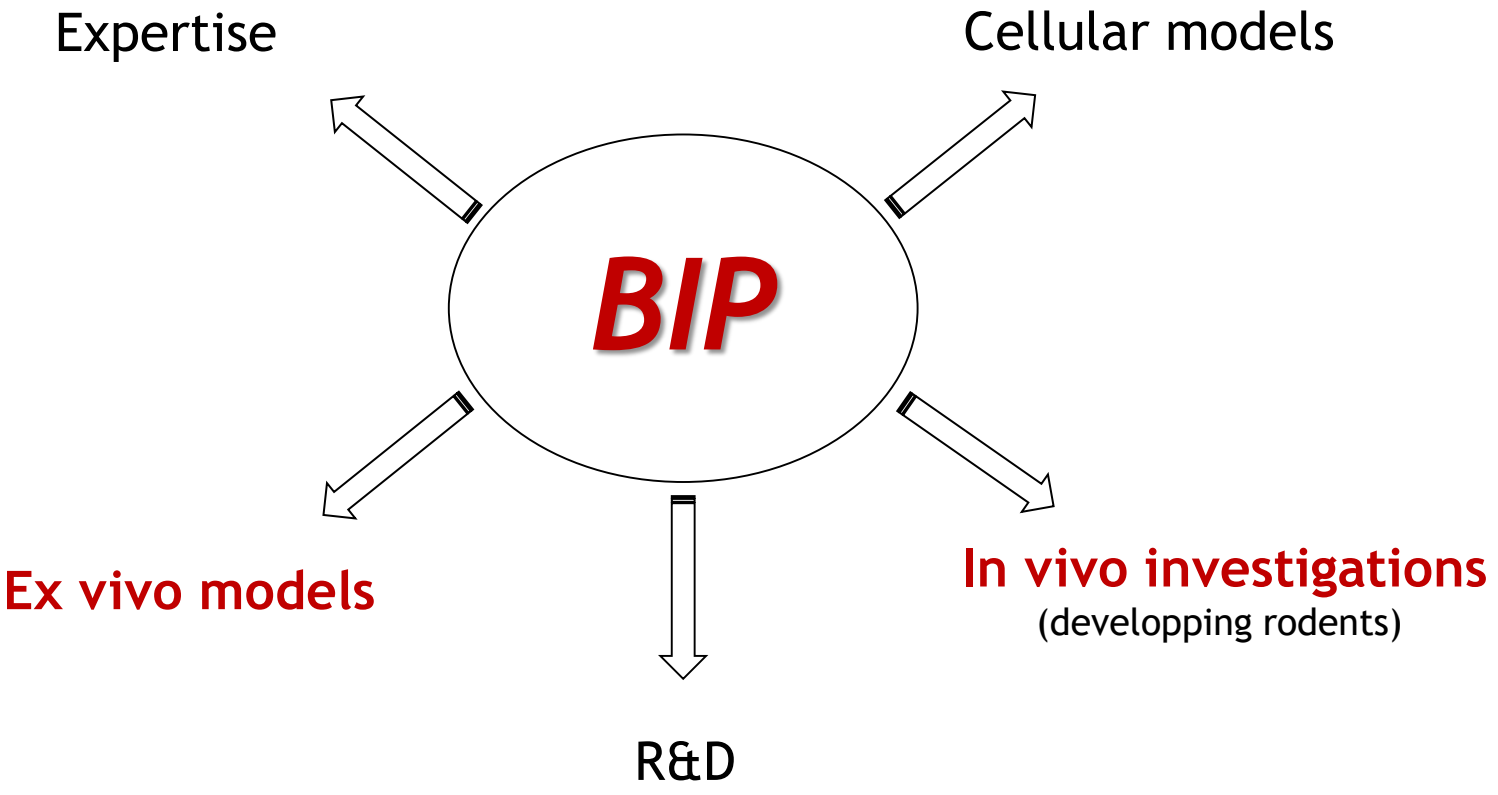
- *Quantitative analysis of endosomal sorting following receptor-mediated endocytosis*



- Articles in preparation
- J Neurotrauma. 2021 Feb 15;38(4):385-398,
- One patent deposited by a partner

Blood-brain interfaces exploratory platform

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



Blood-brain interfaces exploratory platform

in vivo Investigations (developing rodents)

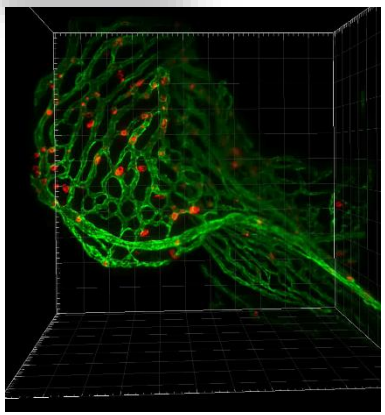
Permeability constants
between Blood and CSF, blood and brain

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

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

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

Cell tracking at the blood-CSF barrier



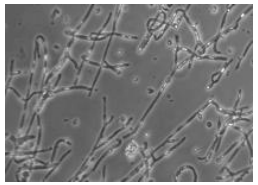
- *Fluids Barriers CNS. 2022 Jun 7;19(1):47*
- *Acta Neuropathol Commun 2020, 8:4*
- *J Neurosci. 2018, 38:3466-3479*
- *Fluids Barriers CNS 2015, 12:8.*

Blood-brain interfaces exploratory platform

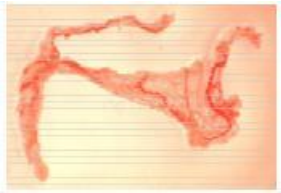
Ex vivo models of blood-brain interfaces

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

- Molecular studies
  (transcriptomic, proteomic)



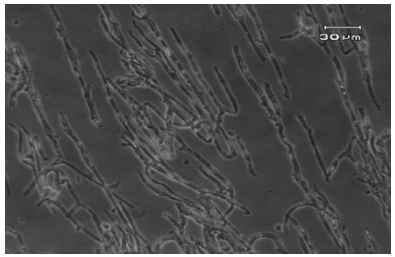
- Functional studies
  (uptake, metabolism, secretion)

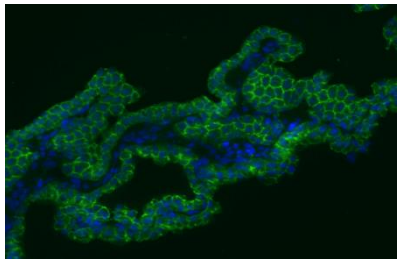


Sci Rep 2019, 9:5998; Am J Physiol Cell Physiol 2018, 315:C445-C456; J Neurosci. 2018, 38:3466-3479; Front Neurosci 2015, 9:123; Fluids Barriers CNS 2013, 10:25; PLoS One 2013, ;8:e65629; Histochem Cell Biol; 2012,138:861-79; J Neurochem 2011, 117:747-56; …

➢ **Primate tissue bank** *Partnership :* CYNBIOSE.

Macaca fascicularis *(mRNA, proteins, paraffin sections, cryopreserved tissue, validated through quality control* Front Pharmacol, 2025, Mar 12:16:1523819





Analytical tools

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

Blood-brain interfaces exploratory platform

Instrumentation available on the facility

- *Analytic*
  - ***HPLC*** *(equipped with 2 detectors Dual UV and fluorescence, a mass detector (available in September 2024), automatic refrigerated sample holder, Shimadzu)*
  - ***Liquid Scintillation*** *(^{14}C, ^{3}H) (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*
  - **Lightcycler** *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, Discoveric-Bio, *Gaoma, Merck-Serono, Neurogen, Novartis, Servier,* SAAT Université Grenoble-Alpes, SAAT Toulouse, 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

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.

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