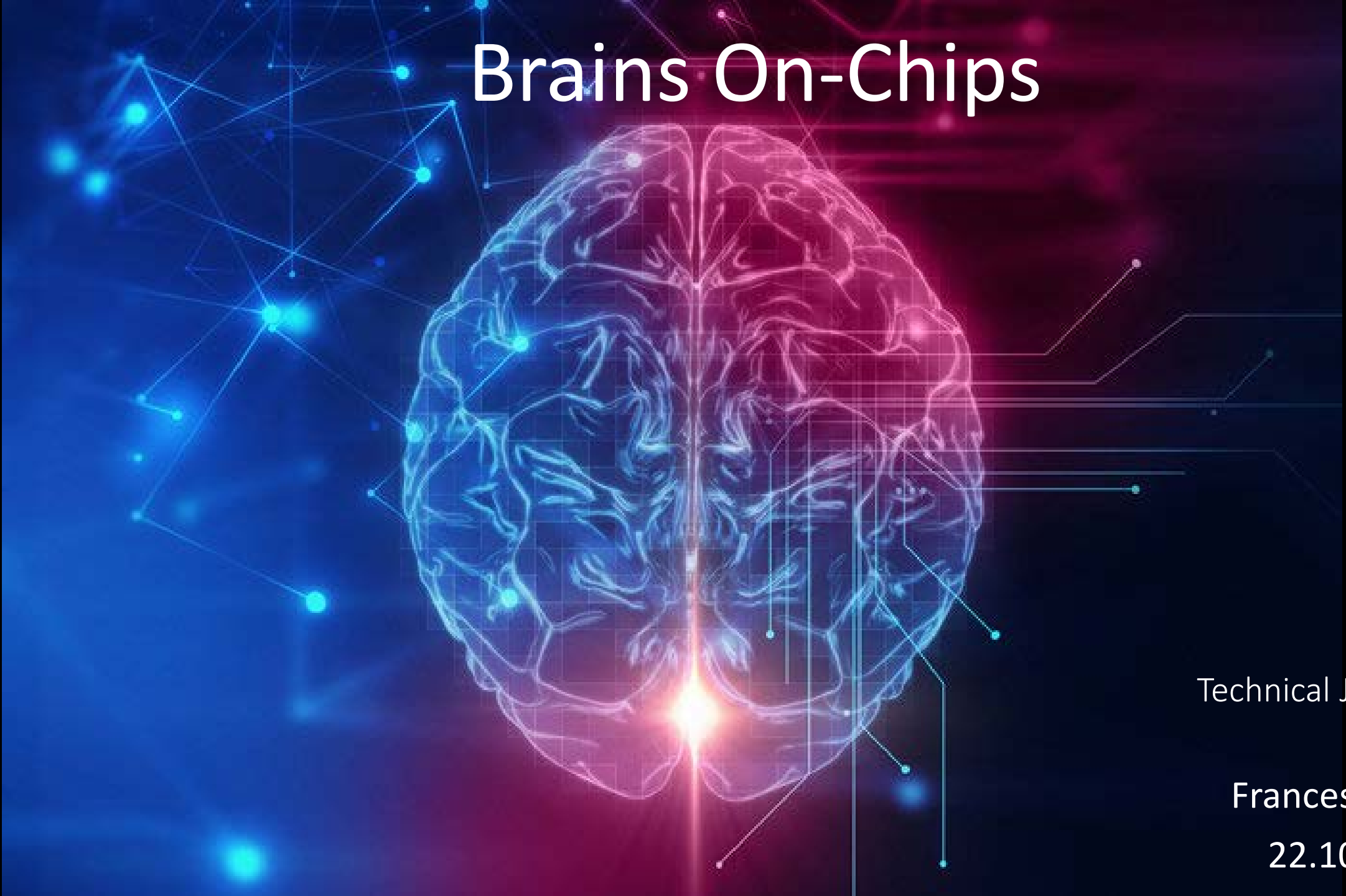
Brains On-Chips



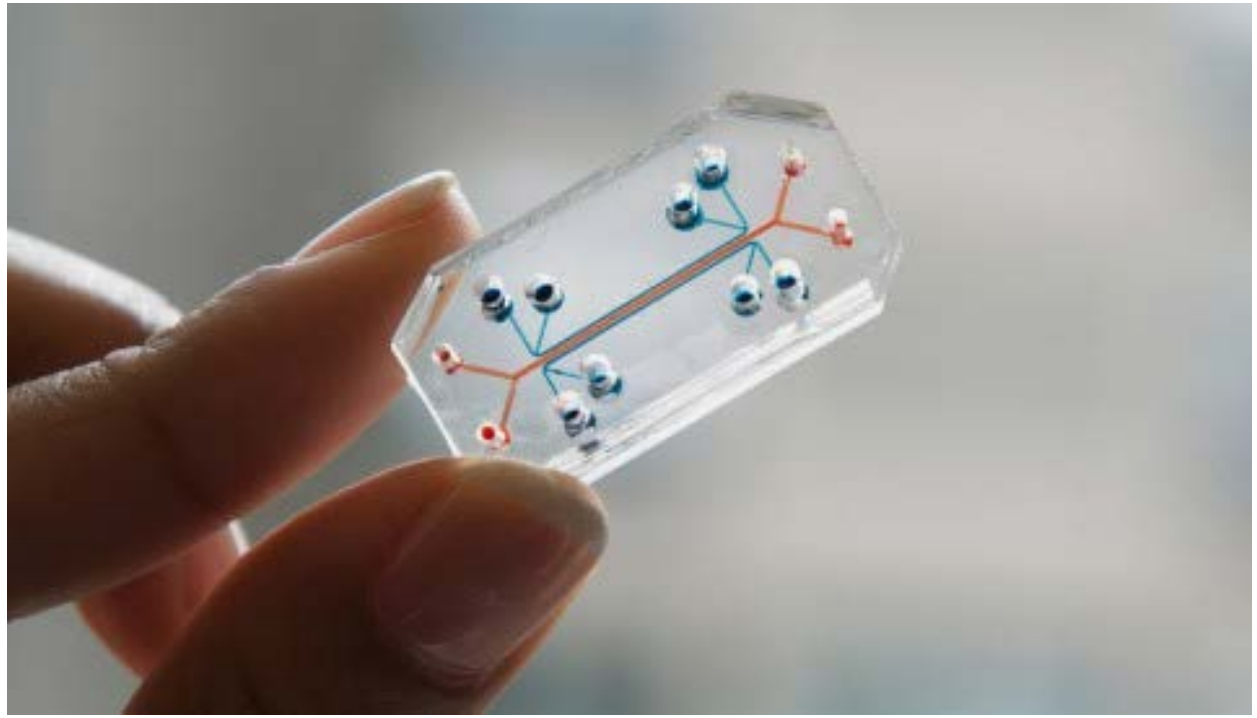
Technical Journal Club

Francesca Catto

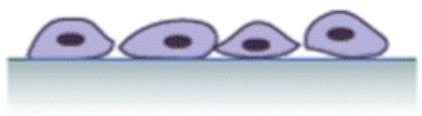
22.10.2019

What is an organ-on-chip?

- It is a multichannel 3-D microfluidic cell culture chip which simulates the physiological activities of the organ.
- They are translucent and provide a window into the inner workings of the organs.
- The idea is to create the smallest functional unit in a microenvironment that imitates the human body.

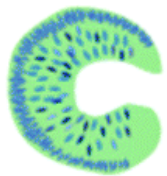


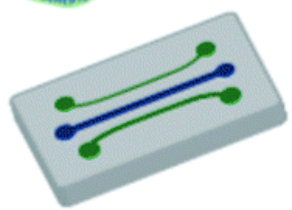
Biological Model Systems



2D cell culture

3D cell culture





Organoids
Organ-on-a-chip

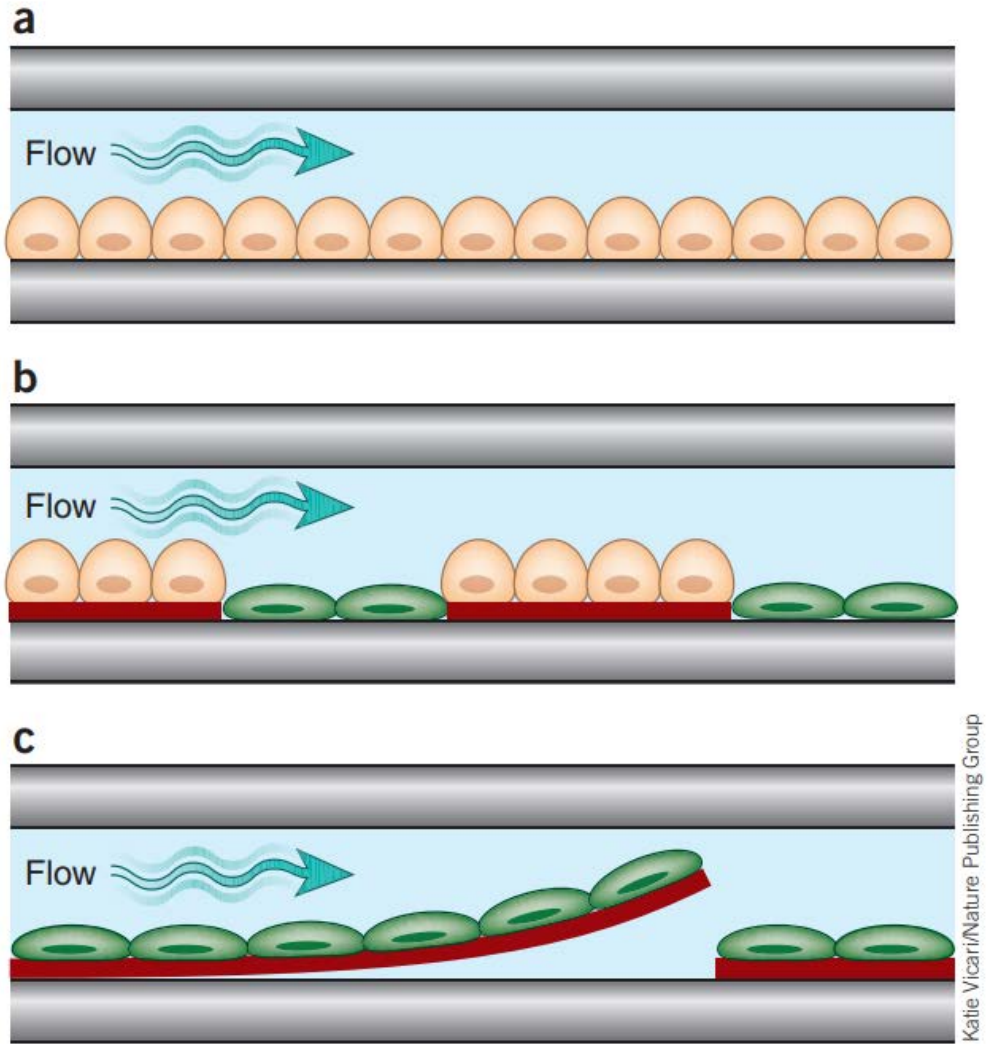
Model organisms

Humans

Experimental Tractability

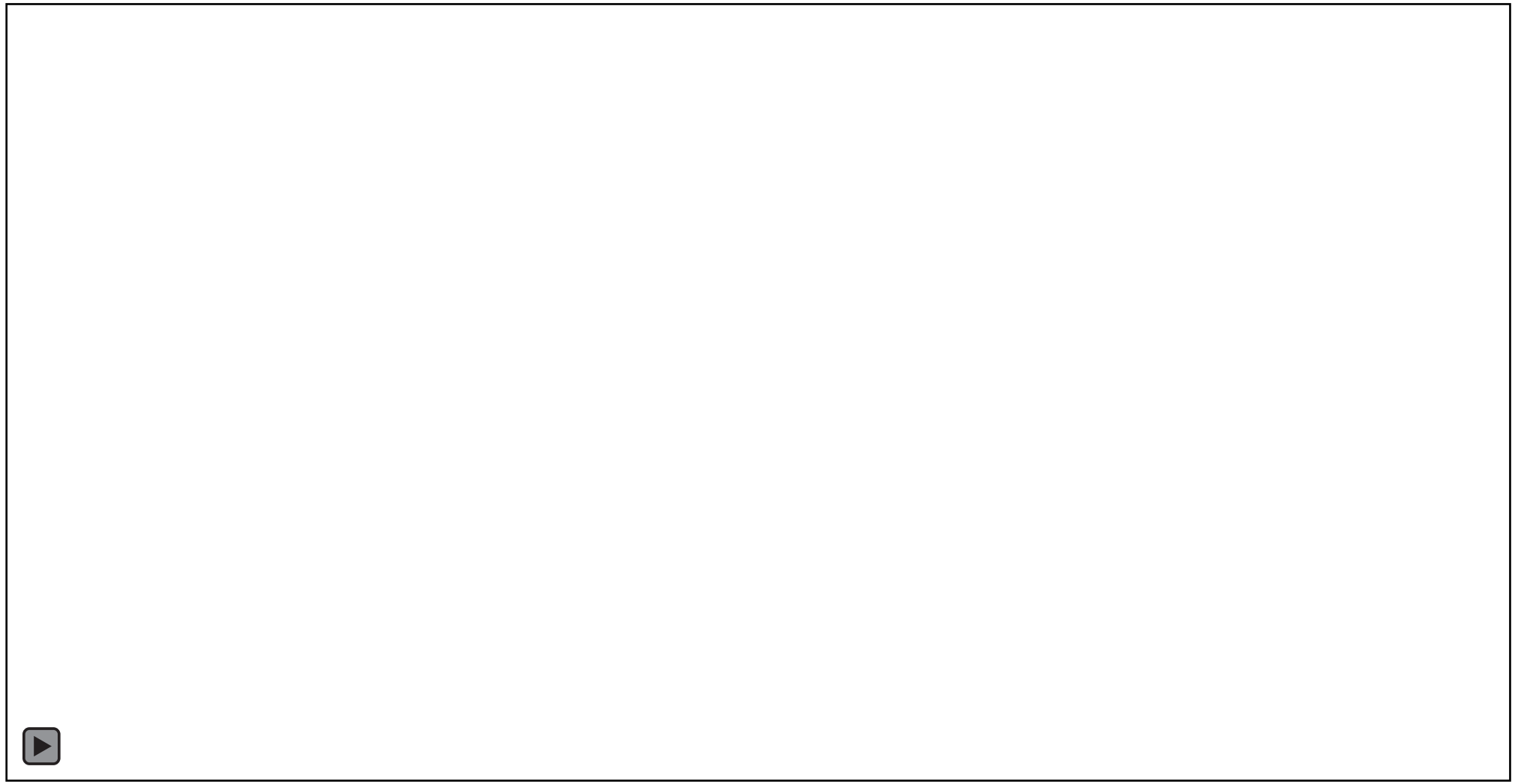
Physiological Relevance

Microfluidic Organ-on-chip culture technology



- Cells of a single type are cultured as a monolayer on a planar rigid (e.g., glass) or flexible (e.g., polydimethylsiloxane, PDMS) substrate on one side of a microfluidic channel through which medium is perfused.
- Cells of two types are cultured in direct juxtaposition by micropatterning ECM adhesive islands within the microfluidic chamber that preferentially support one cell population (e.g., hepatocyte).
- Cells in a tissue construct engineered with ECM are cultured in a microfluidic channel. In this example, microcontact printing of ECM in a linear pattern on a thin PDMS layer coated over the substrate is used to orient muscle cells to create an anisotropic muscle tissue layer.

Microfluidic **Organ-on-chip** culture technology



Paper #1

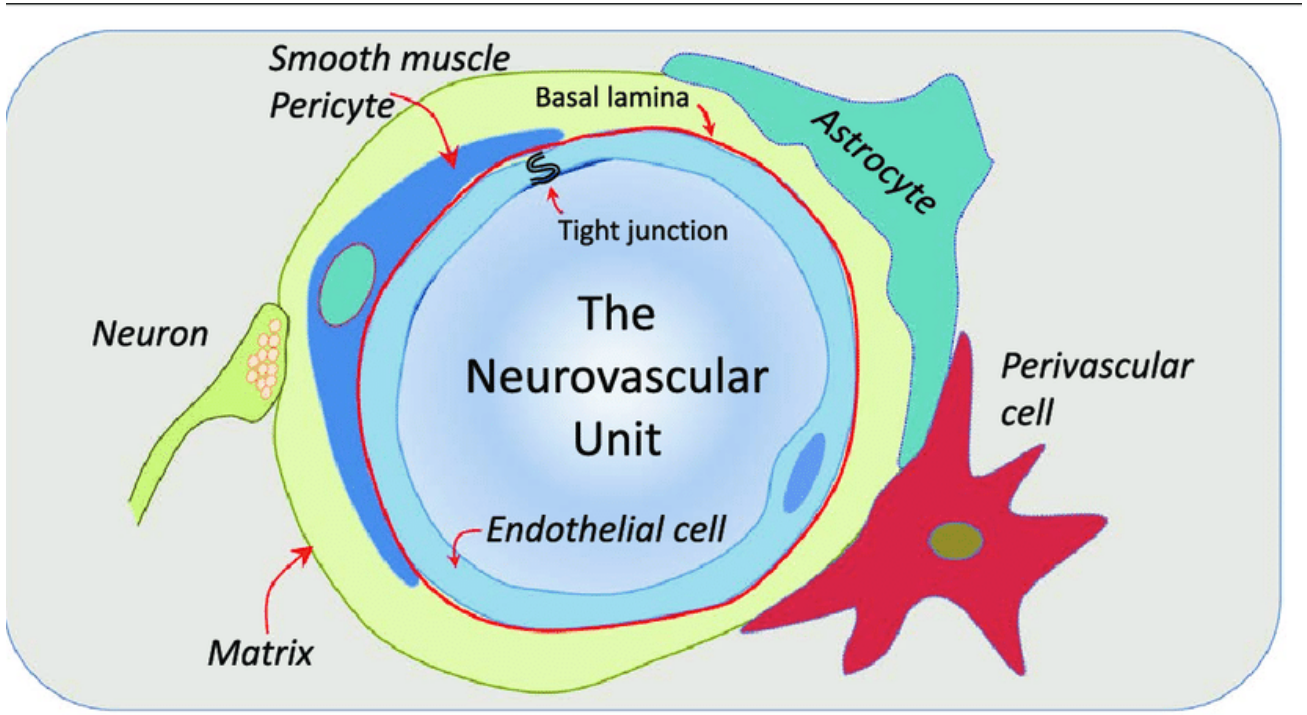
nature biotechnology

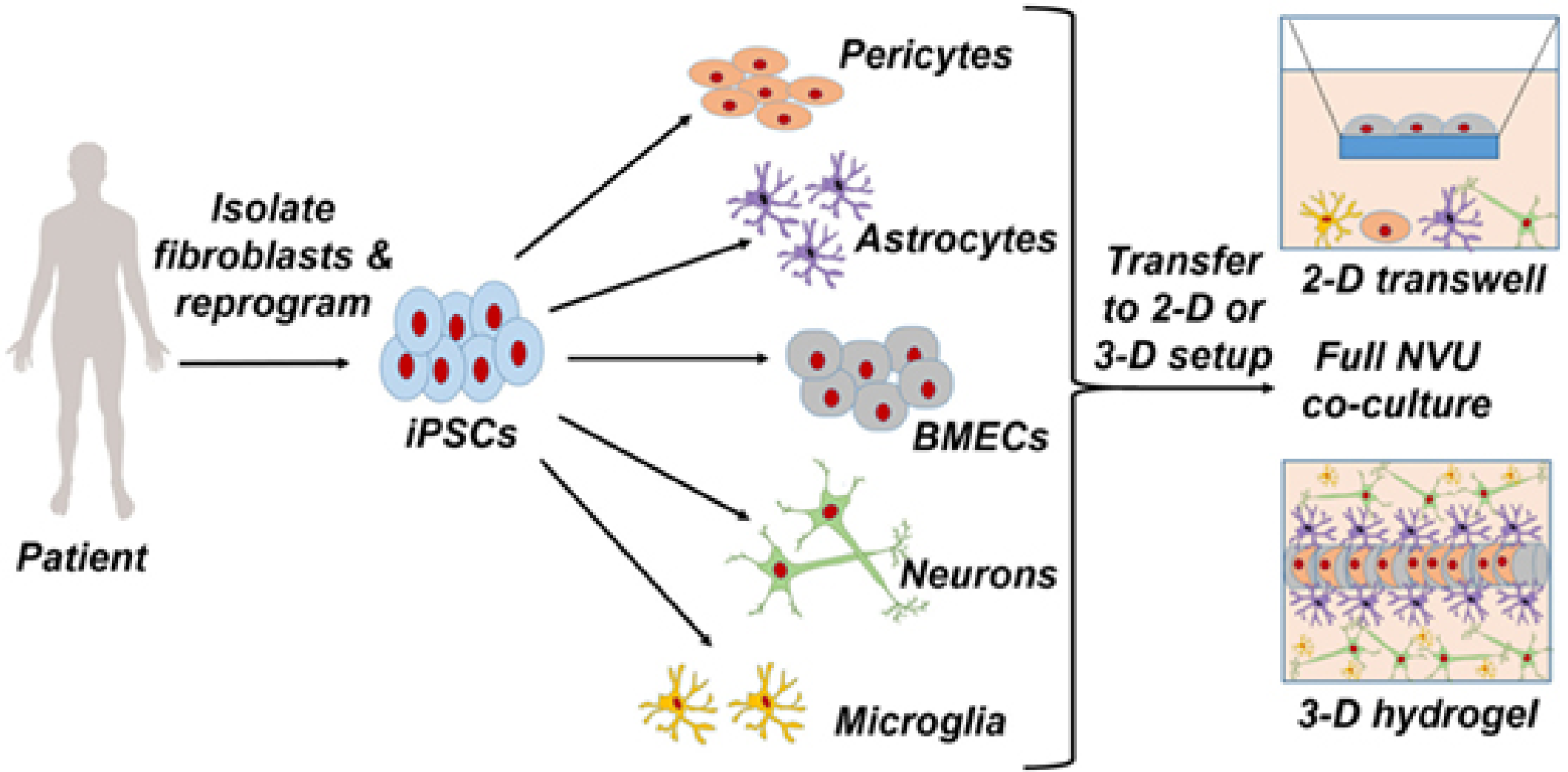
A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells

Ben M Maoz[1–5,14], Anna Herland[2,6,7,14], Edward A FitzGerald[2,14], Thomas Grevesse[1,2], Charles Vidoudez[8], Alan R Pacheco[2,9], Sean P Sheehy[1,2], Tae-Eun Park[2], Stephanie Dauth[1,2], Robert Mannix[2,10], Nikita Budnik[1], Kevin Shores[1,2], Alexander Cho[1,2], Janna C Nawroth[1,2], Daniel Segrè[9,11], Bogdan Budnik[12], Donald E Ingber[2,10,13] & Kevin Kit Parker[1,2]

Neurovascular Unit

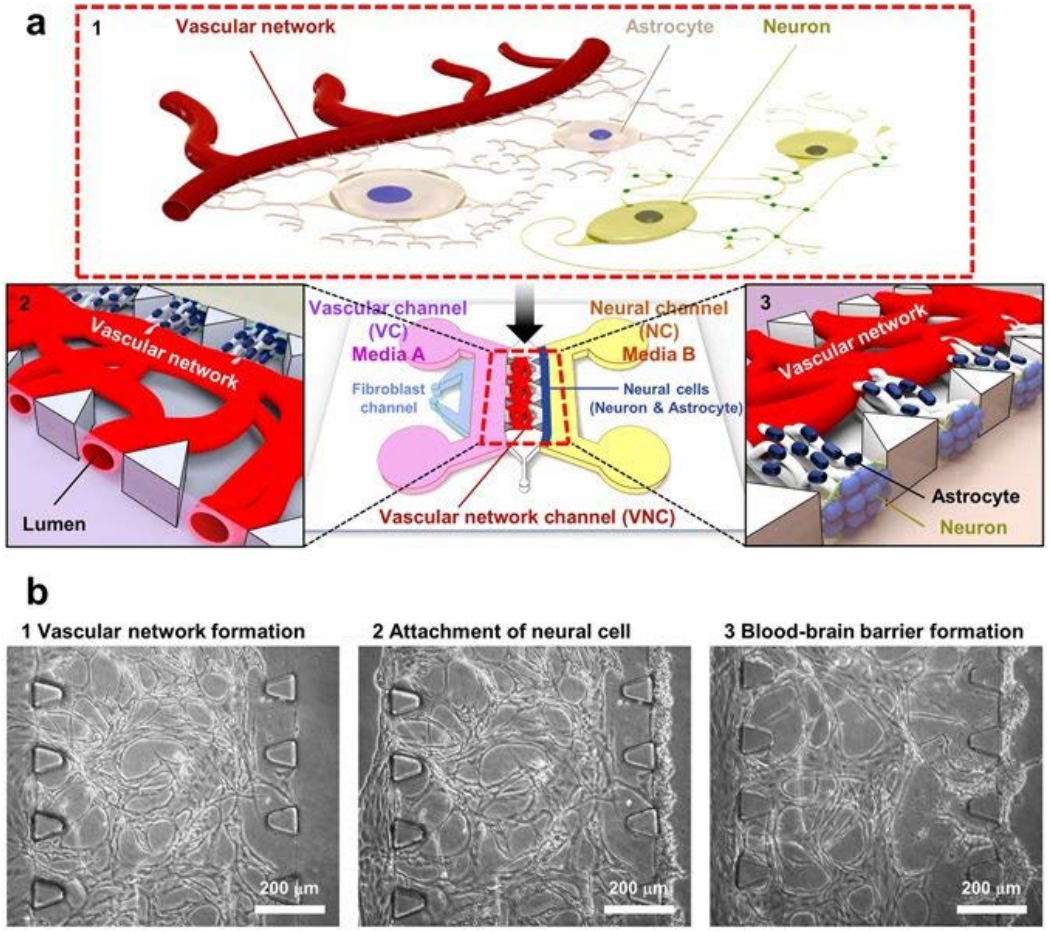
- NVU regulates dynamic influx and efflux of nutrients, metabolites and drugs between Systemic circulation and CNS.
- Current models are limited in their ability to resolved spatial and temporal dynamics of NVU.

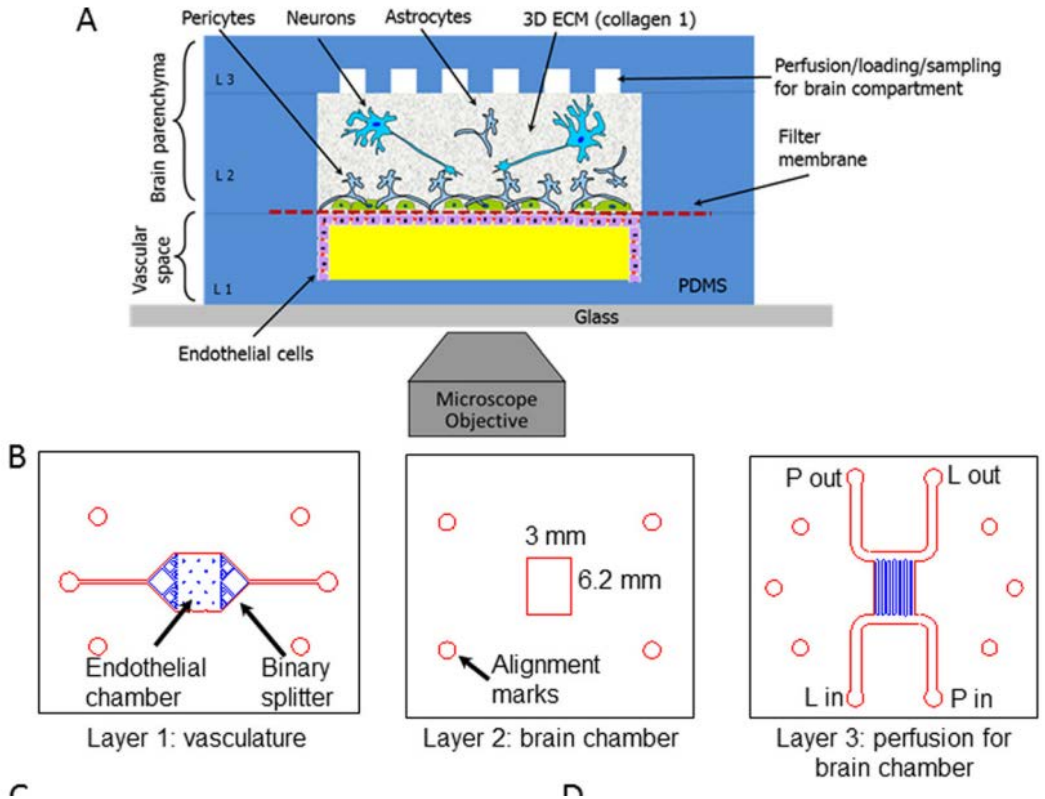



Patient
Isolate fibroblasts & reprogram
iPSCs
Pericytes
Astrocytes
BMECs
Neurons
Microglia
Transfer to 2-D or 3-D setup
2-D transwell
Full NVU co-culture
3-D hydrogel

NVU- Organs on Chips

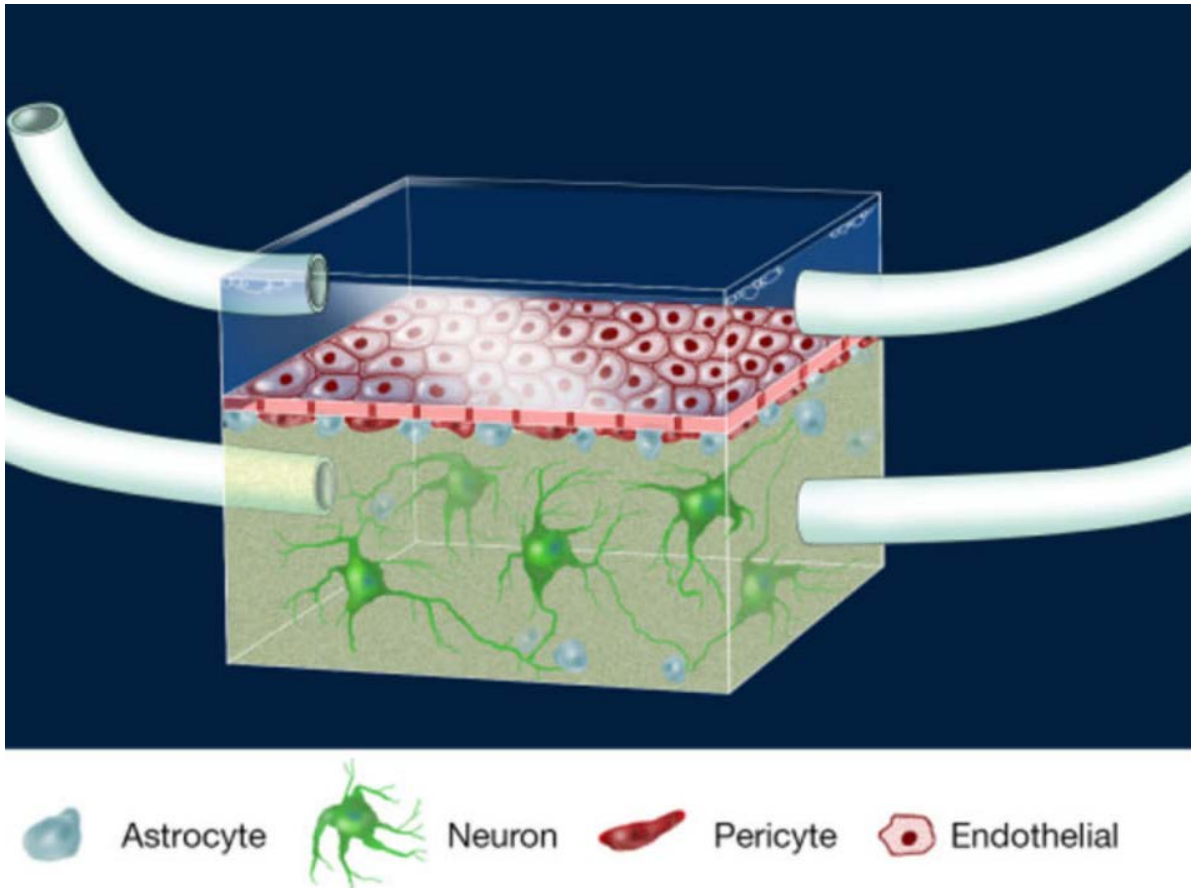
In vitro models of the human BBB and the surrounding perivasculature have been created using both conventional culture methods and microfluidic organ chips





Brown et al., 2015

Recent models of NVU on chips



The interacting cell types were contained in a single flow device.

Max of two inlets and two outlets.

Preventing dissection of the contributions of the individual cell types or subcompartments to the function of the NVU.

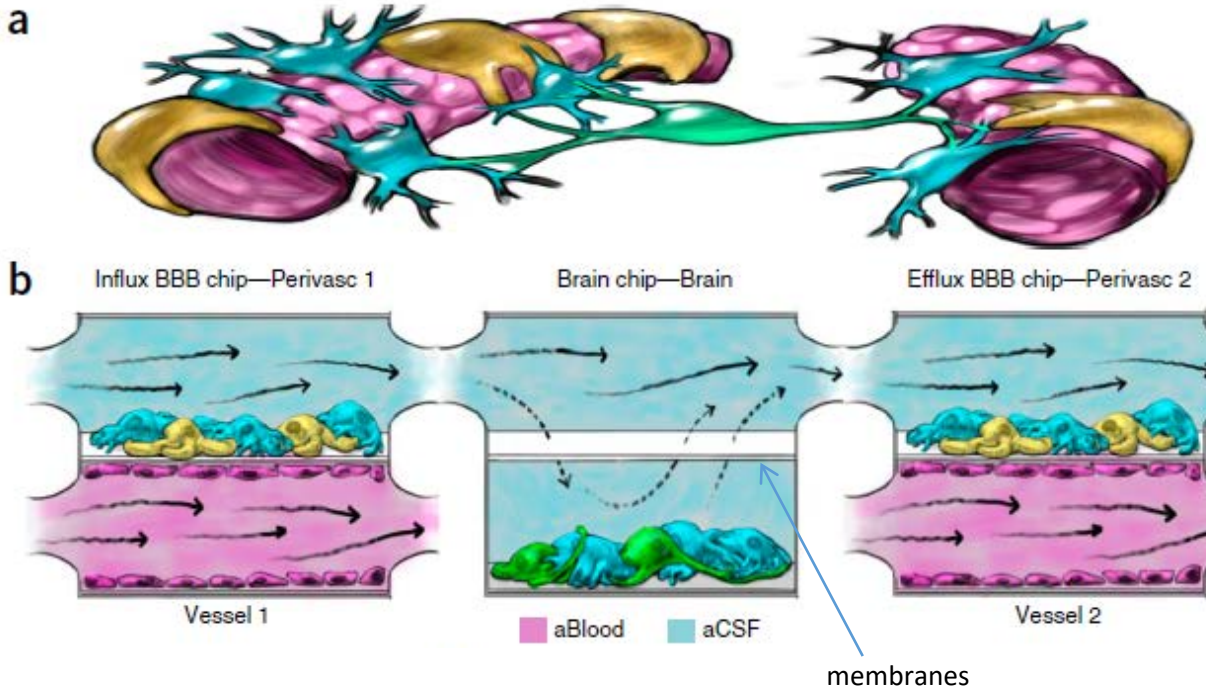
Problems:

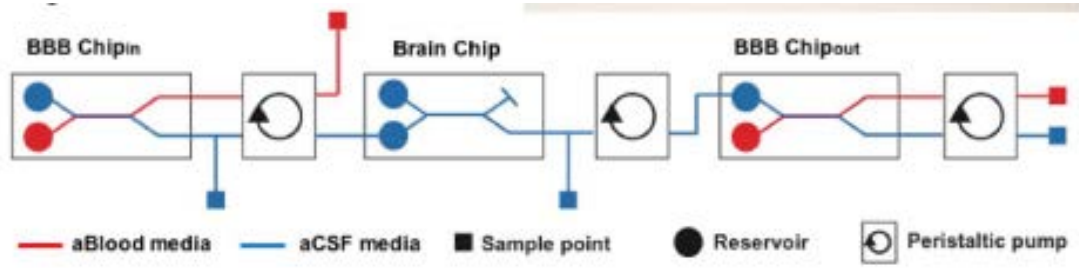
. Contributions of cell types remain unknown

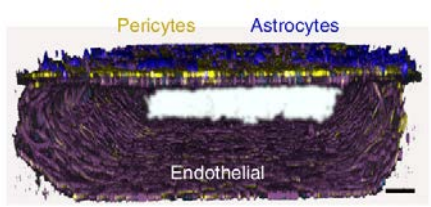
. Does it really replicate a NVU?

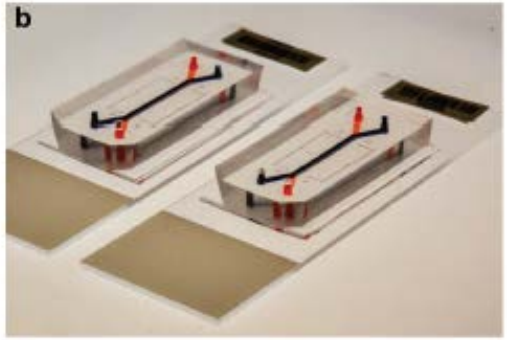
Brown et al., 2016

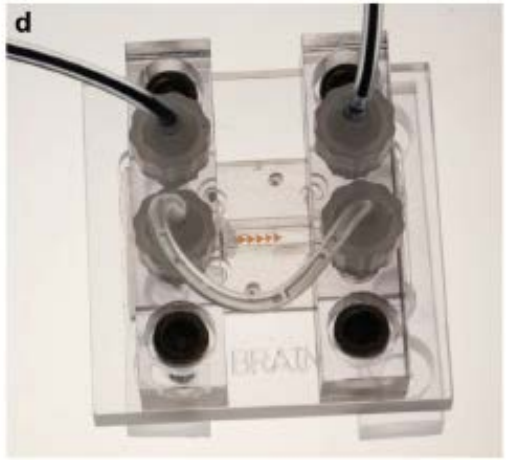
Combination of Chips: Novel NVU











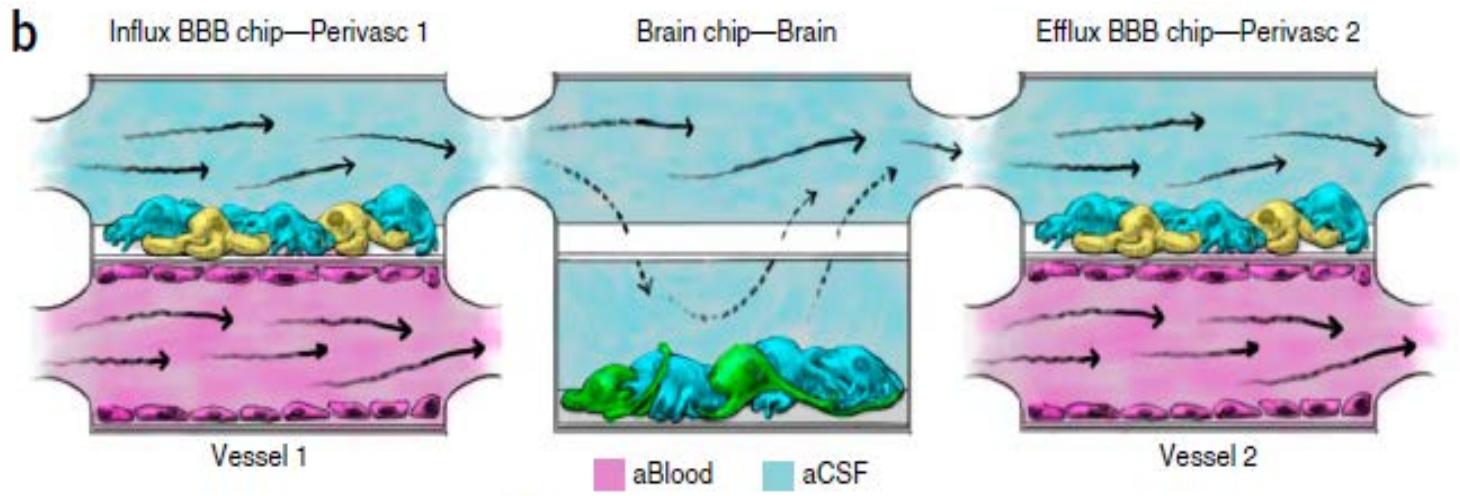
V1/2- Human brain microvascular endothelial cells (hBMVECs).

PV1/2- primary brain microvascular pericytes and astrocytes were cultured

Brain chip: 60% glial cells, 40% neurons (glutamatergic, GABAergic,dopaminergic)

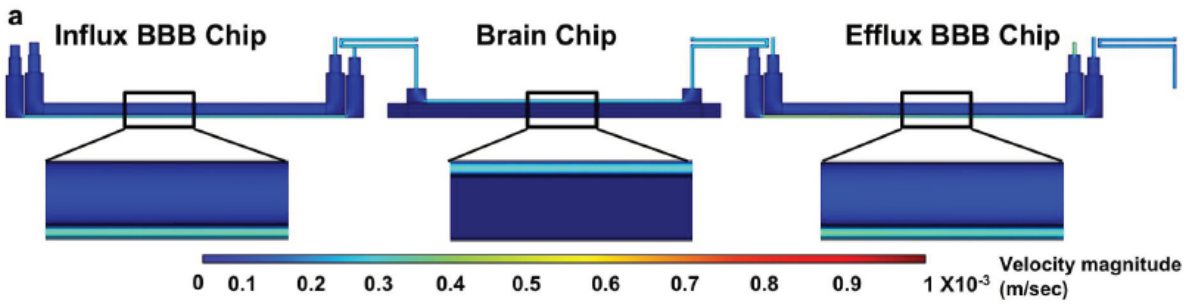
Figure 1

What do they model?



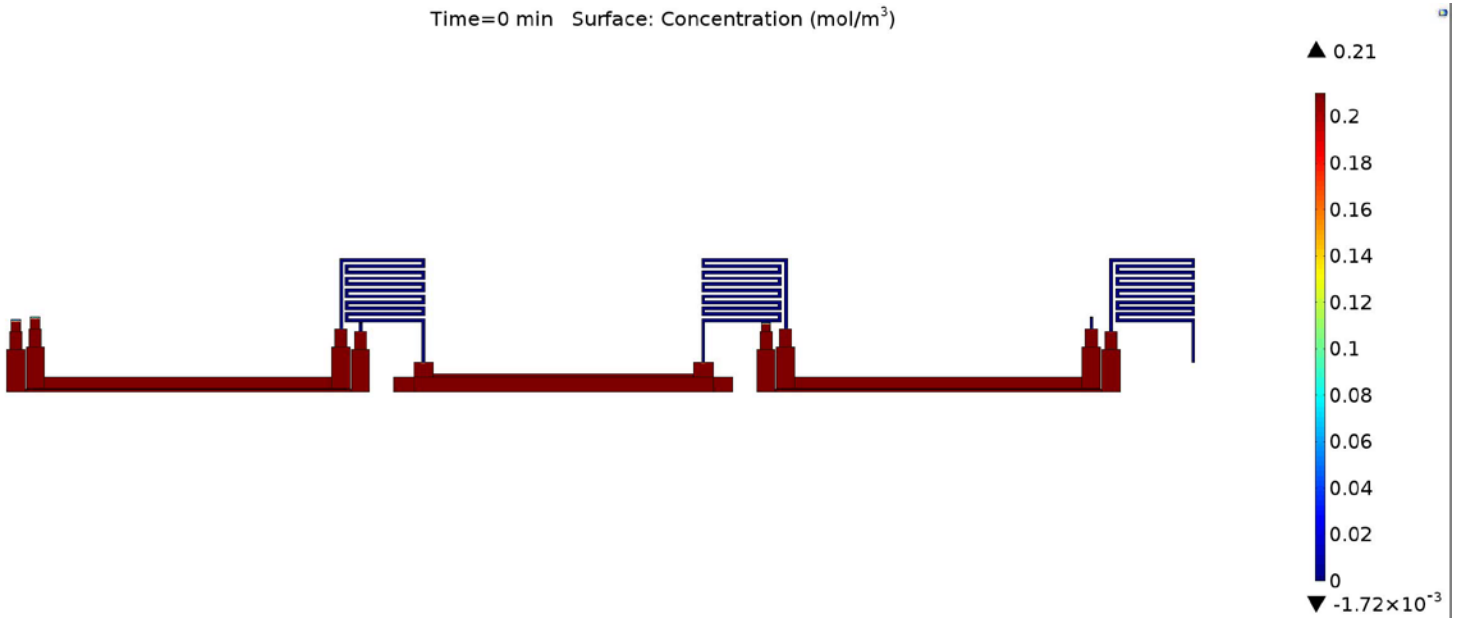
- BBB influx chip modeled influx of compounds from the blood to the brain parenchyma
- BBB efflux chip modeled efflux from the brain to the blood

Flow rate and stress on cell types mimics in vivo conditions



Shear stress: 25 fold more stress on cells in brain chip.

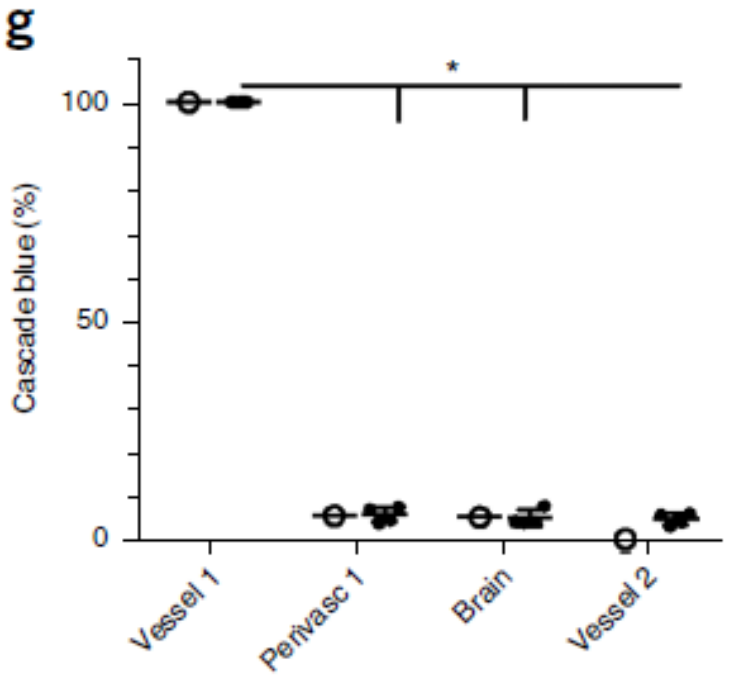
At applied flow rate of 0.06mL/min maintained physiologically relevant oxygen levels throughout the NVU.



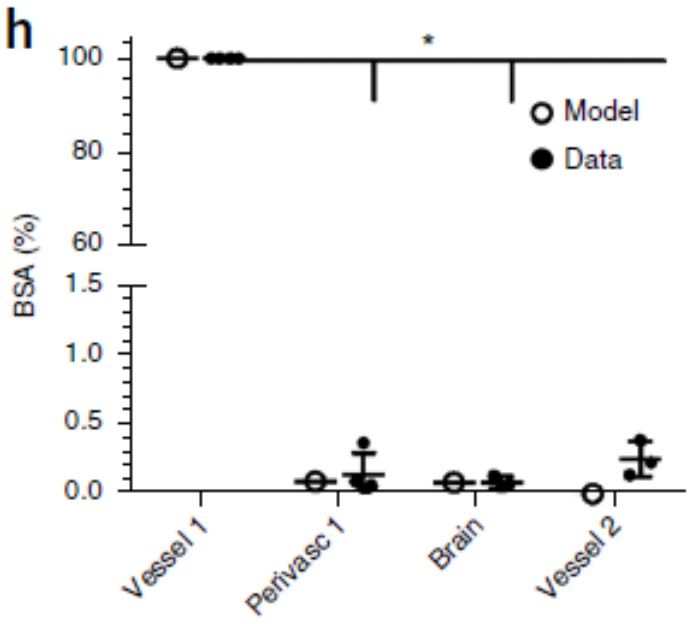
Supplementary fig 1

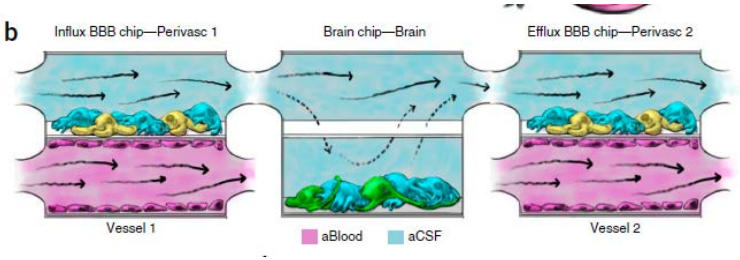
Cultured endothelium functions as a barrier

Small molecule diffusion



Large molecule diffusion





Cascade blue diffusion into PV1 was measured.

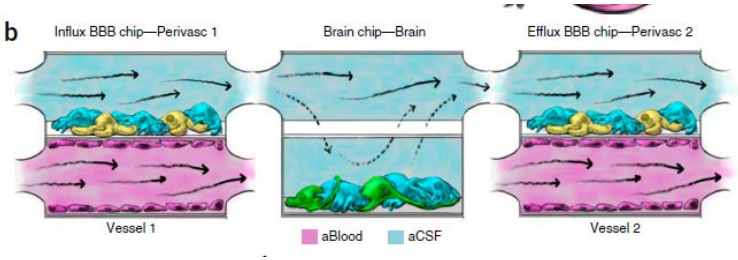
Cascade blue: 530 Da

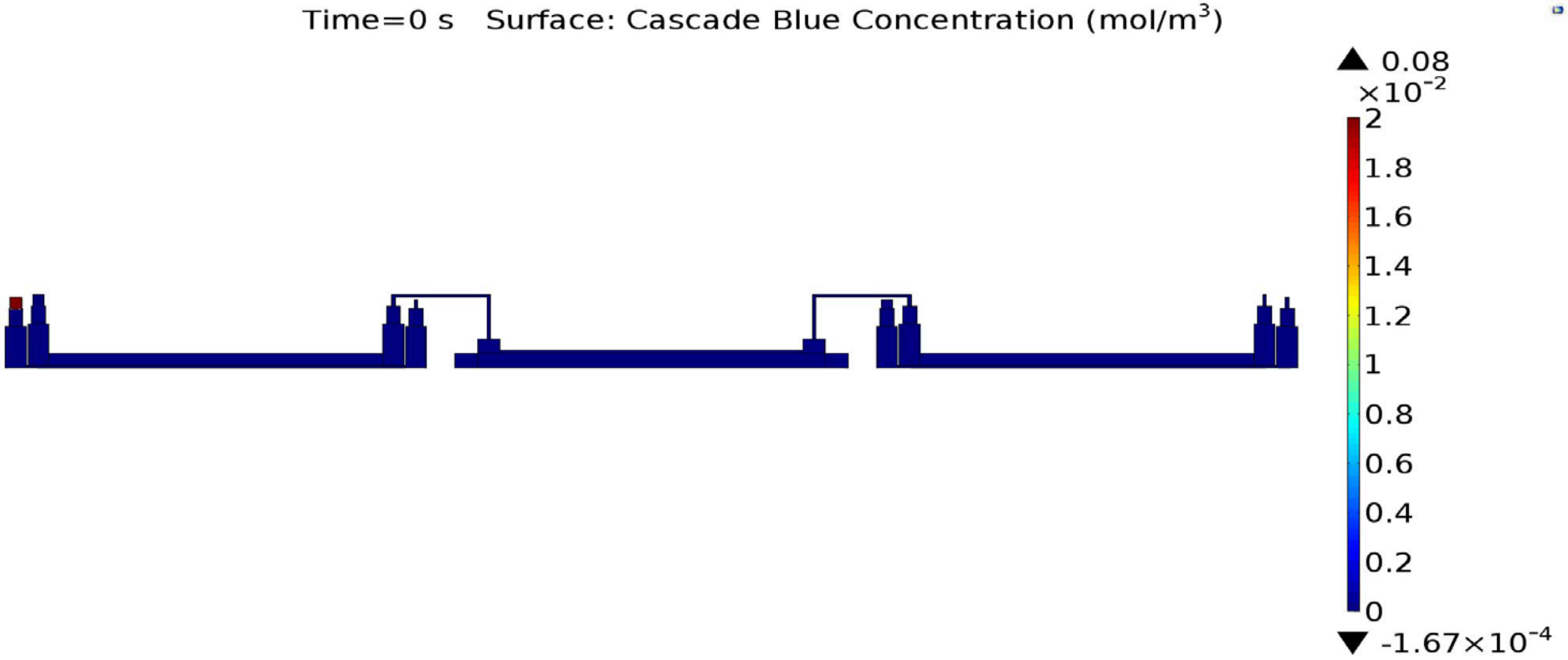
Alexa 555- BSA into PV1 was measured.

BSA-555: 67kDa

Figure 1

Cultured endothelium functions as a barrier





Results

Results_1

Fluidic coupling leads to phenotypic modulation of cells in NVU

Label free proteomic analysis was performed in all chambers and the data obtained was compared with the uncoupled Systems.

1500-2000 proteins were identified in each compartment
--> **significant changes in protein expression in endothelium, perivasculature and neurons in the coupled system.**

color scales indicate log 10 normalized mass abundance:
- blue representing low-abundance proteins
- red representing high-abundance proteins.

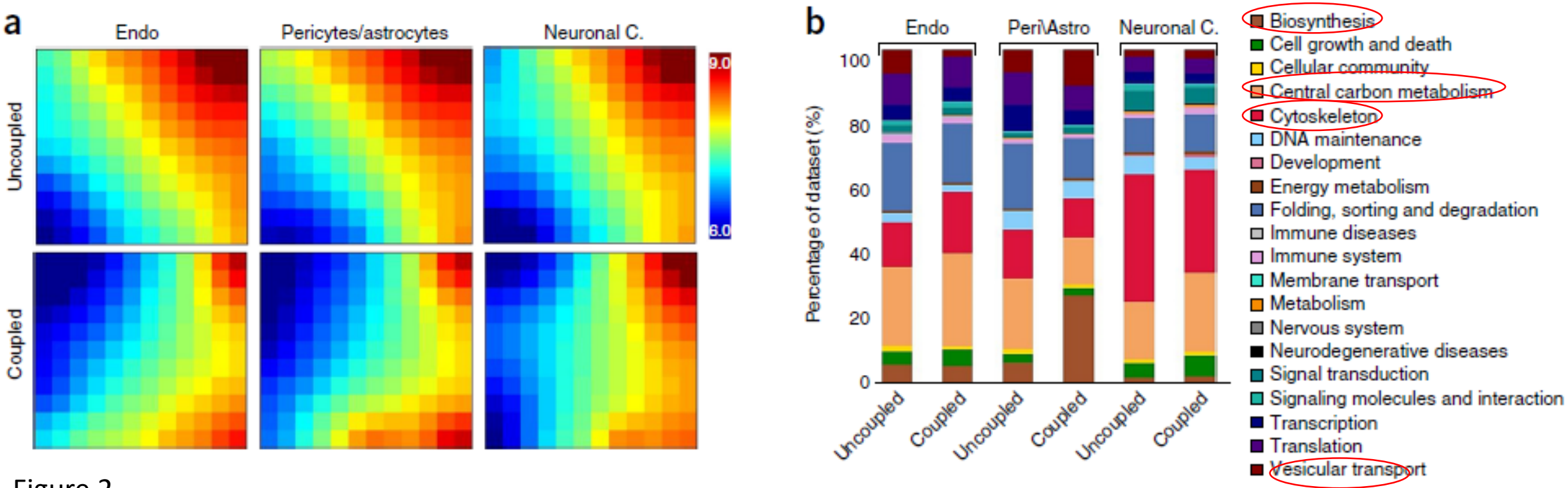
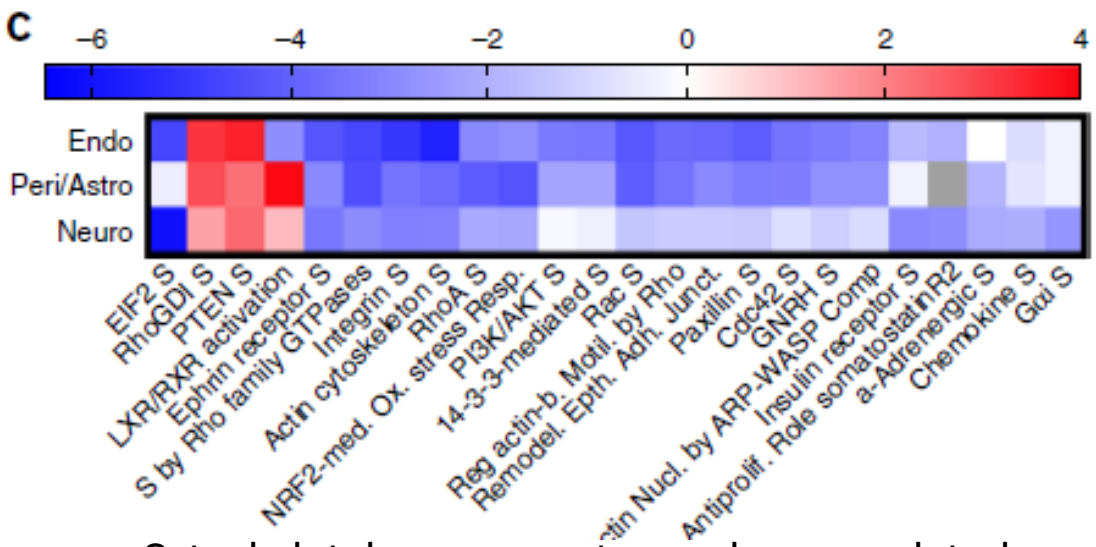


Figure 2

Changes in signaling pathways in coupled systems

Canonical pathway analysis

Fluidic coupling downregulated Rho family GTPases, integrin, paxillin, Rac and actin cytoskeleton signaling in all compartments.

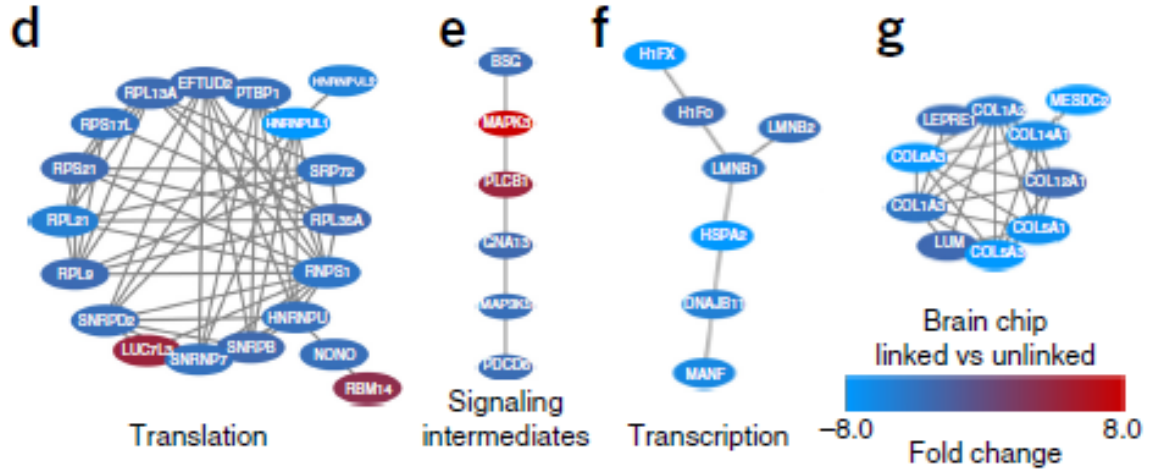


Cytoskeletal components are downregulated in coupled system compared to uncoupled

Conclusion: Cells in coupled systems are less proliferative and less migratory

Protein clusters

Increase in translation, signal transmission intermediate molecules and transcription, and a fourth with downregulation of extracellular matrix proteins.

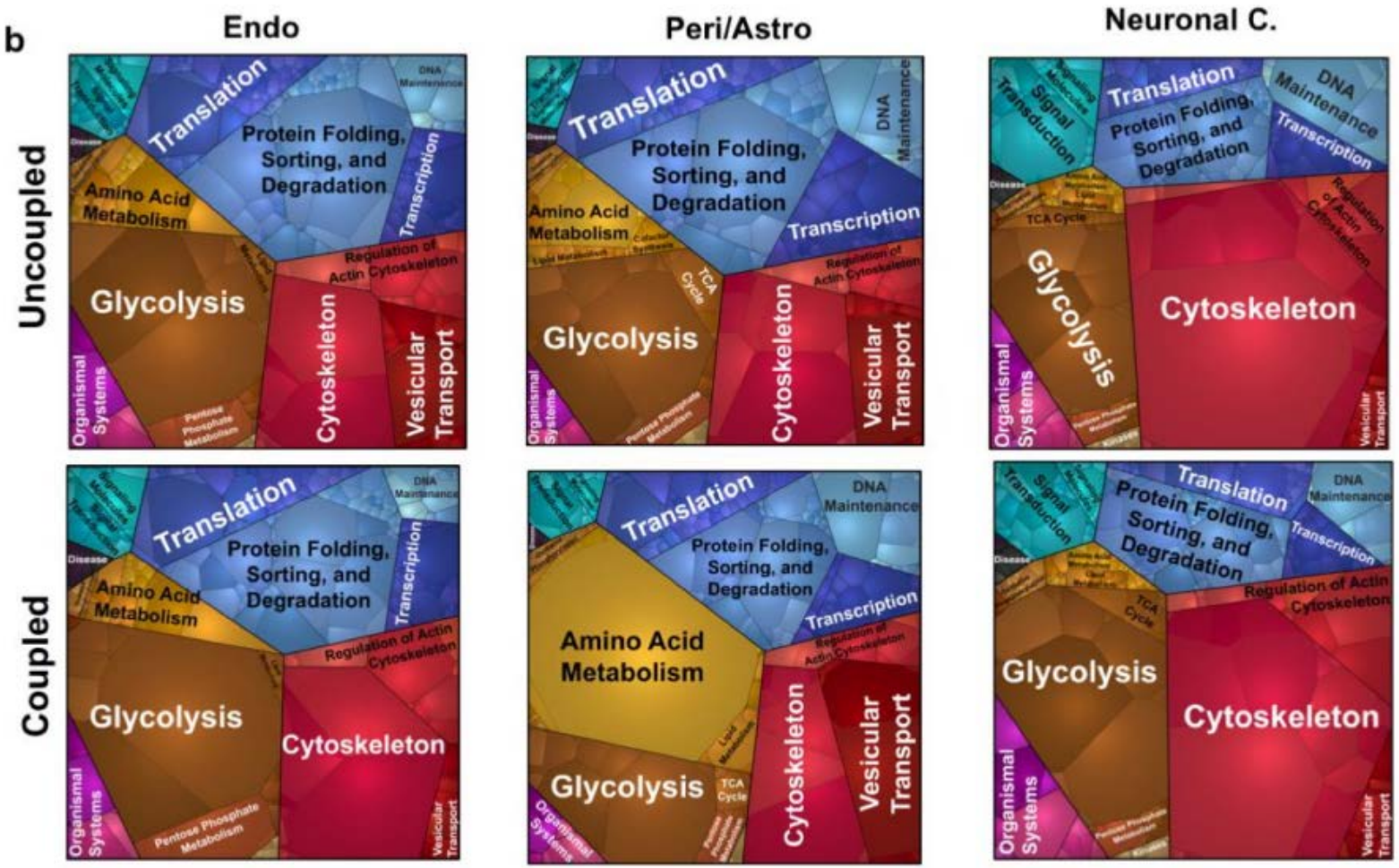


These changes may correspond to reduced cellular stress and astrocyte reactivity in the coupled brain chip.

Conclusion: Cells may be less stressed

Figure 2

Changes in signaling pathways in coupled systems



Brain chip more dominated by cytoskeletal function processes in both coupled and uncoupled chips.

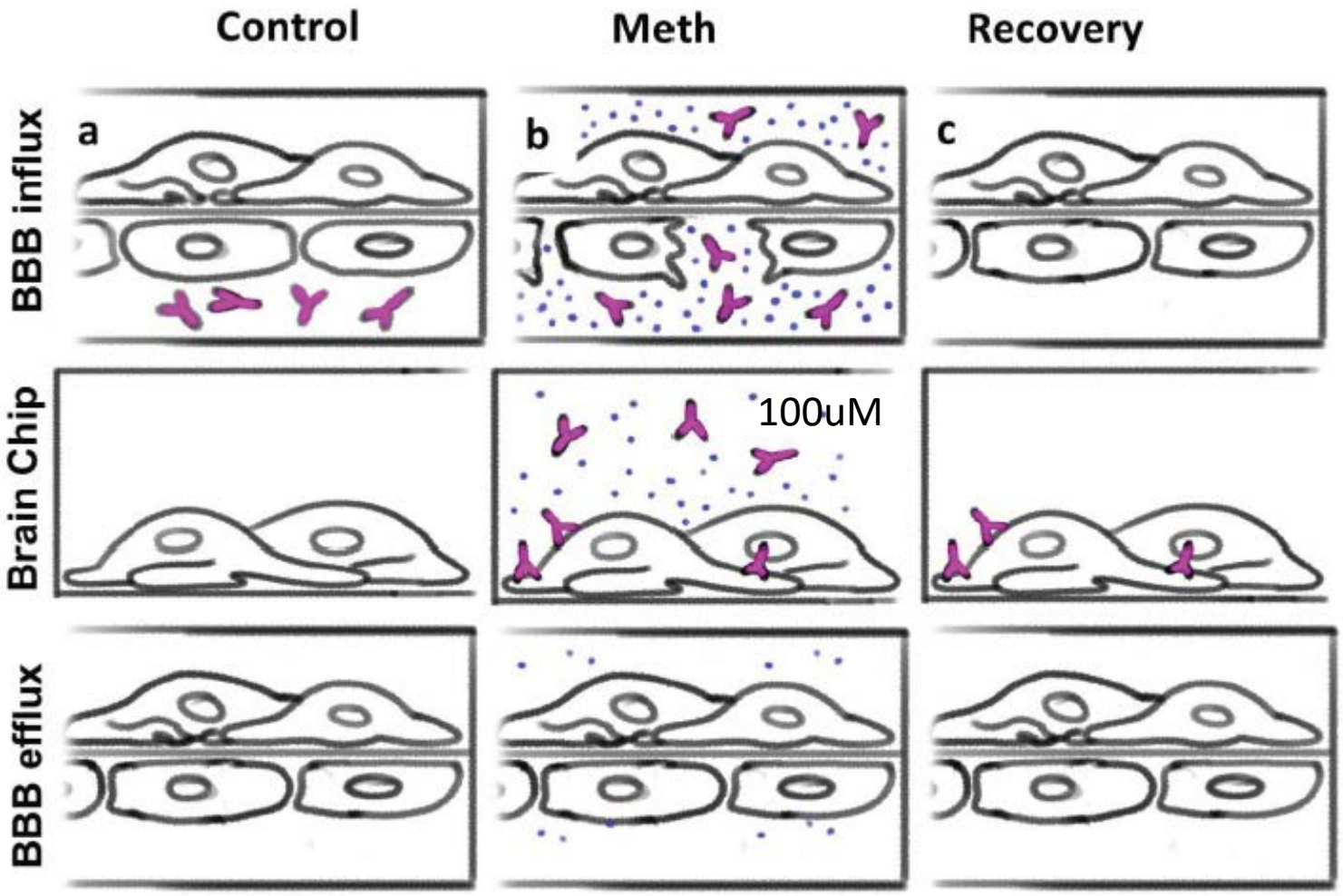
Consistent with neuronal expression pattern in the human brain proteome.

Supplementary figure 5b

Can NVU be used for drug-modeling studies?

- Methamphetamine (Meth) is known to induce reversible disruption of the BBB after acute administration in vivo and in vitro as well as in chronic abusers.

- Treat V1 with 1.5mM Methamphetamine and analyze the BBB permeability.
  - Use CB and BSA-555 to quantify Permeability.
- Effect on neurons measured by anti GluR2 Antibody.



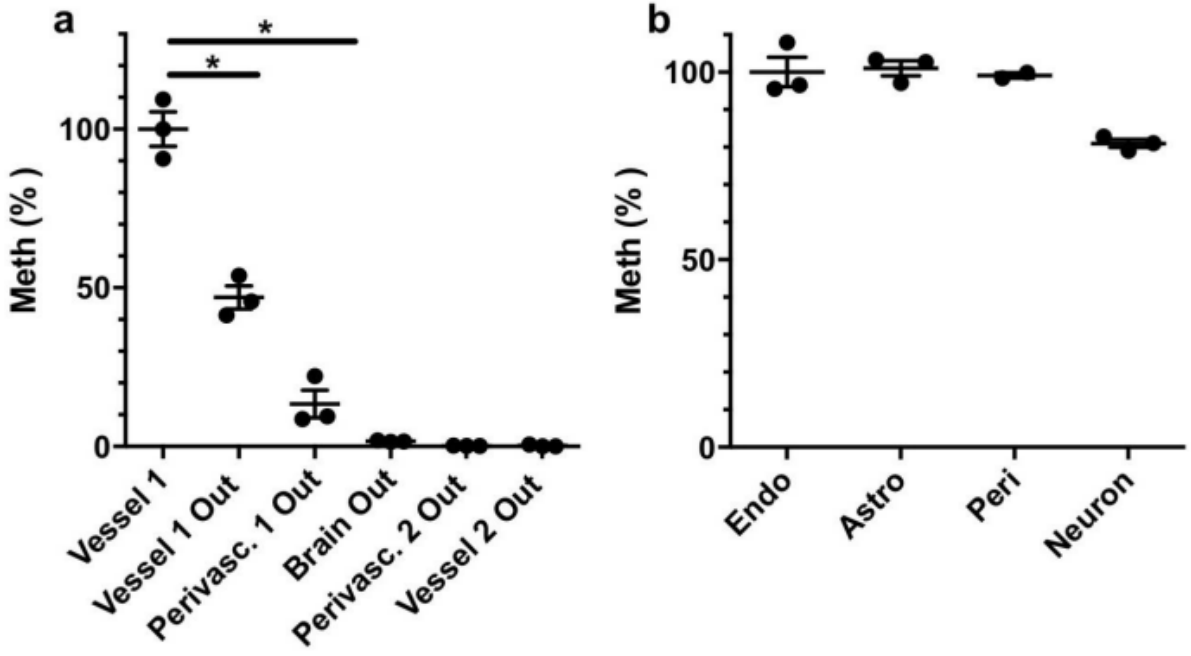
Supplementary figure 6

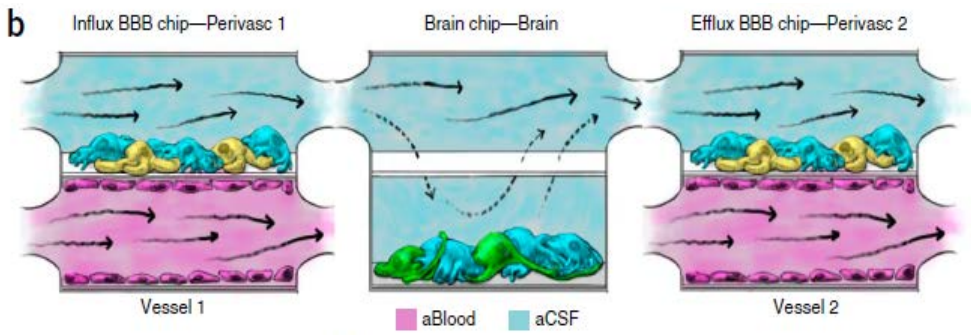
Can NVU be used for drug-modeling studies?

Mass balance of Meth in the coupled NVU microfluidic system and Meth uptake by cells in well plates.

BBB-Brain-BBB Chips

well-plates





Supplementary figure 8

N=3 representing independent NVU systems,

Can NVU be used for drug-modeling studies?

Endothelial cell jnctions: VE-Cadherin (VE-CAD, green
Endothelial monolayer stained for VE-CAD (white)
anti-GluR2 (magenta)
Betatubulin in neurons (green figure g)

Control conditions:

- the endothelium formed a barrier similar to the BBB in vivo --> indicated by the presence of a continuous pattern of VE-cadherin-containing cell–cell junctions (Fig. 3a,b) and lack of GluR2 Ab penetration and staining in the brain compartment (Fig. 3c).
- the endothelium, pericytes and astrocytes of the BBB efflux chip demonstrated similar morphology as those in the BBB influx chip (Fig. 3d).

Meth conditions:

- barrier breakdown, as indicated by disruption of cell–cell junctions and lower expression of VE-cadherin (Fig. 3e,f).
- increased influx of GluR2 Ab into the brain chip and, consequently, enhanced staining of the neurons (Fig. 3g)
- no detectable effect on the cell–cell junctions of the endothelium in the BBB efflux chip (Fig. 3h).

Withdrew of the Meth:

- the barrier breakdown in the BBB influx chip was reversible and the disrupted endothelial cell–cell junctions re-formed (Fig. 3i,j) -->partial recovery of endothelial barrier function.
- anti-GluR2 Ab already bound to the neurons was not washed out of the brain chip (Fig. 3k).
- the BBB efflux chip remained unaffected (Fig. 3l).

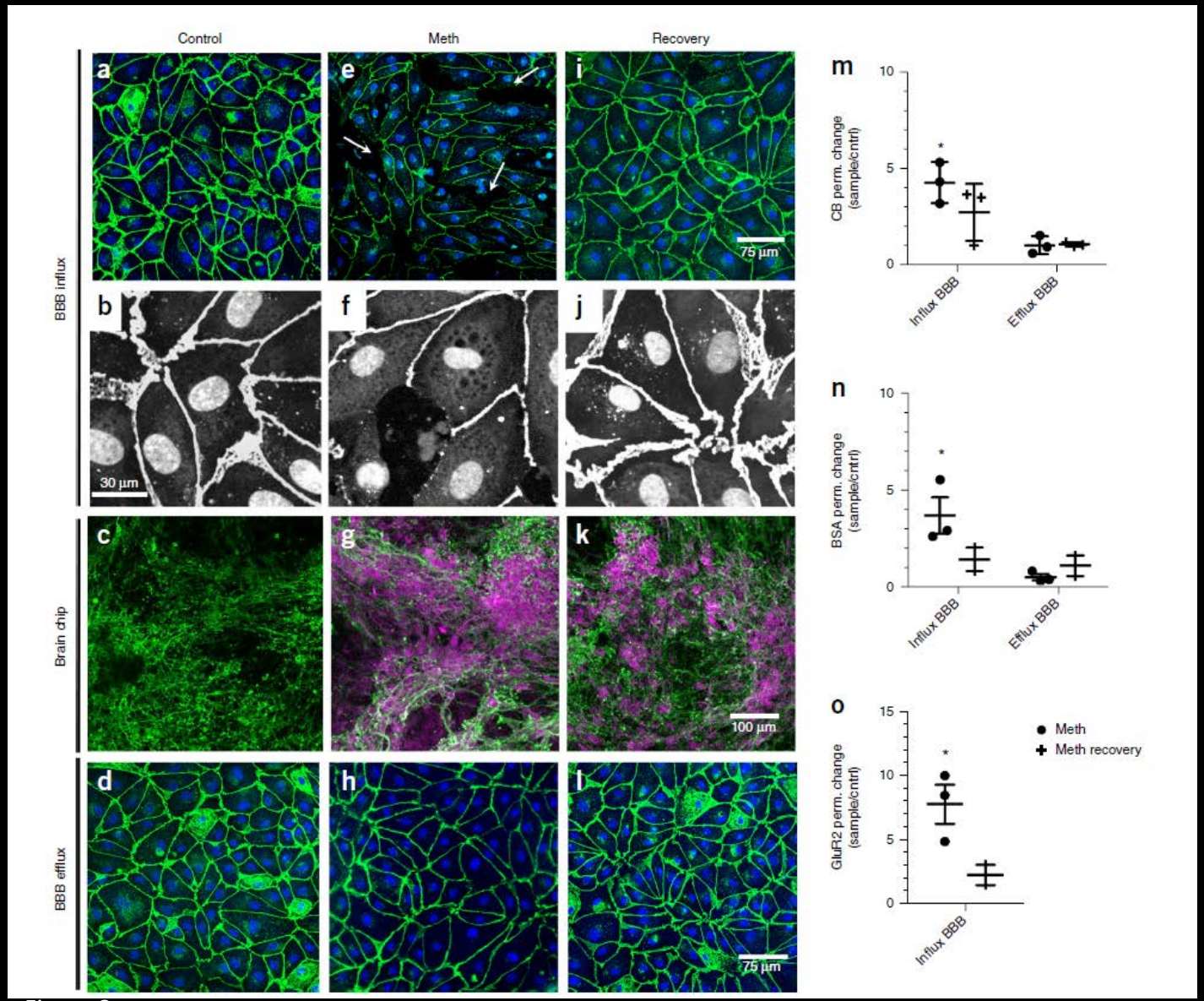


Figure 3

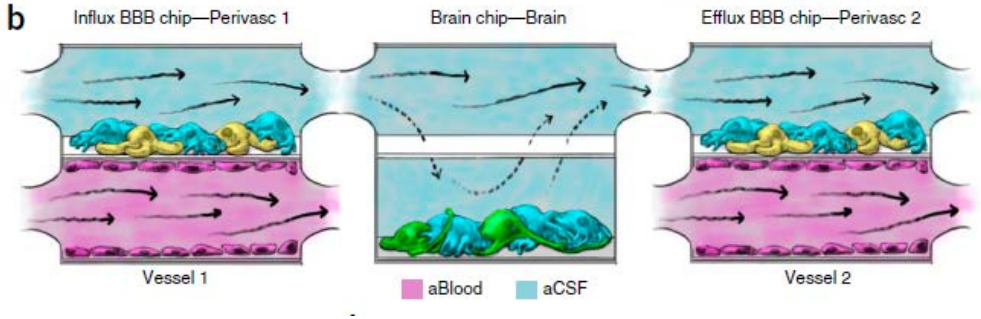
Can NVU be used for drug-modeling studies?

Conclusion:

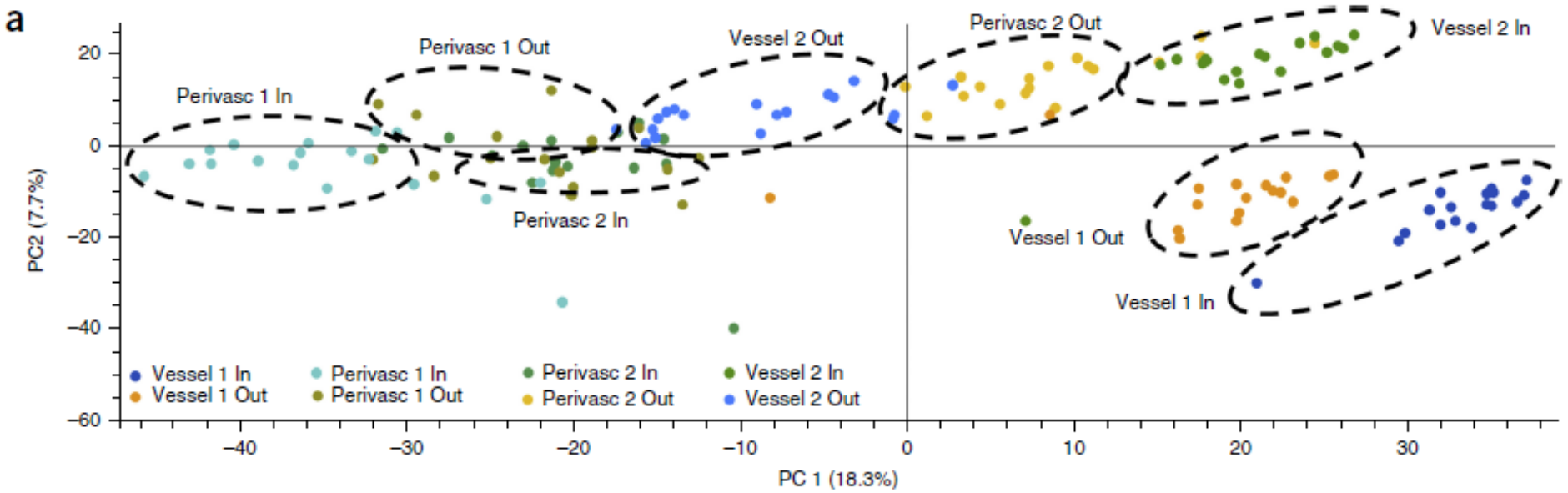
the NVU system effectively mimics the previously reported ability of acute Meth administration to produce reversible breakdown of the BBB in both animals and humans.

Untargeted metabolic analysis of the secretome from each compartment

Understanding the molecular basis of the communication between the cells, vasculature and brain parenchyma



Principle component analysis (PCA) was used to assess the expression variations and abundance of all the metabolites (up to ~3,000 MW) detected in the different compartments of the NVU system by untargeted MS.



- Inflow (Vessel 1 in) and outflow (Vessel 1 out) of the vascular channel of the BBB influx chip.
- Inflow (Perivasc 1 in) and outflow (Perivasc 1 out) of the perivascular channel of the same BBB chip
- Inflow (Vessel 2 in) and outflow (Vessel 2 out) of the vascular channel of the second BBB efflux chip, and the inflow (Perivasc 2 in) and outflow (Perivasc 2 out) of the perivascular channel of the BBB efflux chip.

Each compartment expresses different secretome which can seperated easily by PC analysis.

Figure 4

Secretome alters upon treatment with methamphetamine

Metabolic pathways related to regulation of neutrotransmitters:

- dopamine (e.g., tyrosine biosynthesis IV and l-dopachrome biosynthesis)

- histamine biosynthesis

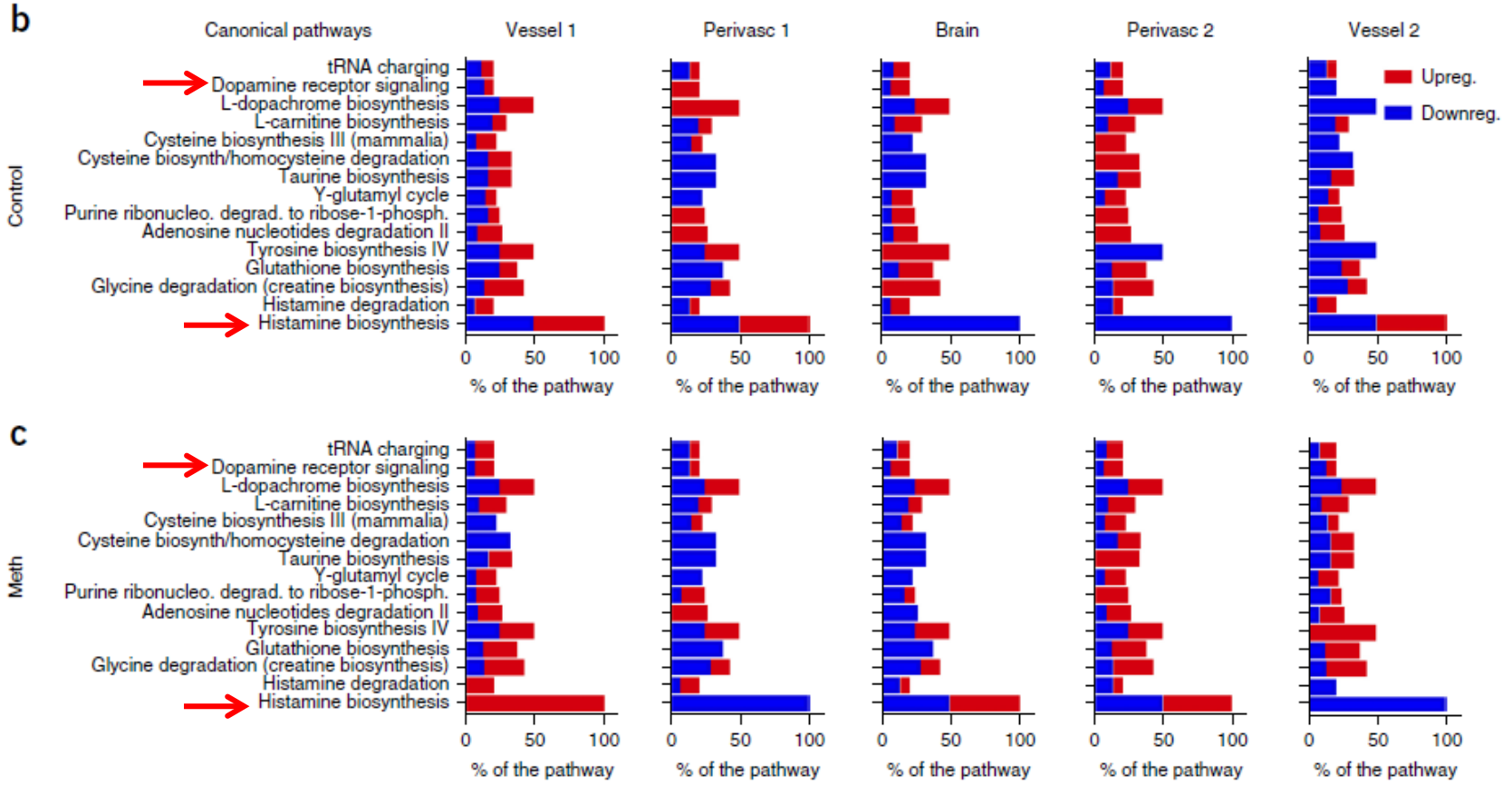


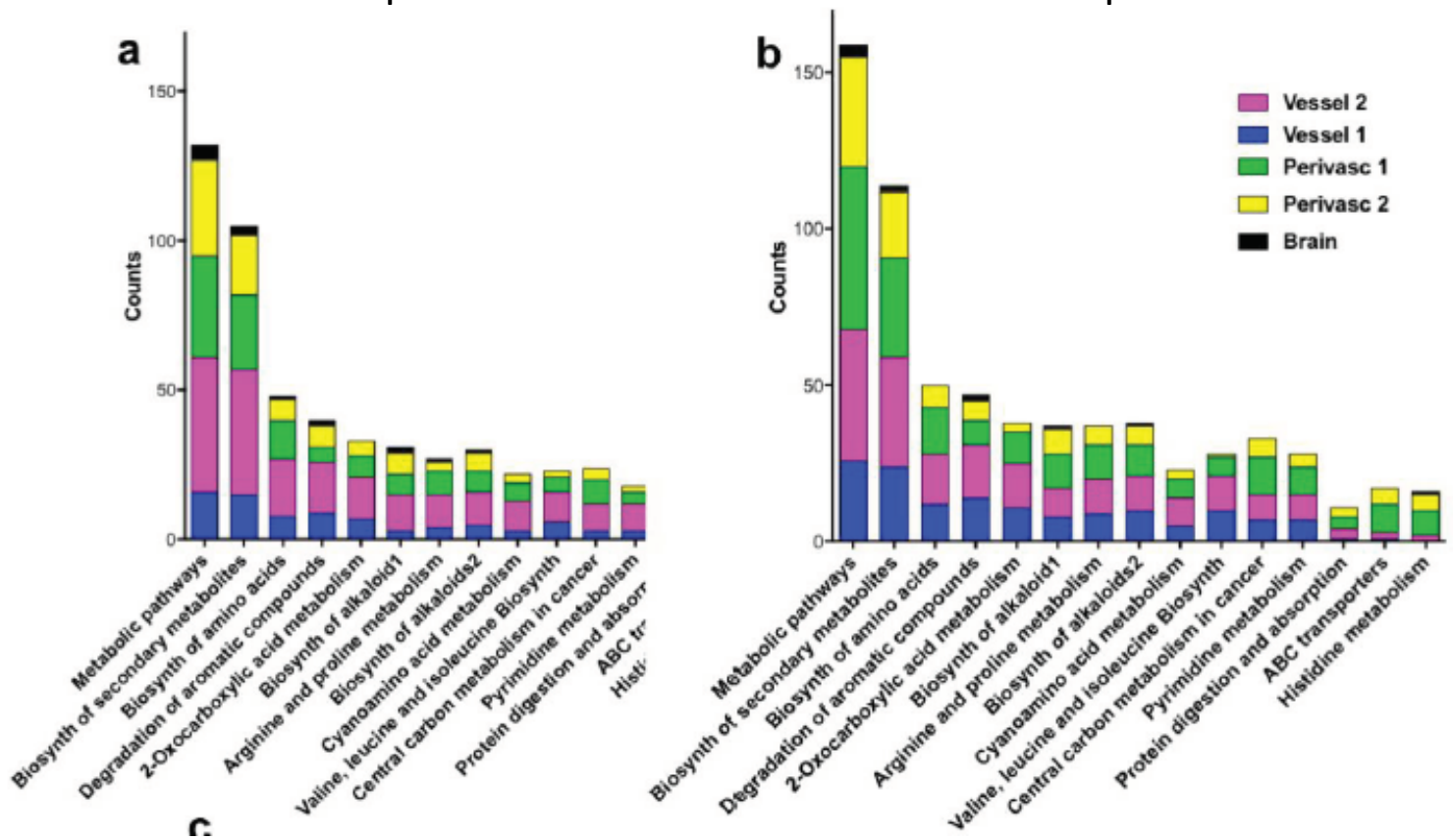
Figure 4

→ perivascular cells are more susceptible to Meth in terms of the higher number and variety of metabolic alterations they express compared to the neighboring endothelium

Results_3

Brain secretes least amount of the metabolites

-Methamphetamine +Methamphetamine



MS analysis (Compound Discoverer) _ Supplementary Fig. 10

basal maintenance of neurons (glutathione biosynthesis, l-carnitine biosynthesis, glycine degradation)

neuronal protection by the vasculature and perivasculature (l-cysteine degradation, cAMP regulation)

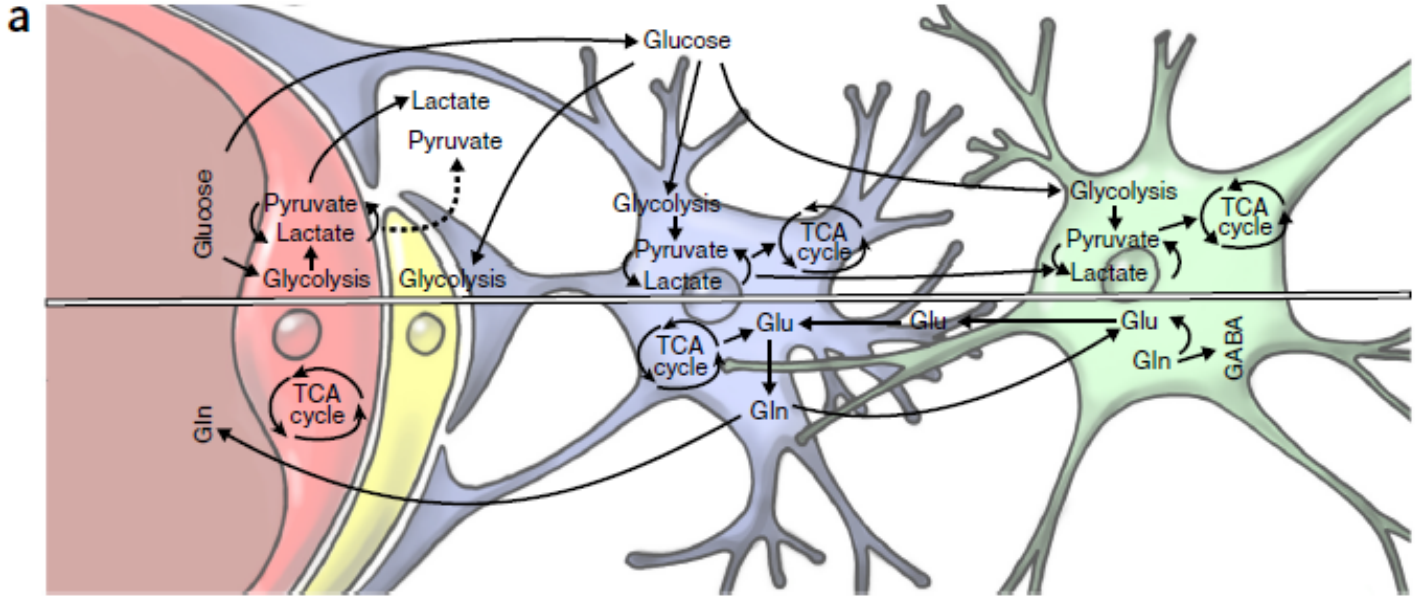
neurotransmitter levels (adenosine nucleotides degradation).

Demonstration of how the secreted metabolome of the BBB provides chemical cues that help to maintain neuronal functionality.

Metabolite transport across the different comparments

- In NVU, Glutamate and GABA are coupled to pyruvate, lactate levels via TCA cycle.
- But where are the metabolites produced?
- Where basal energy shuttle and amino acid conversion take place?

→ Inject C13-labeled glucose into V1 chamber and monitor ist uptake in all chambers



- Endothelial cells and astrocytes primarily depend on glycolysis.
- Neurons typically rely on lactate and pyruvate as energy sources.
- **What is the metabolic flux in pericytes?**

Representation of how glucose and glutamate (Glu)–glutamine (Gln) cycles are thought to interplay with all four cell types of the NVU—endothelial cells, pericytes, astrocytes and neurons.

Figure 5

Metabolites in different compartments

Study the metabolic coupling among these various cell populations of the NVU

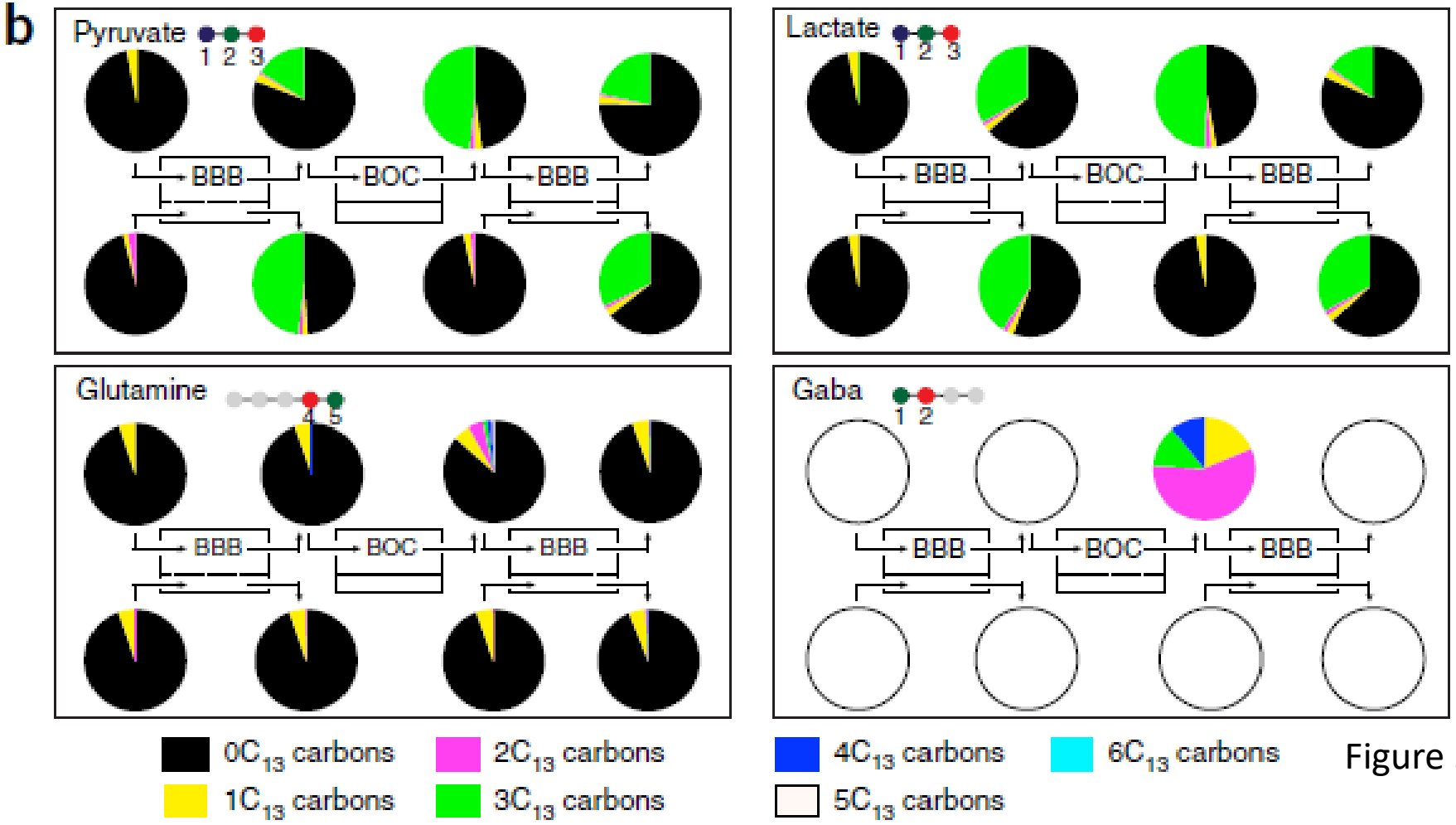
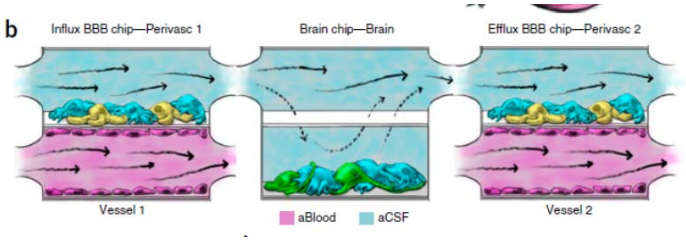


Figure 5

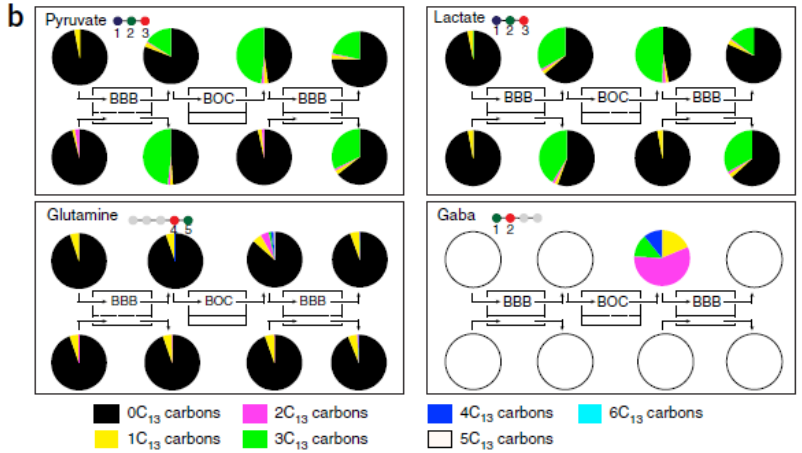
Supplied only C 13 -labeled glucose (6C 13) exclusively to the vascular channel of the BBB influx chip and then followed the generation of C 13-labeled metabolites with high-resolution MS in each of the downstream microfluidic compartments

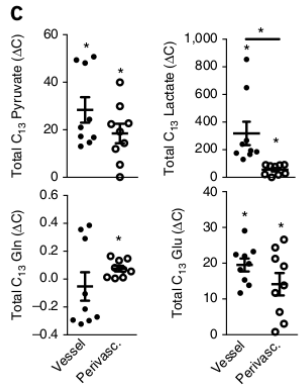


To analyze the metabolic contributions of each compartment of the NVU system to the biochemical pathways associated with glycolysis, the TCA cycle and the glutamine–glutamate cycle

Results_4

Metabolites in different compartments





- **C 13-glucose underwent physiological glucose transport across the BBB** in the influx chip and penetrated into all downstream compartments. (b)
- **Bidirectional glucose fluxes observed in the brain**, with a net glucose flux from blood to brain, as previously observed in vivo. (b)
- Metabolism in each of the compartments resulted in **changes in the amount and ratios of the carbon isotopes of the glucose metabolites**, pyruvate, lactate, glutamine and glutamate. (b)
- **Glycolysis** occurred **in all compartments** of the NVU system. (c)
- **Glutamate synthesis in both the vascular and perivascular compartments** of the BBB chip and brain chip (c)
- C 13 -labeled neurotransmitter, **GABA**, was **exclusively produced by the cells of the brain chip**, which also confirms the functionality of the resident neurons.

Results_4

Coupled systems show uptake of metabolites

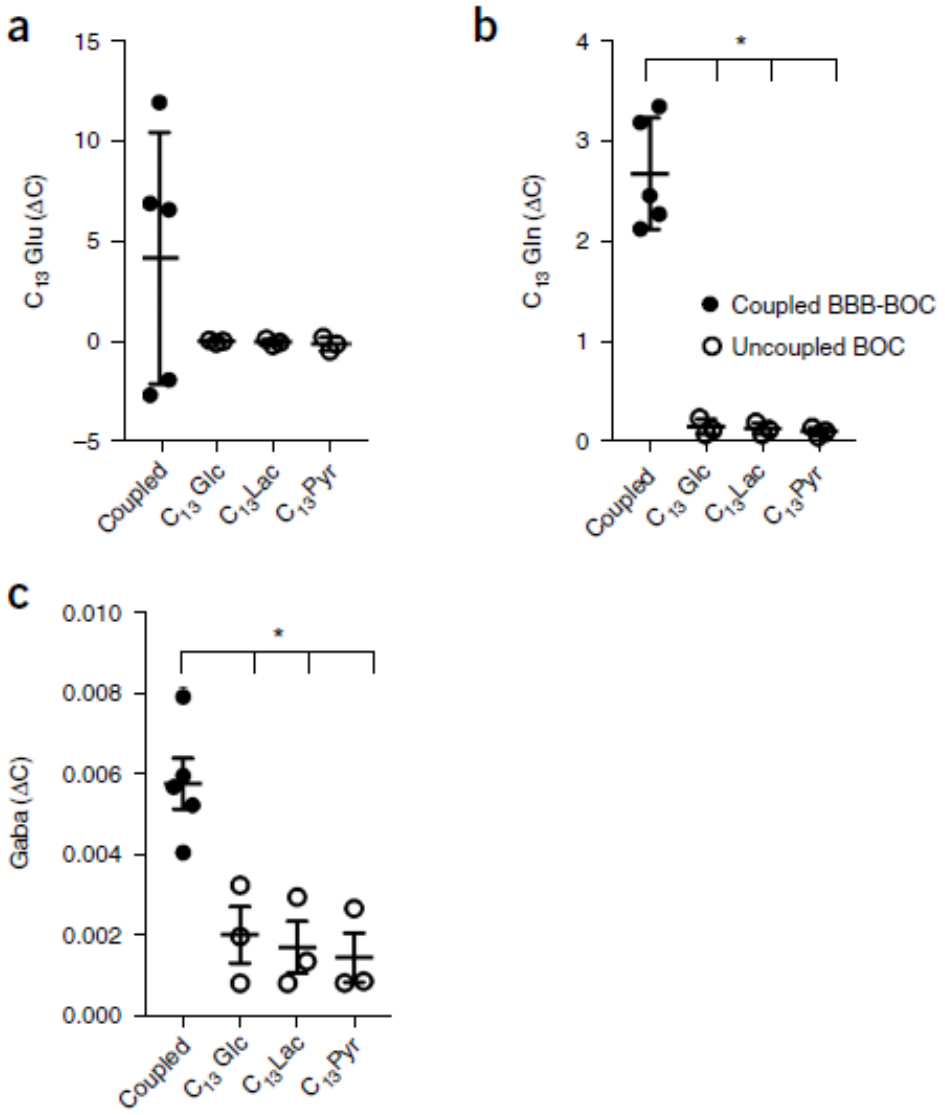


Figure 6

- C 13 -glutamine secretion was significantly higher in coupled brain chips (b) → metabolites from the vascular endothelium and perivascular cells directly influence glutamine production by astrocytes and neurons in the brain chip.

(Glutamate transferred to the brain chip is likely to be taken up by astrocytes, converted to glutamine and shuttled to the neurons, which then synthesize intracellular glutamate, as they do in vivo).

- The levels of GABA synthesis in the coupled brain chip were significantly higher than in the uncoupled chips (c)

↓

Factors produced by vascular endothelium and/or cells of the perivascular niche can indeed influence neurotransmitter synthesis in the brain neuronal compartment.

What they have demonstrated:

- Linked organ chip system **mimics metabolically critical physiological functions** of the NVU, including prolonged and increased expression of metabolic enzymes.

- The **reversible effects of the neuroactive drug**, Meth, on the human BBB can be mimicked in vitro --> Meth exerted its effects preferentially on the influx BBB, whereas we did not detect any changes in the efflux BBB.

- Discovery of **previously unknown metabolic interactions** between the microvasculature and brain neurons --> these interactions significantly increased the neuronal synthesis and secretion of important neurotransmitters, including glutamate and GABA, as a result of direct neuronal utilization of vascular metabolites.

Summary

. Linked organ chips mimics metabolically critical physiological functions.

. Contribution of cell types can be analyzed

. Contribution of metabolites can be analyzed

.Effect of drugs can be studied in a more relevant setting.

Questions

. Does the vasculature itself directly effects the metabolites and neuronal metabolism?

. Reproducibility?

. If opposite results to animal models are observerd, which model to be trusted?

Paper #2

nature COMMUNICATIONS

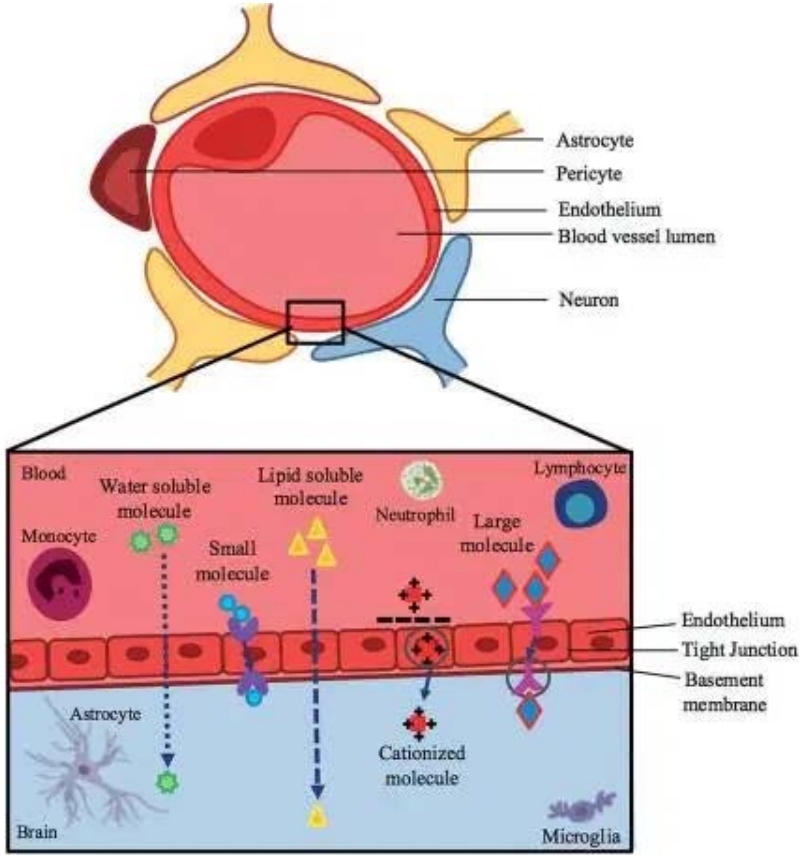
ARTICLE

https://doi.org/10.1038/s41467-019-10588-0 OPEN

Hypoxia-enhanced Blood-Brain Barrier Chip recapitulates human barrier function and shuttling of drugs and antibodies

Tae-Eun Park[1,6,9], Nur Mustafaoglu[1,9], Anna Herland[1,7,8], Ryan Hasselkus[1], Robert Mannix[1,5], Edward A. FitzGerald[1], Rachelle Prantil-Baun[1], Alexander Watters[1], Olivier Henry[1], Maximilian Benz[1], Henry Sanchez[1], Heather J. McCrea[2], Liliana Christova Goumnerova[2], Hannah W. Song[3], Sean P. Palecek[3], Eric Shusta[3] & Donald E. Ingber[1,4,5]

Blood Brain Barrier (BBB)



BBB is a unique and selective physiological barrier that **controls transport between the blood and the central nervous system (CNS)** to maintain homeostasis for optimal brain function.

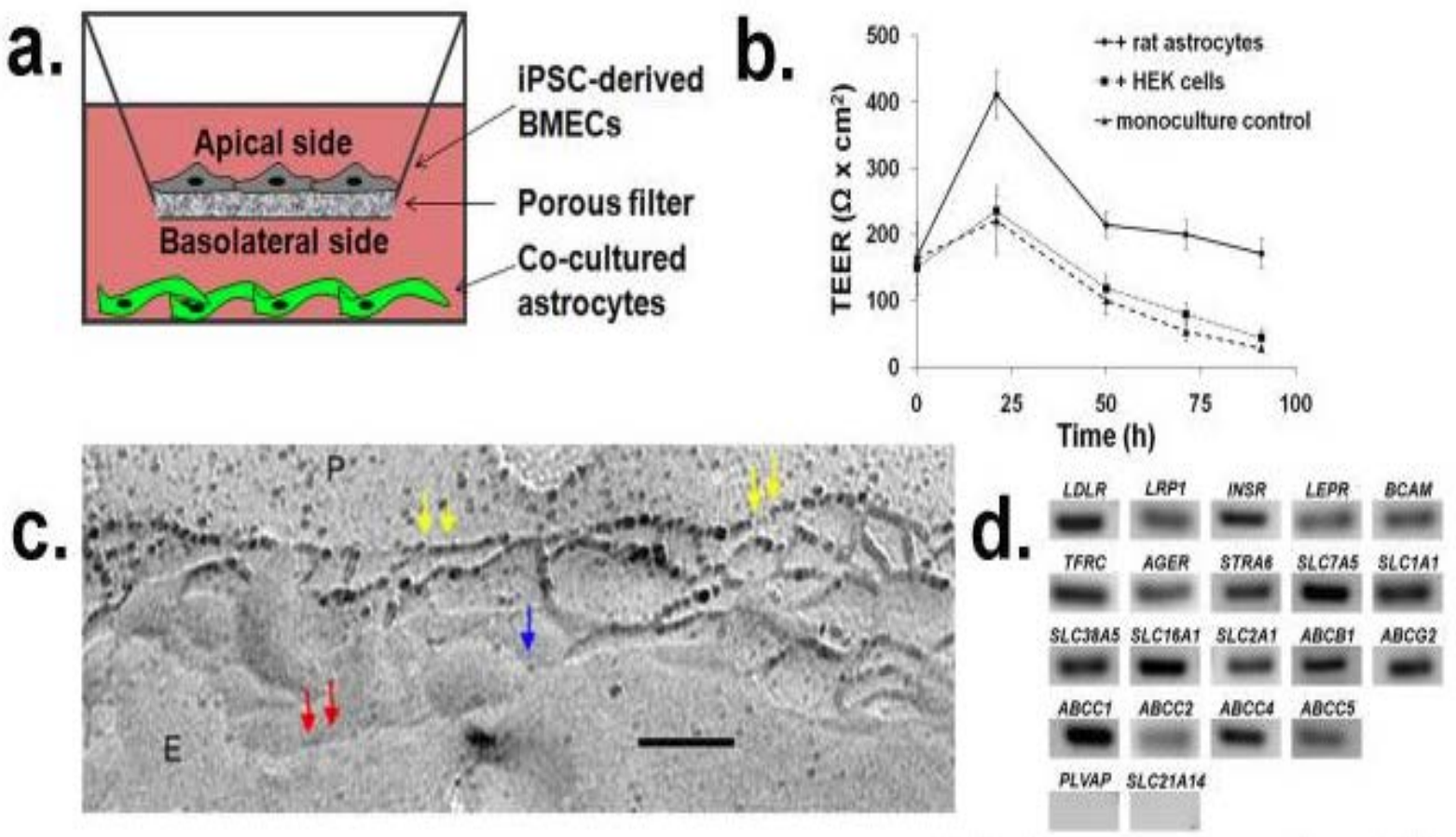
The BBB is composed of:

- brain microvascular endothelial cells (BMVECs)
- pericytes
- astrocytes

- Dysfunction of the BBB observed in many neurological diseases.
- The efficacy of drugs designed to treat neurological disorders is often limited by their inability to cross the BBB.

Need for a human BBB model that could be used to develop new and more effective CNS-targeting therapeutics and delivery technologies.

iPS-BMVECs for studies on targeted delivery to the CNS



Limitations:

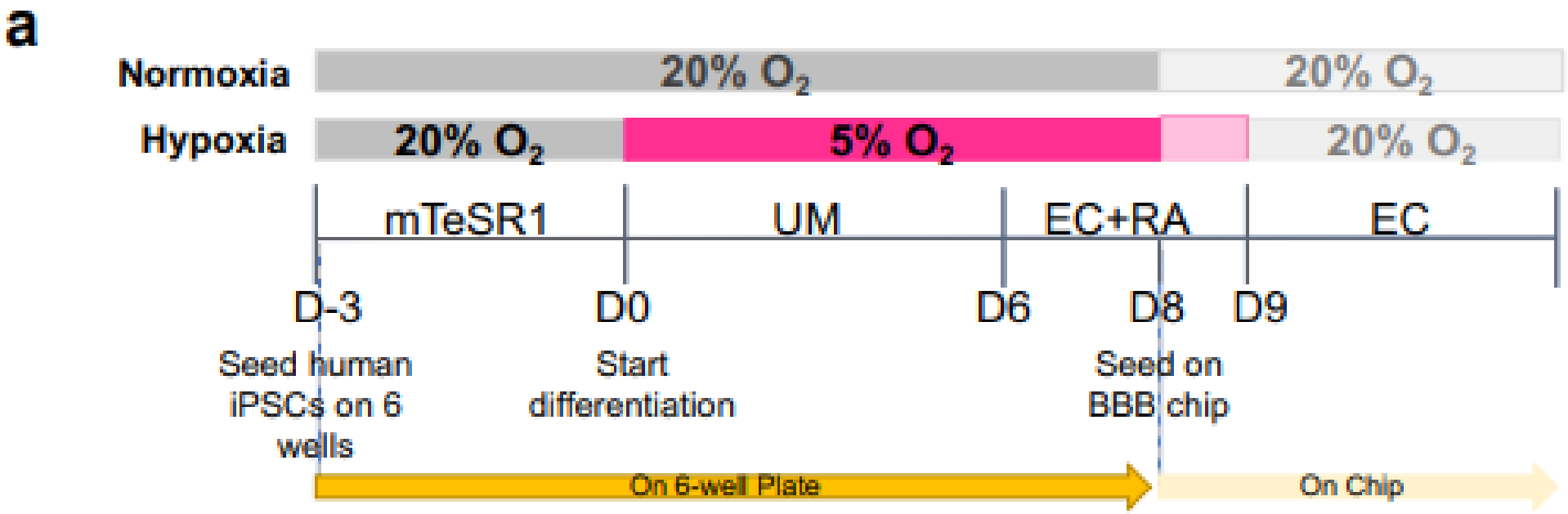
- they can only maintain these high TEER levels for~2 days
- the expression of efflux pumps in these iPS-BMVECs does not fully mimic those of human brain endothelium in vivo

Lippmann, E. S. et al. Derivation of blood-brain barrier endothelial cells from human pluripotent stem cells. Nat. Biotechnol. 30, 783–791 (2012).

Developmentally inspired differentiation of brain endothelium.

- Oxygen availability has been shown to play a vital role in endothelial differentiation from a variety of stem cell sources.
- Culturing iPS cells under similar hypoxic conditions could generate more highly differentiated BMVECs and potentially stabilize their phenotype. (Anon, 2019)

creating human BMVECs

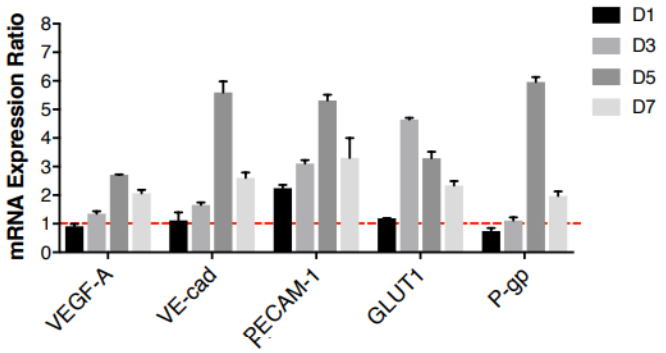


--> successful transition of the iPS cells into a human BMVEC phenotype.

Supplementary Figure 1

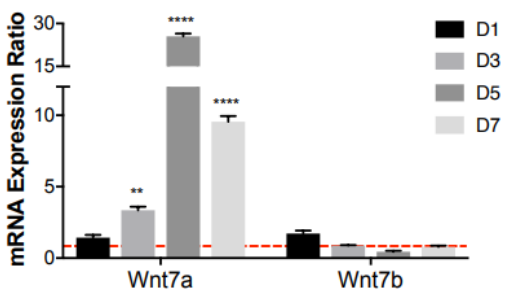
Developmentally inspired differentiation of brain endothelium.

b



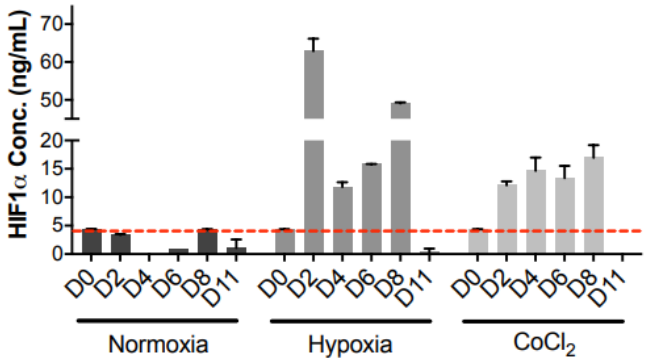
- **VE-cadherin** (vascular endothelial cadherin)
- **PECAM-1** (platelet endothelial cell adhesion molecule)
- influx transporter **GLUT-1** (BBB-specific glucose transporter)
- efflux transporter **P-gp** (permeability glycoprotein)
- **VEGF-A** (angiogenic vascular endothelial growth factor-A),

c



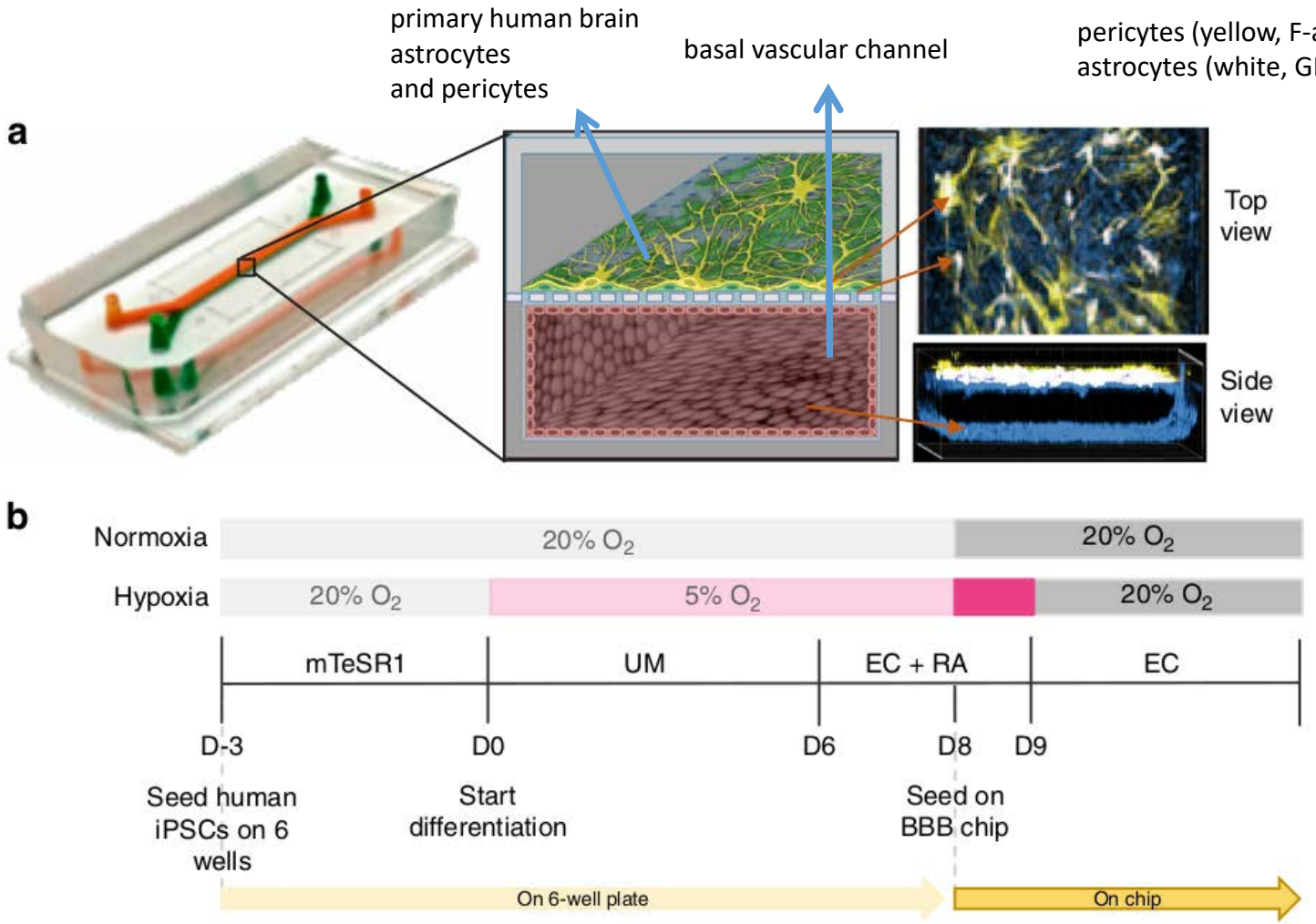
Wnt ligands have been implicated in BBB development in vivo --> Wnt7a mRNA levels increased by over 25-fold with the hypoxic induction protocol compared with normoxic conditions.

d



Supplementary Figure 1

Reconstitution of a human BBB on-chip under hypoxia.



Optically clear, poly(dimethylsiloxane) (PDMS), 2-channel, microfluidic devices containing:

- an upper CNS microchannel separated from a parallel vascular microchannel by a porous (2 µm diameter) polyethylene terephthalate (PET) membrane coated on both sides with an ECM composed of collagen type IV and fibronectin.

Fig. 1a

- Flow control through the vascular channel to maintain physiological levels of fluid shear stress (6 dyne cm −2 at 100 µL h −1)
- Blood-like viscosity control (3–4 cP; modified by adding 3.5% dextran to the medium).

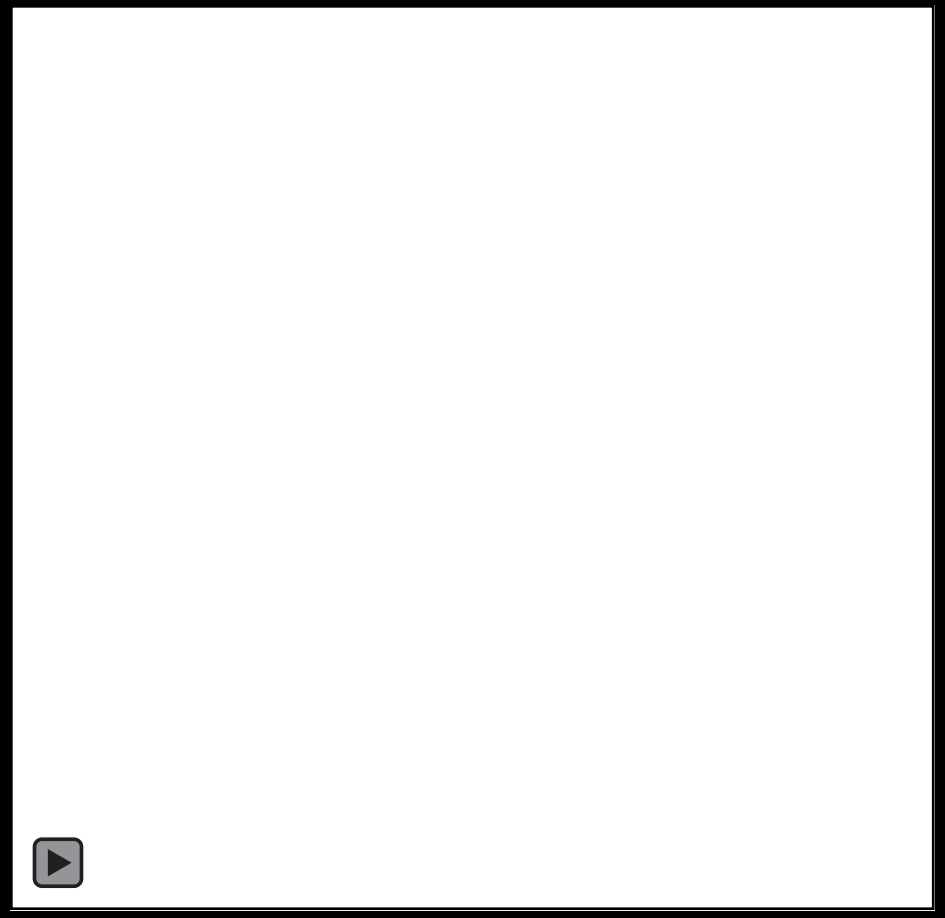
Reconstitution of a human BBB on-chip under hypoxia.

Formation of an iPS-BMVEC monolayer that covered all four walls of the lower channel:

- it creates a vascular lumen

-it is interfaced directly across the porous ECM-coated membrane with primary human pericytes and astrocytes in the CNS channel above.

Reconstitution of a human BBB on-chip under hypoxia.



Astrocytes extended processes through the 2 µm pores of the PET membrane

they come into direct contact with the luminal surface of the brain endothelium below.

Results

Reconstitution of a human BBB on-chip under hypoxia.

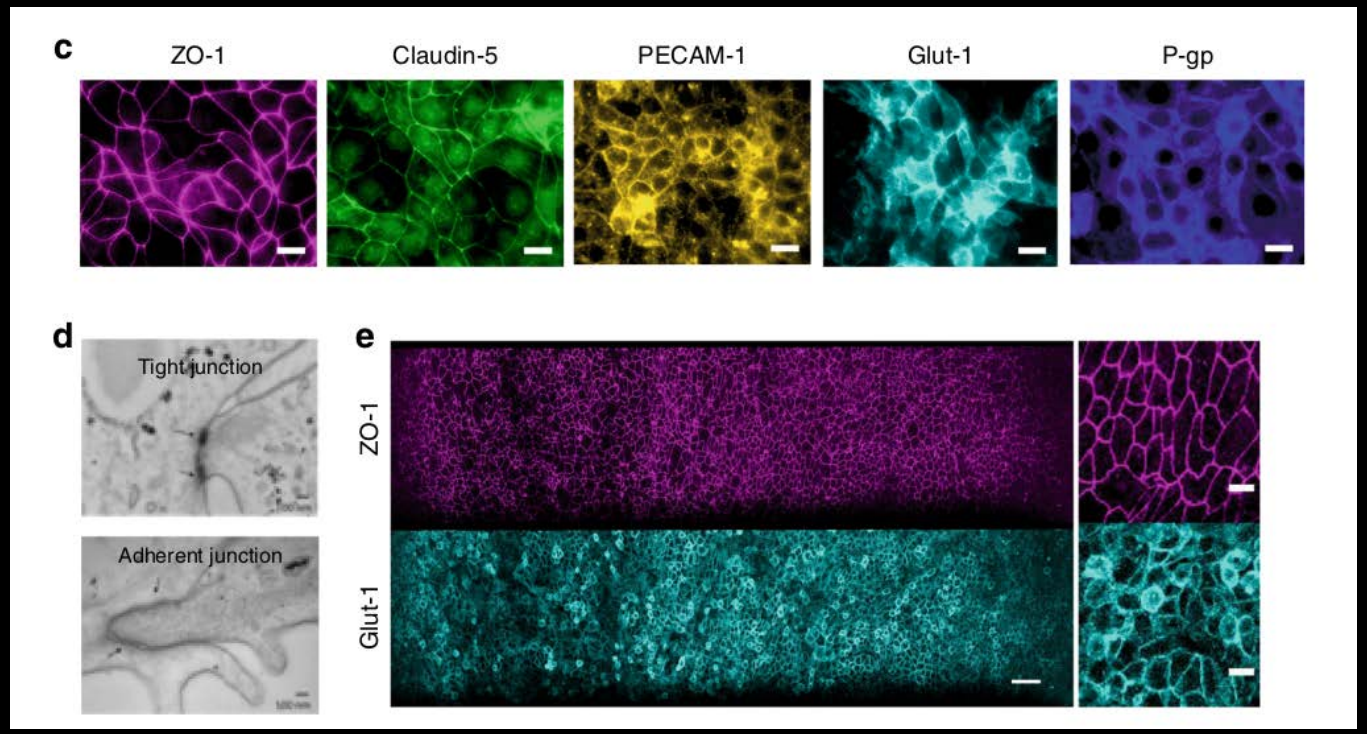


Fig. 1c: hypoxia-induced iPS-BMVECs:

- formed well developed tight junctions containing ZO-1 and Claudin-5
- expressed high levels of the cell–cell adhesion protein PECAM-1 along their lateral borders, as well as GLUT-1 and P-gp transporters on their apical cell membrane.

Fig. 1d: the differentiated iPS-BMVEC monolayer also displayed well-developed tight junctions and adherens junctions.

Fig.1e: BBB Chip maintained its tight junctional integrity and continued to express high levels of ZO-1 as well as the glucose transporter GLUT-1 on their apical surface for at least 1 week in culture.

Results

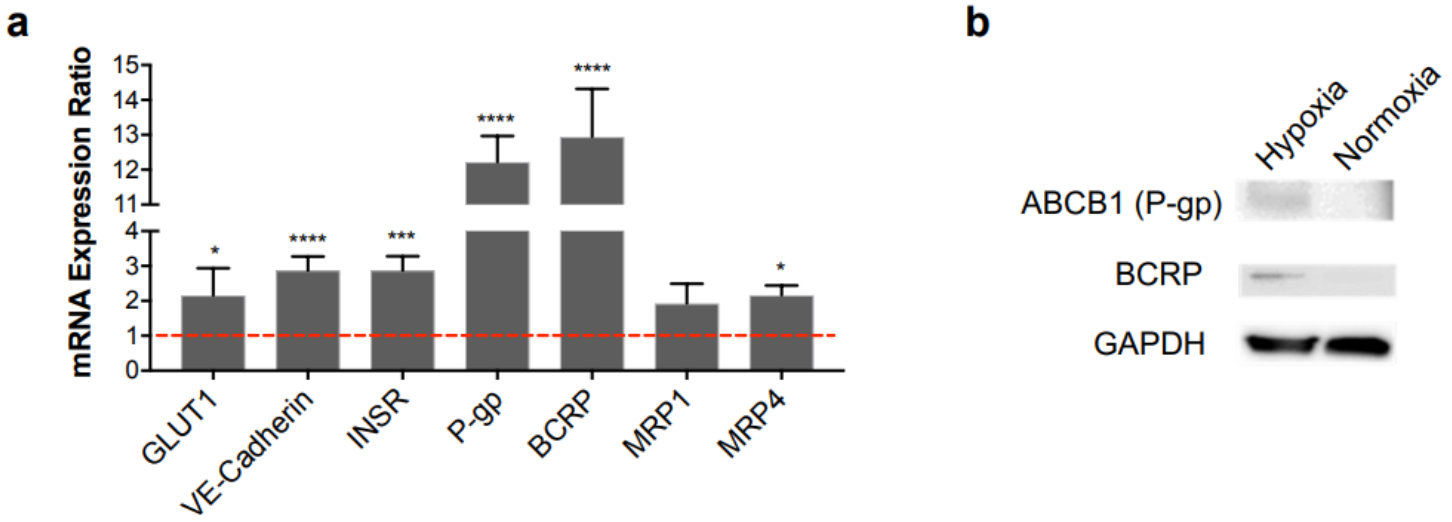
Reconstitution of a human BBB on-chip under hypoxia.

Hypoxia-induced enhancement of brain-specific endothelial cell differentiation:

gene and protein expression levels of representative BBB markers were compared with iPS-BMVECs cultured on-chip differentiated under hypoxic versus normoxic conditions:

→ Statistically significant increases in mRNA expression were observed for genes encoding:

- **GLUT-1**
- **insulin receptor protein (INSR)**
- **BBB efflux transport proteins P-gp**
- **BCRP (breast cancer resistant protein)**
- **multidrug resistance proteins 1 and 4 (MRP1 and MRP4)**
- **Endothelial cell-specific VE-cadherin.**



Supplementary Figure 2

Results

Reconstitution of a human BBB on-chip under hypoxia.

MS analysis of iPS-BMVECs collected from BBB Chips on the 3rd day of seeding revealed:

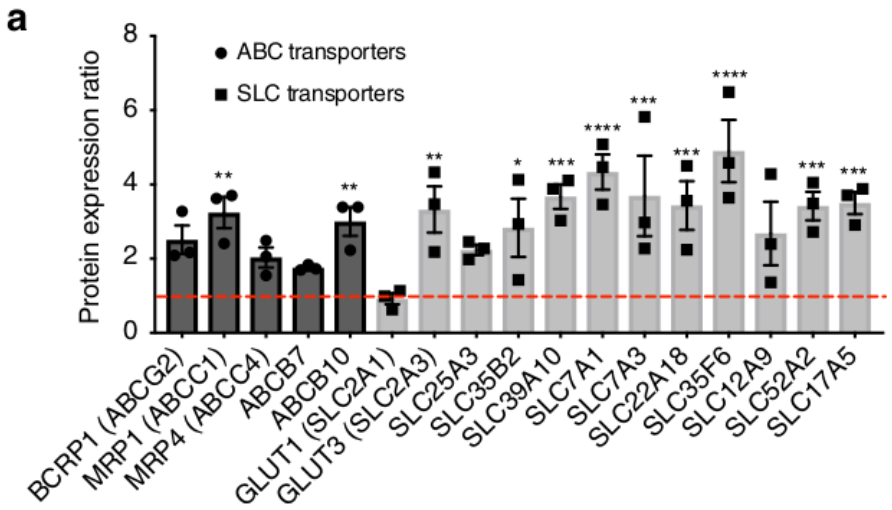
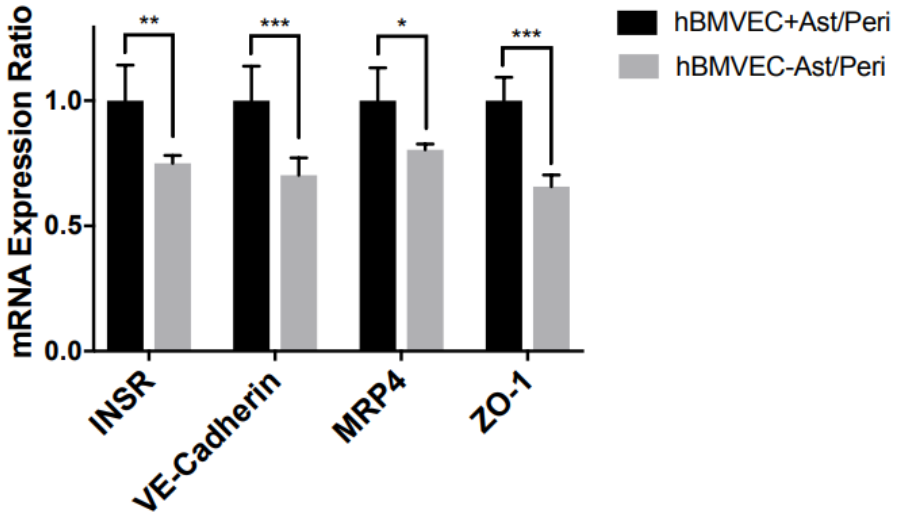


Figure 2

Comparison of mRNA profiles of iPS-BMVECs in mono-culture versus co-culture under continuous flow in the microfluidic BBB Chip.



Supplementary Figure 4

MS analysis of iPS-BMVECs collected from BBB Chips on the 3rd day of seeding revealed:

higher protein levels of ATP-binding cassette (ABC) and solute carrier (SLC) transporter proteins

in hypoxia-differentiated brain endothelium cultured in the BBB Chips vs endothelium differentiated under normoxic conditions

Why?--> To determine the contribution of astrocytes and pericytes to the function of iPS-derived human brain microvascular endothelium.

Result: **The mRNA levels of INSR, VE-cadherin, MRP1, and ZO-1 were all significantly higher in iPS-derived microvascular endothelium when co-cultured with brain astrocytes and pericytes on-chip.**

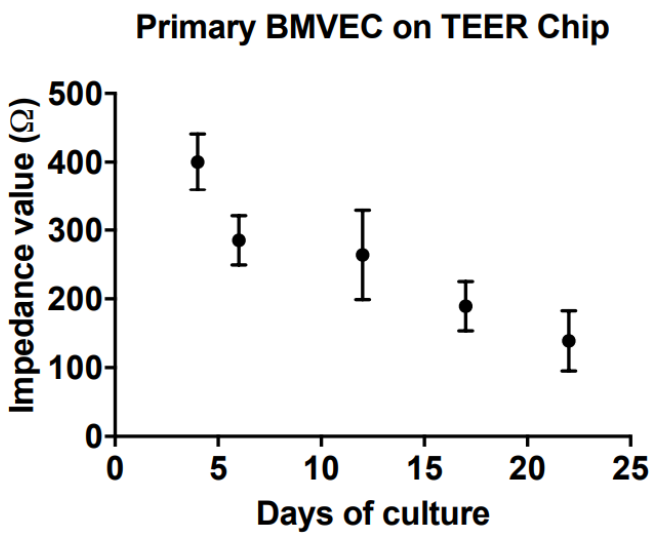
Results

Reconstitution of in vivo levels of BBB function on-chip.

The higher expression of BMVEC-specific surface markers we observed in the presence of pericytes and astrocytes was associated with enhanced barrier function. --> quantified by measuring TEER on-chip using BBB Chips that contained integrated electrodes.

Transepithelial/transendothelial electrical resistance **TEER**

Previously reported BBB chip



Supplementary Figure 5

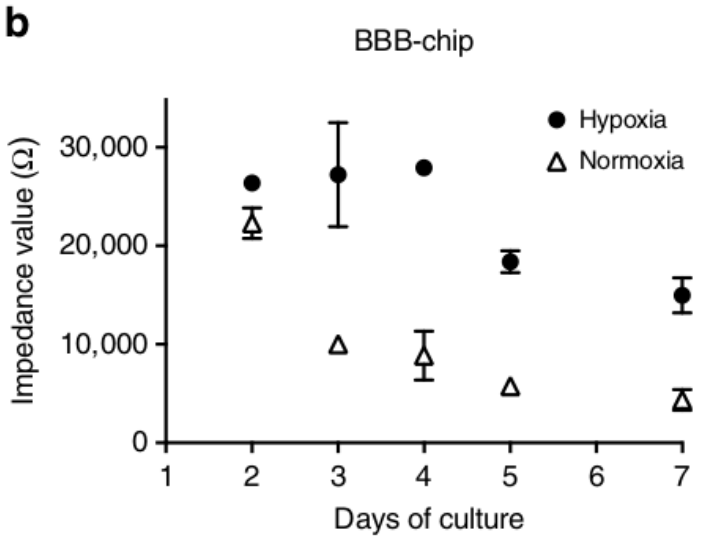


Figure 2

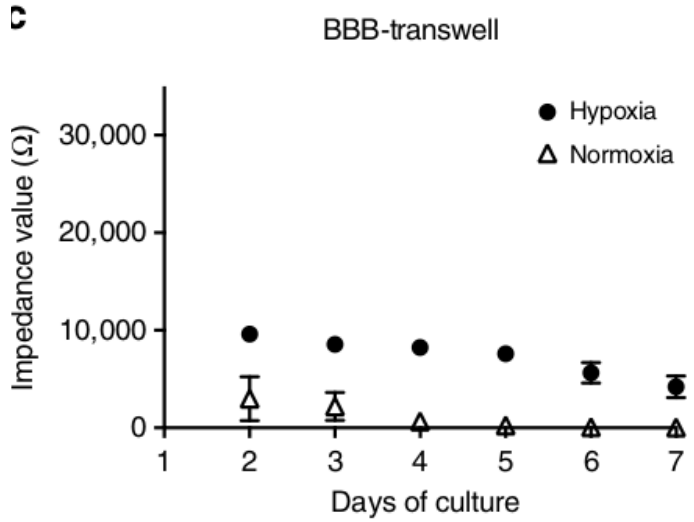
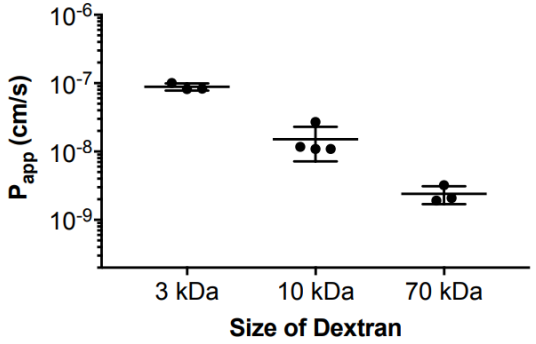


Figure 2

Results

Reconstitution of in vivo levels of BBB function on-chip.

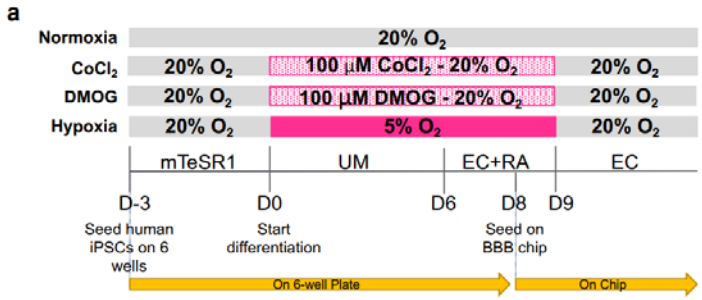
Measuring barrier integrity by dextran tracer

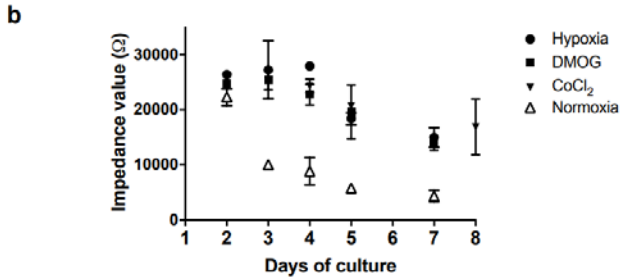


the Papp (apparent permeability) value inversely correlated
with the size of the tracer

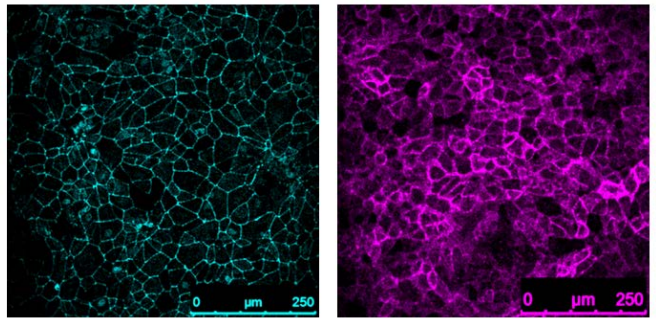
Supplementary Figure 6

Induced normoxic conditions





BBB-Chip
iPSC-derived hBMVECs – $CoCl_2$ differentiated



ZO-1 Glut1

Supplementary Figure 7, 9

- normoxic conditions with the HIF1α inducer CoCl 2 (100 µM),

- normoxic conditions with another chemical hypoxia mimetic, dimethyloxalylglycine (DMOG; 100 µM)

The highly enhanced, in vivo-like level of barrier function (> 20,000 Ω)
obtained with iPS-derived hBMVECs differentiated using CoCl 2
also could be prolonged for more than 2 weeks when cultured
under continuous flow in the microfluidic BBB Chip.

An in vitro model to study molecular trafficking across the BBB

1) Can enhanced human BBB Chip containing hypoxia-differentiated iPS-BMVECs, astrocytes and pericytes recreate a functional metabolic barrier that regulates molecular traffic across the BBB?

2) If yes, does this occur in a more physiologically relevant manner than in chips containing cells differentiated under normoxic conditions?

Results

An in vitro model to study molecular trafficking across the BBB

ABC efflux pumps are expressed on the apical surface of the human iPS-BMVECs induced by hypoxia: what about their functionality and substrate selectivity?

To assess P-gp (efflux pump) activity in the BBB Chip:

pre-treatment with:
- P-gp inhibitor penetration (**verapamil**)

perfusion through the endothelium-lined vascular channel of the BBB Chips of:
- the known P-gp substrates **rhodamine 123** and **DiOC2**.
- the drug **citalopram**

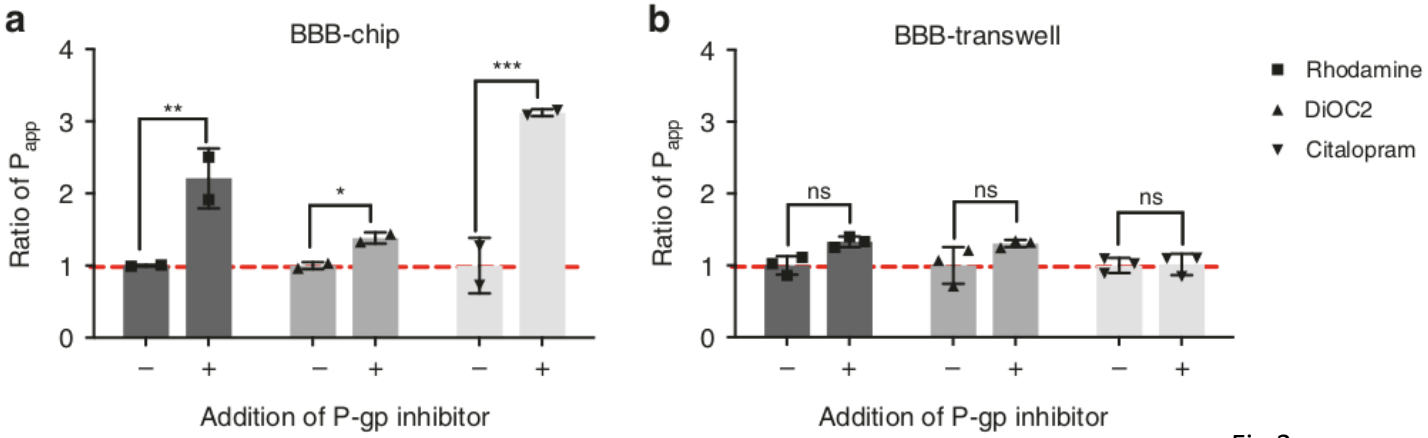


Fig.3

Significant enhancement of the permeability of all three of these molecules through the BBB on-chip when the P-gp efflux pump was blocked by its inhibitor, verapamil, Citalopram was also shown to increase its permeability when P-gp was inhibited in the microfluidic BBB Chip

--> recapitulates similar in vivo findings shown in past animal studies.
--> This is an important result because all past in vitro BBB models failed to identify citalopram as a P-gp substrate.

Results

An in vitro model to study molecular trafficking across the BBB

To analyze the activities of BCRP and MRP1 transporter proteins, the BBB Chips were pretreated with inhibitors of various ABC transporter inhibitors:

- verapamil that target P-gp
- MK571 that target MRPs
- Ko143 that target BCRP

then uptake of fluorescent substrates, rhodamine 123 and DiOC2, was quantified under flow using fluorescent dextran (3 kDa) to monitor barrier integrity.

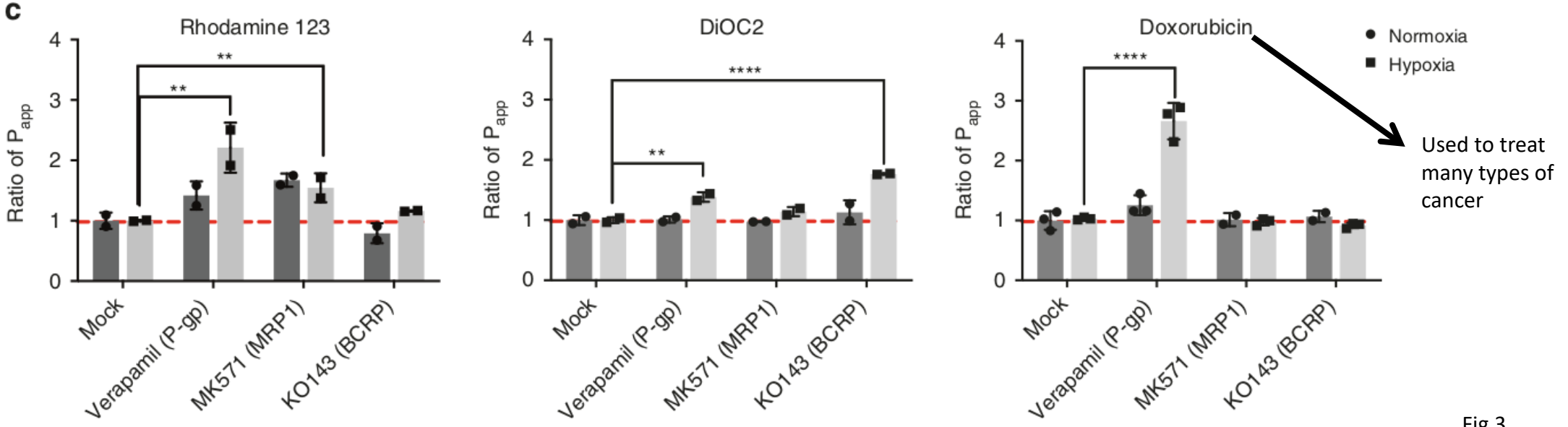


Fig.3

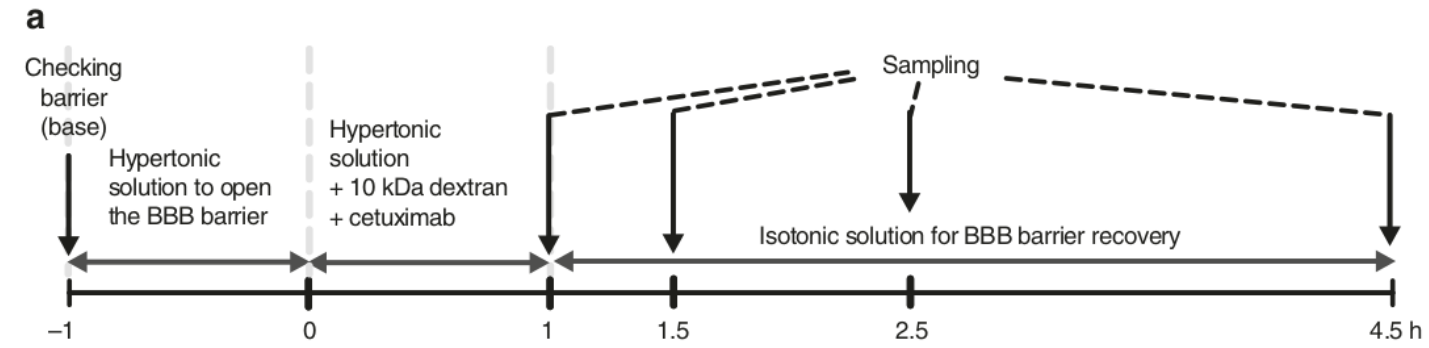
--> While **the iPS-BMVECs differentiated under normoxic conditions** may generate an effective permeability barrier , they **do not fully recapitulate the human BBB's specialized molecular transport** functions that are highly relevant to the drug development process.

--> **Differentiation of iPS-BMVECs in the presence of hypoxia** and positioning of these cells under physiological flow at a tissue-tissue interface with human brain pericytes and astrocytes --> generation of an artificial **human BBB that exhibits higher substrate specificity and functionality of the efflux barrier** than previously reported in any in vitro BBB model.

Results

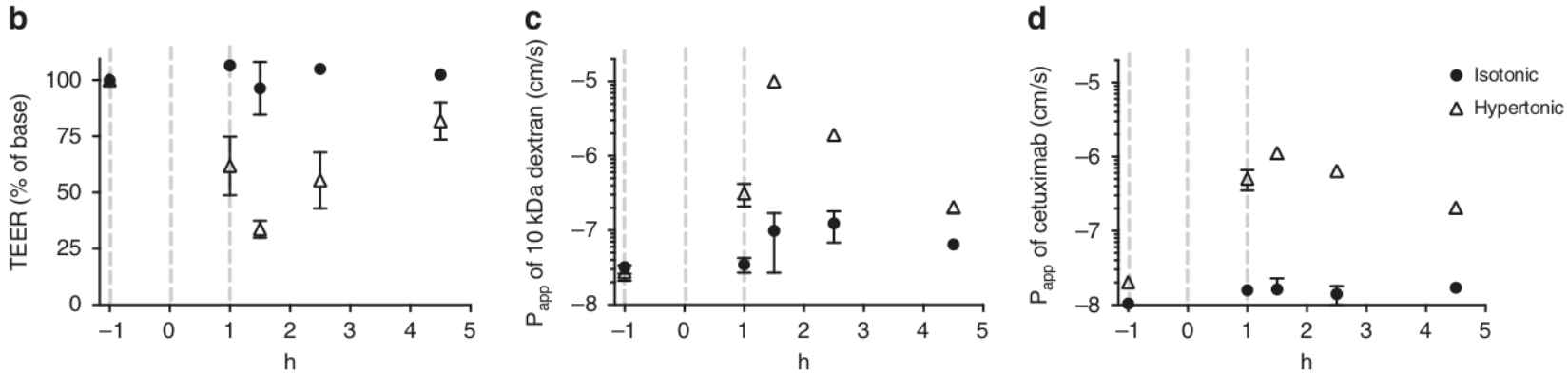
Reversible osmotic opening of the human BBB on-chip.

Is it possible to deliver large antibodies, such as those used as cancer therapeutics, by opening the barrier with hypertonic mannitol on chip?



- Hypertonic (485 mOsmol L−1) mannitol solution was flowed through the endothelium-lined vascular channel of the BBB Chip for 1 h to open the BBB

- Additional hypertonic medium containing 10 kDa dextran and the anti-cancer therapeutic
antibody, cetuximab, was flowed for an additional hour through the same channel.



The permeability of dextran (Fig. 4c) and the antibody (Fig. 4d) decreased to the normal range within 4 h

resulting in full recovery of barrier integrity.

Fig.4

This is the first demonstration of delivery of a clinically approved antibody drug by reversible osmotic opening of the human BBB in vitro.

Recapitulation of BBB-shuttling activities on-chip

Can this model be used to directly assess the human BBB-penetrating capacity of peptides, nanoparticles, and antibodies in vitro?

- Angiopep-2 is a small peptide ligand of LRP-1, expressed by iPS-BMVECs
- It penetrates the BBB in vitro and in vivo.
- The brain penetrating capacity of multiple drugs has been shown to be enhanced when modified with Angiopep-2.

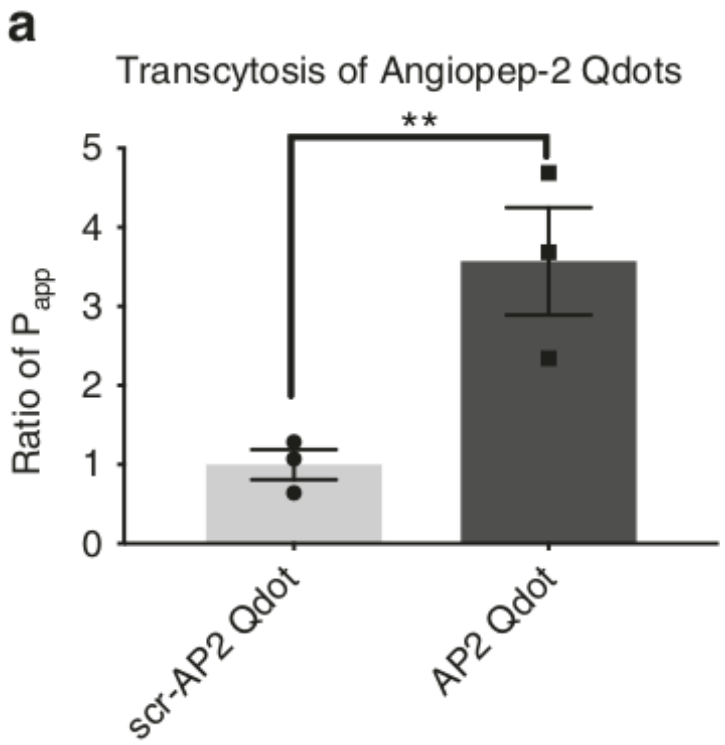
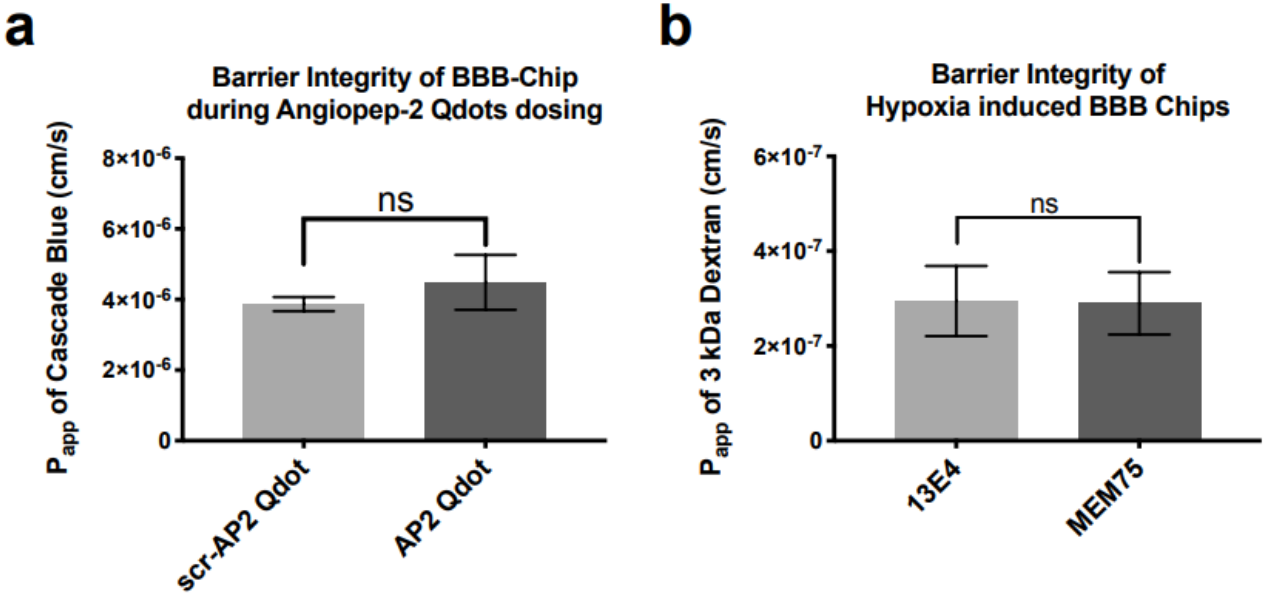


Fig.5



Supplementary fig.11

Old models

Model	Disadvantage
3D human BBB Chip models **using immortalized, primary, or iPS-derived BMVECs.**	**Fail to generate in vivo-like barrier function**.
Microfluidic BBB Chip with human iPS-BMVECs **derived under normoxic conditions and cultured under continuous flow, but with no pericytes.**	Active barrier function specifically mediated by **active efflux pumps was not fully assessed.**
Single channel microfluidic BBB model utilizing iPS-BMVECs that provides a tight **barrier function for up to 6 days.**	**Difficult to carry out TEER measurements**, explore the contributions of parenchymal cells, or **test** for the transcytotic **delivery of different drugs from capillary channels to brain side.**
A self-organized microvascular model of the BBB utilizing human iPS-BMVECs and human primary astrocytes and pericytes cultured **within a fibrin gel.**	**Fibrin gel makes it difficult to incorporate electrodes** for TEER measurements for barrier functions, and to measure trans-BBB permeability values.

What is new?

- By mimicking the **hypoxic microenvironment** of the developing brain during differentiation of human iPSCs into BMVECs, they could **enable differentiation of endothelial cells that recapitulate human-relevant physiological BBB properties** when the cells are interfaced with human brain astrocytes and pericytes in a 2-channel microfluidic BBB Chip.

- This is the **first human BBB Chip model that permits analysis of the BBB-penetrating activities of known BBB shuttle peptides**, as well as analysis of the TfR-based antibody shuttling mechanism.

- **In vivo-like barrier function for 2 weeks** compared with 2–3 days in past studies) and technical capabilities (e.g., real-time measurements of barrier function using integrated TEER electrodes).

Conclusions

- It allows for improved **drug screening** by better recapitulating the in vivo environment of the BBB.

- It may therefore prove useful for **development of drugs or delivery vehicles that selectively cross the BBB** and target the CNS.

- It can be used for **modeling CNS diseases in vitro using patient-derived iPSCs** to investigate improved brain therapies and advance personalized medicine.

Thank you!

BACK UP SLIDES

Coupled systems show uptake of metabolites

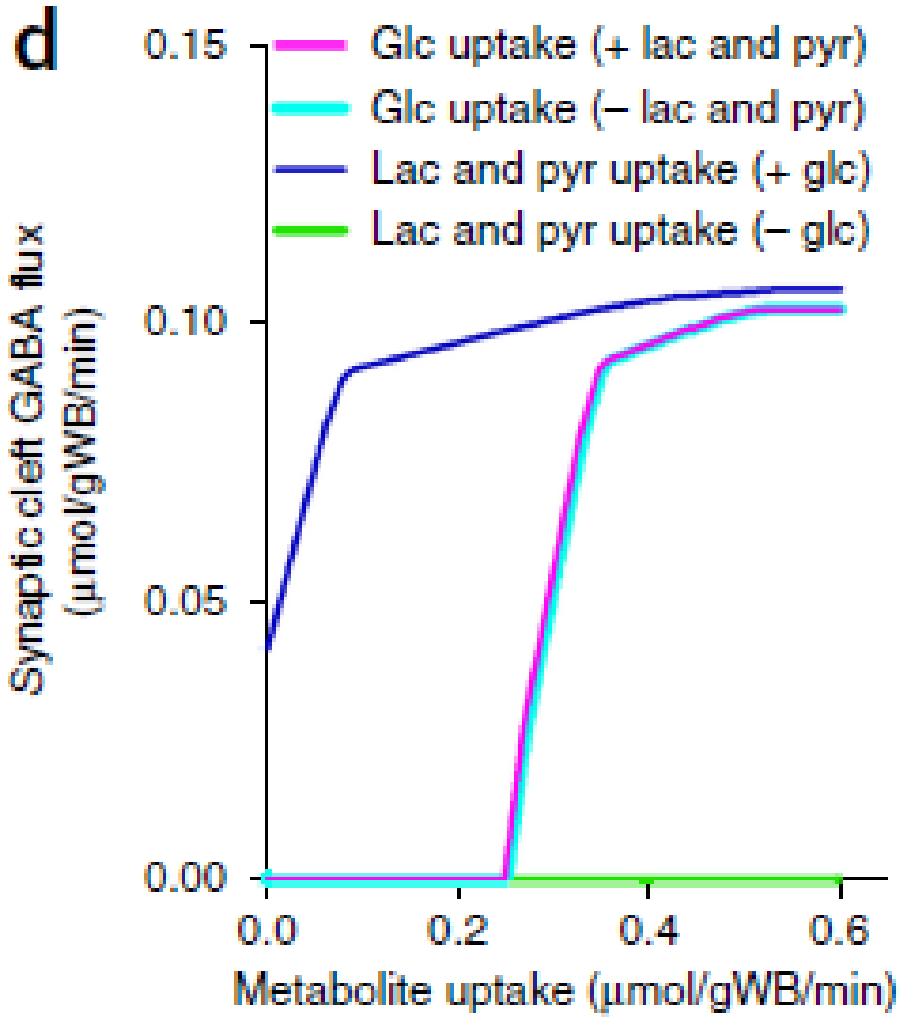


Figure 6

To identify how metabolites from the vascular and perivascular compartments influence GABA production:

modelling of the metabolic flux of GABA turnover in the synaptic clefts at contact points between astrocytes and neurons.

free exogenous pyruvate and lactate, which is not directly supplied in the cleft by astrocytes or taken up by astrocytes and directly supplied to the neurons, can contribute to neurotransmitter synthesis.

Results

Recapitulation of BBB-shuttling activities on-chip

Can this model be used to directly assess the human BBB-penetrating capacity of peptides, nanoparticles, and antibodies in vitro?

- The transferrin receptor (TfR) on BMVECs also has been shown to function as a molecular shuttle.
- It can mediate differential penetration of anti-TfR antibodies through the BBB that differ in their binding affinity.
- The anti-TfR antibody MEM75 has been shown to penetrate into the brain more efficiently than the 13E4 antibody in vitro.

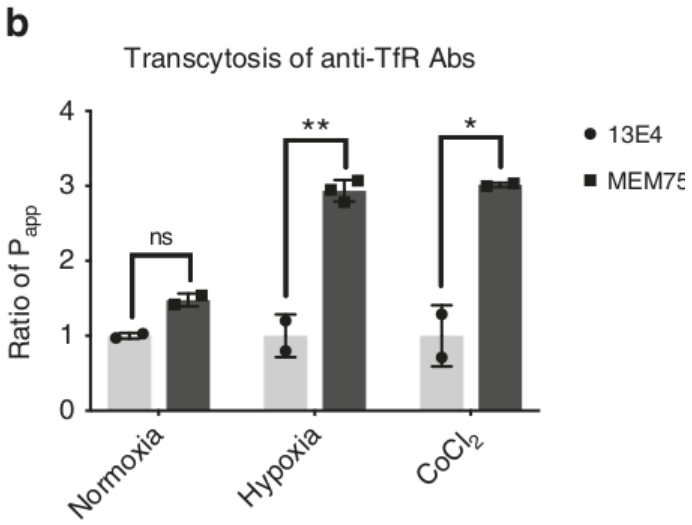
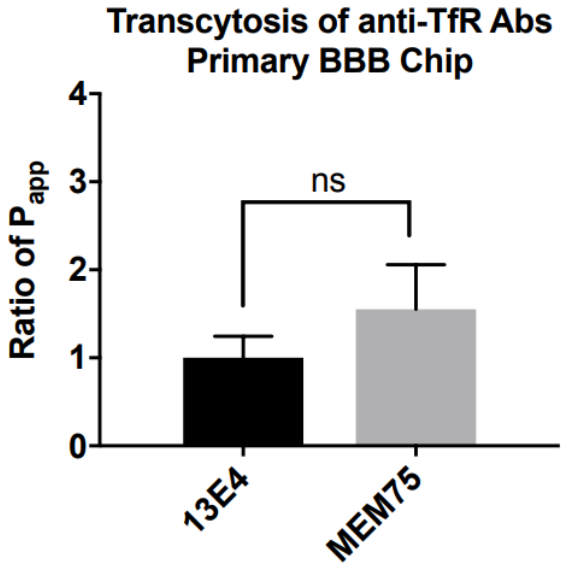
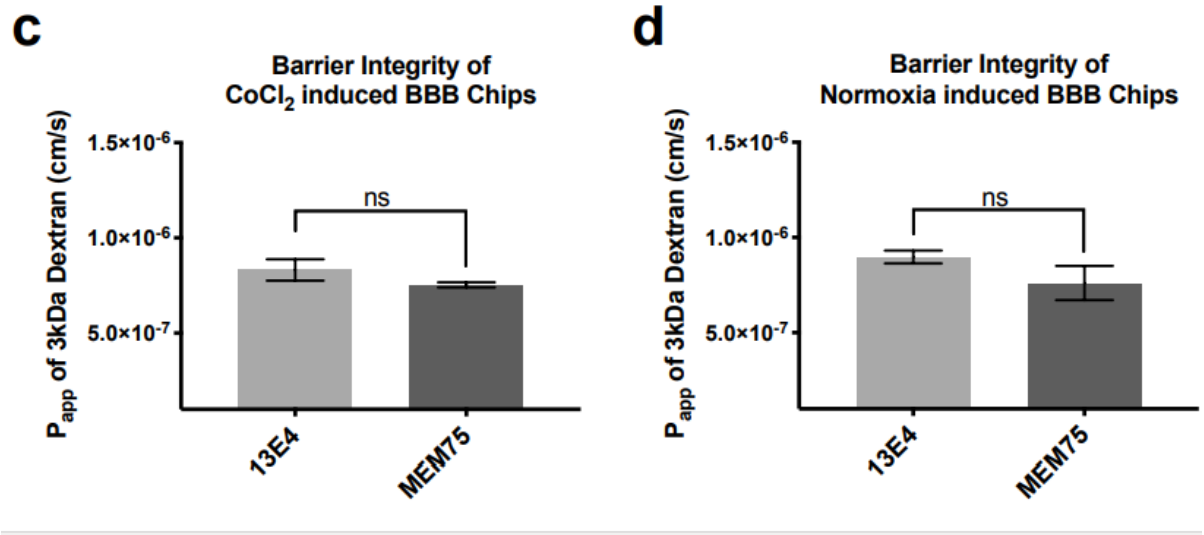


Fig.5



Supplementary fig.12



Supplementary fig.11

threefold higher penetrance of MEM75 into the CNS channel relative to the 13E4 antibodies.

Discussion

Their new model: the hypoxia-enhanced BBB Chip

- The differentiated human brain microvascular endothelium also experiences dynamic fluid flow and shear stress that significantly influence:

- BBB structure and function
- tightly regulated transcellular transport activities that are crucial for drug development studies for 2 weeks

- The 2-channel design of the human BBB Chips also enables direct and independent access to both the parenchymal and vascular compartments, as well as real-time TEER measurements for quantitative assessment of barrier function, which greatly facilitates trans-BBB shuttling studies.

- Induction of HIF1α appears to be the essential trigger for the generation of an enhanced in vivo-like BBB Chip as similar induction of iPS-BMVEC differentiation could be obtained by addition of small molecule inducers of HIF1α (CoCl 2 and DMOG).

- It is able to mimic transporter-mediated drug efflux including appropriate substrate specificity.

- It recapitulated in vivo interaction of P-gp and citalopram under physiological flow, which could not be reproduced under static conditions in past 3D models.