THE 2ND BARCELONA BLOOD-BRAIN-BARRIER (B4) CONFERENCE

BOOK OF ABSTRACTS

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Dr. Monica Mir[1], Ms. Anna Panteleeva [1], Dr. Sujey Palma-Florez [1], Ms. Carla Arroyo [1], Dr. Anna Lagunas Targarona [1], Prof. Josep Samitier Martí [1]

1. *IBEC*

Tracing proteomes across the blood-brain barrier

Wednesday, 1st October - 09:00: (Auditorium 1) - Keynote speakers

Mr. Andrew Yang [1]

1. *Gladstone Institutes, Gladstone Institute of Neurological Disease, San Francisco, CA, USA*

TBC

Regulation of claudin-5 at the BBB in health and disease

Wednesday, 1st October - 10:00: (Auditorium 1) - Invited Speaker

Prof. Matthew Campbell [1]

1. *Trinity College Dublin*

TBC

3D bioprinted human blood brain barrier: a high-throughput in vitro platform for neurodegenerative diseases modeling and drug screening

Wednesday, 1st October - 11:10: (Auditorium 1) - Oral

Ms. Gal·la Vinyes i Bassols [1], ***Prof. Josep Samitier Martí*** [2], ***Dr. Anna Lagunas Targarona*** [2]

1. *Institute for Bioengineering of Catalonia & University of Barcelona,* ***2.*** *IBEC*

The highly selective permeability of the blood-brain barrier (BBB) is crucial for protecting the brain from toxic substances, yet it simultaneoulsy poses a substantial obstacle to central nervous system (CNS) drug delivery, severely limiting the effectiveness of therapies targeting the brain. This challenge is reflected in the clinical landscape, where nearly 80% of drug candidates for neurodegenerative diseases (NDDs) fail in trials, resulting in the lowest approval rate among all therapeutic areas. To address the pressing need for more predictive and physiologically relevant NDD models, we introduce a three-dimensional (3D) bioprinted human BBB platform that versatility recapitulates both physiological and pathological CNS conditions *in vitro*. Leveraging a novel, microvalve-based embedded 3D bioprinting approach, our automated system enables high-throughput fabrication and precise spatial placement of cells with excellent viability. Using a low-viscosity bioink composed of fibrinogen, alginate, and brain microvascular endothelial cells (BMECs), we reproducibly generate ring-patterned scaffolds—up to 48 constructs in just 9 minutes—by droplet deposition into a crosslinking bath. After alginate removal, BMECs proliferate and self-organize within the fibrin matrix, forming intricate, branching vascular networks within a week. This versatile platform also supports the integration of additional neural cell types, enabling comprehensive studies of neurovascular interactions and the dynamic crosstalk between neural and vascular compartments. Furthermore, the system allows for the targeted introduction of drugs, nanoparticles, or neuroimaging agents into the luminal compartment, facilitating a wide range of applications including investigations of brain physiology, disease mechanisms, drug efficacy and safety, and neuroimaging probe development. Collectively, our findings establish a robust, scalable, and cost-effective 3D bioprinted BBB model that offers significant potential to bridge the gap between preclinical research and clinical translation in neurodegenerative disease drug development.

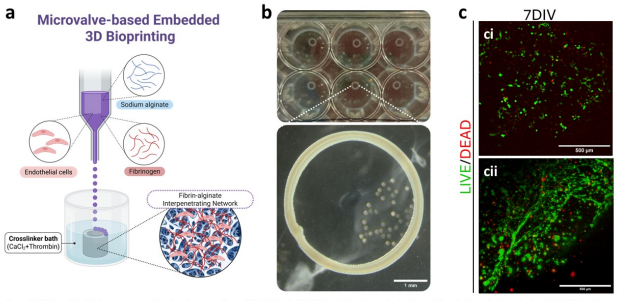


Figure 1. 3D bioprinted fibrin rings support brain microvascular endothelial cells (BMECs) proliferation and migration. ***a)*** *Novel microvalve-based embedded 3D bioprinting strategy. A low viscous bioink consisting of fibrinogen, alginate and BMECs is ejected in droplets into a crosslinker bath following a ring pattern.* ***b)*** *Axial plane of 3D bioprinted polymerized fibrin/alginate structures in a 48 well plate. 48 reproducible constructs are bioprinted within 9 minutes.* ***c)*** *LIVE/DEAD cell viability assay of 3D bioprinted BMECs in fibrin/alginate (ci) and fibrin only (cii) rings. BMECS form self-assembled branching vascular networks in fibrin only matrix rings 7 days post-printing. DIV: Days in vitro.*

Figure1 3dbp fibrinrings bmecs.jpg

Defining mechanisms of blood-brain barrier dysfunction in neurodegenerative diseases using advanced organ-on-a-chip models

Wednesday, 1st October - 11:25: (Auditorium 1) - Oral

Prof. Mootaz Salman [1]

1. *University of Oxford*

Neurodegenerative diseases are complex, multifactorial conditions and remain a leading cause of disability and mortality worldwide. Our research addresses a central question: *How does inflammation-driven blood-brain barrier (BBB) dysfunction contribute to the initiation and progression of neurodegeneration and can we intervene to stop it?*

Compelling evidence indicates that BBB breakdown is not merely a consequence of neurodegenerative diseases such as Parkinson's, Alzheimer's, and cerebral small vessel disease, but may precede and accelerate their onset. Critically, this barrier disruption is often detectable before the appearance of clinical symptoms, making it a promising early target for therapeutic intervention.

Our work focuses on dissecting the cellular and molecular crosstalk between the principal components of the BBB; endothelial cells, astrocytes, and pericytes , under neuroinflammatory and mechanobiological conditions relevant to human disease.

We have developed dynamic 3D microfluidic BBB-on-a-chip models using patient-derived induced pluripotent stem cells (iPSCs) and human primary cells. These physiologically realistic systems allow us to recreate and monitor barrier behaviour under disease-relevant stressors and to study the mechanisms of neurovascular failure at high resolution.

Our platform offers a powerful and scalable tool for studying lifelong brain health, uncovering the early drivers of BBB dysfunction, and supporting the discovery of new therapeutic targets for dementia and related central nervous system disorders.

Integrating endothelial networks in cerebral organoids model the human neurovascular unit and reduce necrotic core

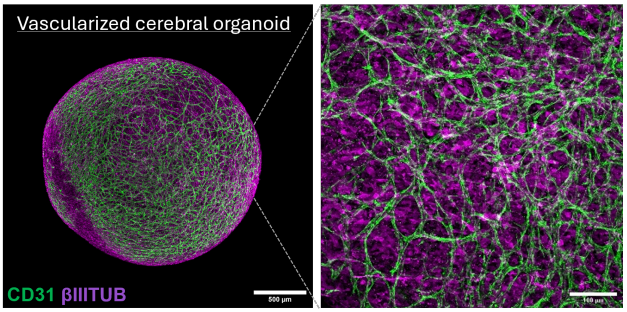
Wednesday, 1st October - 11:40: (Auditorium 1) - Oral

Mr. Josep Fumado Navarro [1], ***Dr. Siobhan Crilly*** [1], ***Prof. Abhay Pandit*** [1], ***Dr. Maria Bernabeu*** [2], ***Dr. Mihai Lomora*** [1]

1. *University of Galway,* ***2.*** *European Molecular Biology Laboratory (EMBL, Barcelona)*

Cerebral organoids (COs) are multicellular, self-organized *in vitro* 3D systems that are derived from stem cells through a specific patterning to resemble the premature morphological and physiological features of the brain [1]. These emerging models are attractive for developmental biology, disease modelling and drug screening, and as tools to reduce animal experimentation in neuroscience. However, the lack of vascularity limits their potential. The vascularization of COs poses several technical challenges, including the simultaneous differentiation of two tissues of different origin, insufficient support for both organoid and vascular counterparts, limited penetration of the vascular networks within the microtissue, and the absence of luminal perfusion [2,3]. To mitigate some of these issues, we devised an encapsulation approach in which human brain microvascular endothelial cells (HBMVECs) were delivered to developing COs from a progressively-degrading surrounding biomaterial. HBMVECs were selected for their natively specialized characteristics, which may enhance interactions with neural tissue more effectively than other endothelial cell types. The composition of the media and the concentration of the hydrogel were tuned to promote both neurodevelopment and endothelial network formation. Using this strategy, we identified a higher density of vascular-like networks that expand towards the organoid's centre. By using pathway inhibitors and fluorescent endothelial cells, the origin of these endothelial networks was revealed to be both product of endogenous differentiation and the introduced HBMVECs. Furthermore, typical blood–brain barrier characteristics like astrocytes and pericyte-like cells wrapping the networks and surrounding laminin and collagen IV depositions have been identified. Vascularized COs showed smaller necrotic cores, sustained neuronal activity and appear to be more permeable to an external dye. RNA sequencing confirmed that neurodevelopment is not inhibited while blood vessel development genes were enhanced. Overall, these findings will allow the establishment of a user-convenient protocol for enhanced vascularized COs as the basis for generating suitable platforms for cerebrovascular modelling and drug testing.

1. Lancaster, M., Renner, M., Martin, C. et al (2013). Nature 501, 7467. doi:10.1038/nature12517
2. Cakir, B., Xiang, Y., Tanaka, Y. et al. *Nature Methods* 16,1169-1175. doi:10.1038/s41592-019-0586-5
3. Zhang, Z., Wan, Z., and Kamm, R. D. (2021). *Lab Chip* 21, 473-488. doi:10.1039/d0lc01186j



Vascularized co.png

Epac-1 as a Key Therapeutic Target in cAMP-Mediated Protection of the Blood-Brain Barrier During Inflammation

Wednesday, 1st October - 11:10: (Auditorium 2) - Oral

Mrs. Nuria Seoane [1], Mr. Aitor Picos [1], Dr. Dolores Viña [1], Dr. Manuel Campos-Toimil [1]

1. *Physiology and Pharmacology of Chronic Diseases (FIFAEC), CiMUS, University of Santiago de Compostela*

Blood-brain barrier (BBB) dysfunction under inflammatory conditions is a hallmark of many neurological diseases. Cyclic AMP (cAMP) is a key intracellular second messenger with barrier-enhancing effects, acting through two primary effectors: protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac). In this study, we investigated the protective role of elevated cAMP levels and the contribution of its downstream signaling pathways in preserving BBB integrity under inflammatory conditions.

In the murine brain endothelial cell line bEnd.3, we modeled inflammation using lipopolysaccharide (LPS), which induced a significant increase in reactive oxygen species (ROS), IL-6 expression, and STAT3 phosphorylation, leading to upregulation of ICAM-1 and VCAM-1, increased macrophage adhesion, and a significant reduction in transendothelial electrical resistance (TEER). Tight junction integrity was compromised, with disrupted claudin-5 expression, while occludin and ZO-1 remained unchanged. Confocal microscopy revealed pronounced morphological changes, including cell rounding.

Co-treatment with forskolin (adenylyl cyclase activator) and rolipram (PDE4 inhibitor) restored TEER, reduced ROS, and prevented macrophage adhesion. This combination also preserved claudin-5 localization and endothelial morphology. However, IL-6 expression remained unaffected, suggesting that barrier protection is mediated by structural, not anti-inflammatory, mechanisms.

To dissect the roles of cAMP effectors, we used 8-pCPT-2'-O-Me-cAMP (Epac-1 selective activator) and 6-Bnz-cAMP (PKA activator). Epac-1 activation tended to increase claudin-5 protein levels, reduced ROS production and VCAM-1 expression, leading to restored TEER, whereas PKA activation only decreased VCAM-1 expression. Epac inhibition (ESI-09), but not PKA inhibition (Rp-cAMPs), blocked forskolin/rolipram-mediated TEER recovery.

These findings highlight that the activation of Epac-1 by cAMP plays a dominant role in maintaining BBB structure under both physiological and pathological conditions, while PKA primarily modulates certain pro-inflammatory markers. In conclusion, targeting Epac-1 may offer precise therapeutic strategies to preserve BBB function in neuroinflammatory disorders.

Endothelial cells in Multiple Sclerosis, a personalized model at the crossroad between neuro-inflammation and repair

Wednesday, 1st October - 11:25: (Auditorium 2) - Oral

Dr. Eliana Lauranzano [1], ***Dr. Margherita Ravanelli*** [1], ***Dr. Giuseppe Liberatore*** [2], ***Dr. Luca Lambroia*** [1], ***Mrs. Elisa Faggiani*** [2], ***Dr. Claudia Cutellè*** [2], ***Dr. Paolo Kunderfranco*** [1], ***Prof. Eduardo Nobile-Orazio*** [2], ***Prof. Michela Matteoli*** [3]

1. *IRCCS Humanitas Research Hospital,* ***2.*** *Neuro Center, IRCCS Humanitas Clinical and Research Center, Via Manzoni 56, Rozzano-Milan,* ***3.*** *Humanitas University, Via Rita Levi Montalcini 4, Pieve Emanuele, Milan, Italy*

A complex interplay between the immune system and the brain exists, and it is implicated both in the maintenance of brain physiology and in disease. This intricate crosstalk is key to neuroinflammatory and autoimmune diseases, such as Multiple Sclerosis (MS), yet studying immune interactions at the neurovascular interface remains a challenge due to an unmet need for detailed functional studies employing human disease-relevant tissues and cells.

To bridge this gap, we engineered a personalized human BBB in vitro model using primary endothelial cells (ECs) and autologous immune cells from healthy donors or persons with MS (pwMS), allowing patient-specific insights into immune interactions.

The endothelial identity of ECs was characterized by bulk RNAseq, and validated by multi-color flow cytometry and confocal imaging, confirming the expression of endothelial epitopes and their ability to uptake acetylated-LDL. ECs retained their fingerprint ex vivo, thus maintaining the features and recapitulating the specialized properties of the subject they originated from. Cultured ECs, under pro-inflammatory conditions, upregulated adhesion molecules, a key feature to study the phenotype of T cell subset specifically transmigrating in MS. Also, ECs were co-cultured with primary human astrocytes growing on opposite sides of a matrix-coated permeable membrane, and the integration into the NVU platform ameliorated barrier properties. Autologous lymphocytes were used to perform subject-specific transmigration studies across the NVU model. We evidenced a different transmigration capacity of T cells isolated from the same person with MS (pwMS) when treatment-naïve relapsing and at remitting follow up stage by scRNAseq. We set up and validated a multicolor flow cytometry panel to immunophenotype the signature of T cell subsets transmigrating across the autologous in vitro model in a prospective cohort of pwMS and controls.

By integrating these approaches, we provide insight into the immune-brain axis, with the final ambition to improve the understanding of neuro-immune interactions and their involvement in brain pathophysiology.

Funding: Italian Multiple Sclerosis Foundation, Italian Ministry of Health.

Investigation of the blood-brain barrier in a vascular disease cohort using novel MRI T1-mapping methods

Wednesday, 1st October - 11:10: (Sala Blava 2) - Oral

Mr. Scott French [1], ***Dr. Summan Zahra*** [1], ***Ms. Haley Wiskoski*** [1], ***Dr. Juan Arias*** [1], ***Dr. Francesca Vitali*** [1], ***Dr. Edward Bedrick*** [1], ***Dr. Raza Mushtaq*** [2], ***Dr. Maria Altbach*** [1], ***Dr. Theodore Trouard*** [1], ***Dr. Craig Weinkauf*** [1]

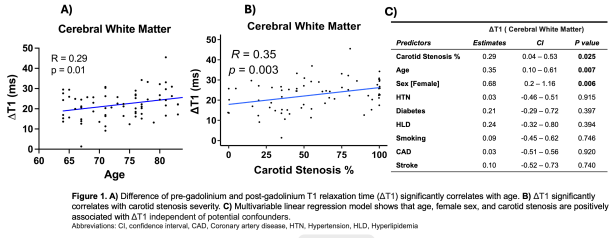
1. *University of Arizona,* ***2.*** *Barrow Neurological Institute*

Background: Asymptomatic extracranial carotid artery disease (aECAD) is a prevalent cerebrovascular disease affecting 10-15% of adults over the age of 60. aECAD is associated with neurodegeneration and increased Alzheimer's disease (AD) risk, yet mechanisms for this are unclear. We hypothesize that blood-brain barrier (BBB) dysfunction could be one factor mediating these effects. Using developmental imaging methodologies, we sought to explore the relationship between aECAD and BBB dysfunction through quantitative T1-weighted mapping.

Methods: 69 adults aged 50-85 with a clinical diagnosis of aECAD and/or $\geq$2 cardiovascular risk factors were included. Prior diagnoses of dementia, neurological disorders, and recent stroke (<6 months) were exclusionary. BBB leakage was quantified in white matter using developmental quantitative T1 mapping techniques (Radial TurboFLASH; TR/TE=5.43/2.95ms, TI=30ms, flip angle =10º, FOV=220x220x156mm, voxel =0.9x0.9x4.0mm) on a 3T MRI scanner pre- and post- Gadoteridol injection.

Results: The cohort had a mean age of 72.8±7.4 years and 41% were female. Age and female sex were positively associated with ΔT1 (P<0.05, Figure 1). Carotid stenosis severity positively correlated with ΔT1 (R=0.35, P<0.01), and this relationship persisted after adjusting for potential confounders (β=0.29, P=0.03, Figure 1).

Conclusions: ΔT1 was elevated in patients with aECAD which may indicate BBB dysfunction. However, these data are limited because there was no gold standard available for evaluation of BBB integrity, and ΔT1 is not a validated marker of BBB dysfunction. These warrant further exploration and validation in larger patient cohorts.



French figure1 deltat1 age aecad.png

Disentangling MRI-derived blood-brain barrier leakage into vascular permeability and surface area

Wednesday, 1st October - 11:25: (Sala Blava 2) - Oral

Mr. Damon Verstappen [1], Dr. Joost de Jong [1], Dr. Paulien Voorter [1], Dr. Maud van Dinther [2], Prof. Robert van Oostenbrugge [2], Prof. Julie Staals [2], Prof. Jacobus Jansen [1], Prof. Walter Backes [1]

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Objective

The MRI-derived measure of blood-brain barrier (BBB) leakage rate (K_i=PS), is the product of vascular permeability (P) and vascular surface area (S). These components can oppose each other and lead to ambiguous interpretations of the measured leakage rate. We aim to separate leakage into permeability and surface area.

Methods

45 patients with diagnosed cerebral small vessel disease (cSVD) (69 ± 9 years, 15 female) and 25 elderly controls (68 ± 7 years, 6 female) underwent 3 Tesla MRI. Tissue types were segmented from structural scans using automated software. Leakage rate (K_i) was derived from dynamic contrast-enhanced (DCE) MRI using pharmacokinetic modelling. Cerebral blood volume (CBV) and vessel size index (VSI) were obtained from a hybrid dynamic susceptibility pulse sequence, in which the gradient-echo part is sensitive to all vessels and the spin-echo part to microvessels. P and S were calculated by $P = K_i/S$ and $S=2\times CBV\times \Delta v/VSI$, respectively ($\Delta v$=voxel volume).

To improve microvascular specificity, we excluded voxels with large vessels, voxels adjacent to the ventricles, and omitted the cortex. We evaluated tissue differences in K_i, P and S using an ANOVA test with post-hoc paired t-tests for controls. Disease effects were assessed using a multivariable linear mixed-effects model, corrected for age and sex.

Results

In elderly controls, K_i was greater in deep gray matter (DGM) compared to normal appearing white matter (NAWM), but neither differed from white matter hyperintensities (WMH). P was highest in WMH compared to DGM and NAWM, and similar between DGM and NAWM. S was highest in DGM, followed by NAWM and WMH (figure 1).

For cSVD S decreased and trended towards increased P in WMH, but had no effect in NAWM and DGM. Figure 2 shows parameter maps. Aging increased K_i and P, and decreased S (Table 1).

Conclusion

Higher permeability and lower surface area counterbalance as opposite effects to the leakage rate in normal brain tissue, cSVD and aging. WMH have stronger permeability and reduced surface area, indicating BBB damage and microvascular rarefaction, respectively. Separating leakage rate into permeability and surface area provides a more specific biological view on normal and diseased brain tissue.

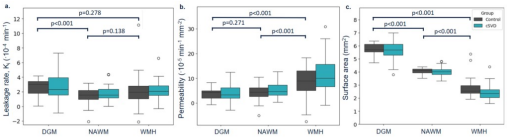


Figure 1: Boxplots of (a) BBB leakage rate (K_i), (b) intrinsic blood-brain barrier (BBB) permeability (P), and (c) surface area (S) in the deep gray matter (DGM), normal appearing white matter (NAWM), and white matter hyperintensities (WMH) for elderly controls and patients with cSVD. The p-values are derived from paired t-tests for controls between tissue regions.

Table 1: Multivariable mixed-effects linear regression model results evaluating leakage rate (K_i), intrinsic permeability (P) and vascular surface area (S) in the deep gray matter (DGM) and white matter hyperintensities (WMH) in reference to normal appearing white matter (NAWM) of elderly controls and patients with cerebral small vessel disease (cSVD).

Predictor	**Leakage rate, K_i ($\cdot 10^{-4}$ min^{-1})**		**Permeability, P ($\cdot 10^{-5}$ min^{-1} mm^{-2})**		**Surface area, S (mm^2)**	
	Coefficient (SE)	p-value	Coefficient (SE)	p-value	Coefficient (SE)	p-value
Intercept (NAWM)	-1.21 (1.21)	0.318	-7.64 (3.58)	**0.033**	4.92 (0.42)	**< 0.001**
cSVD	0.35 (0.36)	0.331	1.07 (1.12)	0.339	-0.06 (0.14)	0.665
DGM – NAWM	1.20 (0.27)	**< 0.001**	-0.49 (0.90)	0.588	1.69 (0.12)	**< 0.001**
WMH – NAWM	0.71 (0.27)	**0.008**	4.33 (0.90)	**< 0.001**	-1.30 (0.12)	**< 0.001**
cSVD x DGM	-0.32 (0.34)	0.335	-0.72 (1.13)	0.523	-0.04 (0.15)	0.814
cSVD x WMH	-0.21 (0.34)	0.535	2.01 (1.13)	0.074	-0.32 (0.15)	**0.034**
Age	0.04 (0.02)	**0.020**	0.18 (0.05)	**< 0.001**	-0.01 (0.01)	**0.028**
Female sex	-0.50 (0.32)	0.121	-1.76 (0.95)	0.064	0.17 (0.11)	0.124

Figure1 and table1.jpg

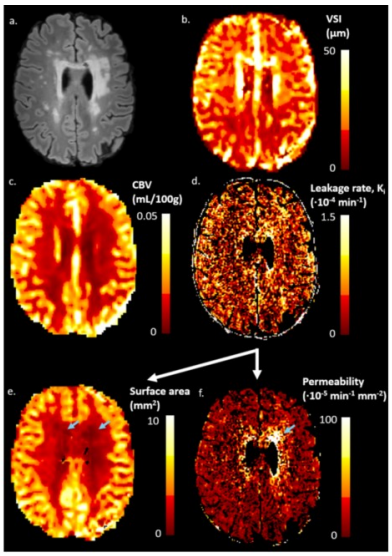


Figure 2: Brain maps for a cSVD patient (60 years old, male) showing: (a) white matter hyperintensities on T2-fluid attenuated inversion recovery (FLAIR) image, (b) vessel size index (VSI), (c) cerebral blood volume (CBV), (d) leakage rate (K_i), which can be split up in (e) vascular surface area (S) and (f) intrinsic vascular permeability (P). Note the reduced surface area in the white matter hyperintensities (blue arrows in panel e) and the strongly increased permeability around the lateral ventricles (blue arrow in panel f)

Figure2.jpg

Amyloid-β PET imaging with a brain-penetrant bispecific version of Lecanemab

Wednesday, 1st October - 11:40: (Sala Blava 2) - Oral

Dr. Sara Lopes van den Broek [1], ***Dr. Ximena Aguilar*** [1], ***Dr. Klas Bratteby*** [2], ***Dr. Thuy Tran*** [2], ***Prof. Stina Syvänen*** [1], ***Dr. Dag Sehlin*** [1]

1. *Uppsala University,* ***2.*** *Karolinska University Hospital*

Overcoming blood-brain barrier (BBB) limitations is critical for antibody-based therapeutic and diagnostic approaches in Alzheimer's disease (AD). Bispecific antibodies, designed to bind both a BBB receptor and a brain target, offer a promising strategy to enhance antibody brain delivery. Here, we developed a bispecific version of the therapeutic amyloid-β (Aβ) antibody Lecanemab (Lecanemab-Fab8D3), engineered to bind the transferrin receptor (TfR) for receptor-mediated transcytosis across the BBB. Fc-silencing mutations (LALA-PG) were introduced to reduce Fc-gamma receptor-mediated interactions with immune cells.

This study focused on evaluating Lecanemab-Fab8D3 as a radioligand for Positron Emission Tomography (PET). The antibody was radiolabeled with PET compatible radionuclides zirconium-89 (^{89}Zr), copper-64 (^{64}Cu), or iodine-124 (^{124}I), while preserving binding properties. PET imaging was performed in AD mice – Tg-ArcSwe - and wild-type controls. Dynamic scans at multiple time points up to 72 hours post-injection revealed cortical, thalamic and hippocampal brain uptake across all three radioligands, with [^{89}Zr]Zr-DFO*-Lec-Fab8D3 and [^{124}I]I-Lec-Fab8D3 achieving the best imaging contrast and highest brain-to-blood concentration ratios both in vivo and ex vivo (Figure A and B). The overall brain signal was highest in the groups with the radiometal-labeled (^{89}Zr and ^{64}Cu) antibodies, both in Tg-ArcSwe and WT mice, likely reflecting the residualizing effect of the radiometals.

Ex vivo autoradiography and immunostaining confirmed specific localization of the antibody to Aβ deposits in the cortex and hippocampus, with low vascular retention, demonstrating successful BBB transcytosis and target engagement (Figure C). Biodistribution analysis showed higher peripheral organ retention for radiometal-labeled constructs compared to radioiodinated constructs, which again is expected to be a result of the residualizing effect of the radiometals.

In conclusion, bispecific Lecanemab-Fab8D3 enabled specific, non-invasive PET imaging of brain Aβ pathology after BBB transcytosis. ^{89}Zr and ^{124}I showed the most favorable imaging characteristics, supporting their use for future clinical translation as companion diagnostics alongside anti-Aβ immunotherapy.

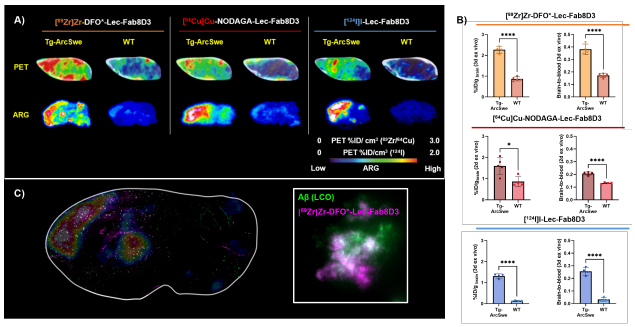


Figure A) Top: Sagittal PET images of the brain 2–3 days post radioligand administration, showing cortical uptake of [^{89}Zr]Zr-DFO*-Lec-Fab8D3 (left), [^{64}Cu]Cu-NODAGA-Lec-Fab8D3 (middle), and [^{124}I]I-Lec-Fab8D3 (right) in Tg-ArcSwe mice. Bottom: Corresponding autoradiography images of brain sections confirm radiotracer accumulation in cortical regions associated with Aβ deposits. **B)** Quantification of ex vivo brain uptake and brain-to-blood ratio 2-3 days post radioligand administration. Top (yellow/orange) represents [^{89}Zr]Zr-DFO*-Lec-Fab8D3, Middle (purple/red) represents [^{64}Cu]Cu-NODAGA-Lec-Fab8D3, bottom represents [^{124}I]I-Lec-Fab8D3 and shows distinct increased brain uptake and brain-to-blood ratios for Lecanemab-Fab8D3 with all three radionuclides. **C)** Overlay of an autoradiography image with the antibody and Aβ staining, illustrating the direct co-localization of radioactive signal with Aβ plaques (left) and an isolated amyloid plaque demonstrating binding of the Lecanemab-Fab8D3 antibody to the plaque. Lec = Lecanemab

Id61 - figa pet images - figb - staining and arg whole brain section - figc costaining amyloid plaque and radioligand.png

Generating a perfusable iPSC-derived Blood-Brain Barrier (BBB) in vitro model to investigate mechanisms of drug delivery to the brain

Wednesday, 1st October - 12:00: (Auditorium 1) - Oral

Dr. Shane Clerkin [1], ***Dr. Philip Dettinger*** [2], ***Dr. Martina Pigoni*** [1], ***Dr. Jose Luis Garcia Cordero*** [2], ***Dr. Roberto Villaseñor Solorio*** [1]

1. *Roche Pharma Research and Early Development, Neuroscience and Rare Diseases (NRD), Roche Innovation Center Basel, Switzerland,* ***2.*** *Roche Pharma Research and Early Development, Institute of Human Biology (IHB), Roche Innovation Center Basel, Switzerland*

The selective nature of the Blood-Brain Barrier (BBB) poses a significant challenge for developing efficacious treatments for neurodegenerative diseases. Therapeutic shuttles targeting the human transferrin receptor (TfR) have been shown to increase antibody uptake into the parenchyma. As the field progresses, and antibody shuttles with increasingly complex modalities continue to advance into the clinic, a comprehensive understanding of their transport mechanisms across the BBB will be crucial. Building on our work focused on establishing a new protocol for the generation of hiPSC-derived endothelial cells with improved BBB identity, we have generated a bioengineered BBB microfluidic chip system that incorporates bidirectional flow over the endothelial layer. We have used this system to conduct functional validation studies using fluorescently labelled dextrans and have demonstrated receptor-mediated transcytosis of BrainShuttle™ conjugates targeting TfR. Additionally, we have generated hiPSC-derived brain pericytes (iPericytes) that possess close transcriptional congruence to primary human brain pericytes. We have integrated the iPericytes, along with hiPSC-derived neurons and astrocytes, into our microfluidic chip to generate a complex perfused co-culture model. We expect that this advanced in vitro iPSC-derived BBB microfluidic system will accelerate the optimization of next-generation brain delivery technologies.

Human iPSC-derived blood-brain barrier model recapitulates key features of neurovascular pathophysiology

Wednesday, 1st October - 12:15: (Auditorium 1) - Oral

Mr. Henrique Nogueira Pinto [1], ***Ms. Nine R. Kok*** [1], ***Ms. Lois Kistemaker*** [2], ***Ms. Manon Karsten*** [1], ***Mr. Jannis Heuer*** [1], ***Mr. Arthur Ermakov*** [2], ***Ms. Susanne van der Pol*** [1], ***Mr. Mike de Kok*** [1], ***Dr. Stephanie D. Beekhuis-Hoekstra*** [1], ***Dr. Nienke M. de Wit*** [1], ***Prof. Elly M. Hol*** [2], ***Prof. Helga E. de Vries*** [1]

1. *Department of Molecular Cell Biology and Immunology, Amsterdam UMC location Vrije Universiteit Amsterdam, Amsterdam, The Netherlands,* ***2.*** *Department of Translational Neuroscience, University Medical Center Utrecht Brain Center, Utrecht University, Utrecht, The Netherlands*

The blood-brain barrier (BBB) is a dynamic system that protects the brain from blood-borne insults. BBB breakdown is an early hallmark of multiple neurodegenerative diseases, forming an attractive target for treatment. Yet, the lack of human models that recapitulate critical aspects of the BBB hampers the discovery and testing of potential therapeutics. Therefore, we developed a method, inspired by the BBB development, to generate human induced pluripotent stem cell (hiPSC)-derived brain microvascular endothelial cells (hiBMECs) with a comprehensive BBB phenotype.

HiBMECs developed tight junctions, expressed functionally active BBB transporters, such as P-glycoprotein, and formed a leak-tight barrier, indicated by elevated transendothelial electrical resistance and low fluorescein permeability. Transcriptomically, hiBMECs clustered closely with primary and freshly-isolated adult human BMECs, and were enriched in multiple BBB markers and related pathways. Regulon analysis revealed key transcription factors involved in BMEC commitment, and shed light into the synergistic mechanisms of pericyte co-culture and Wnt/β-catenin pathway activation. Cytokine stimulation elicited an inflammatory response via induction of cell-adhesion molecules, pro-inflammatory cytokines and antigen presentation molecules.

To integrate the BBB with other neural populations, recapitulating the complexity of the neurovascular unit (NVU), we co-cultured hiBMECs with a cortical organoid (cOrg) slice in a Transwell system. Here, astrocytes from the cOrg migrated towards the BBB compartment and established cell-cell contacts with hiBMECs. Thereafter, to better mimic the BBB microenvironment, we developed a membrane-free NVU-on-chip, where hiBMECs formed an endothelized BBB vessel, and cOrgs attached and outgrew towards the BBB channel.

Altogether, a novel method to generate hiPSC-derived BMECs with BBB properties was developed to overcome the limitations of current protocols. HiBMECs were integrated with cOrgs in a physiologically relevant NVU model, suitable for drug delivery studies and disease modelling.

This work was funded by NWO Human Measurement Models 2.0 CONNECT Grant #18957.

Establishing Multicellular Blood-Brain Barrier Infection Models for Antiviral Drug Discovery

Wednesday, 1st October - 12:30: (Auditorium 1) - Oral

Dr. Marisol Zuniga [1], ***Dr. Alexander Herrmann*** [1], ***Dr. Matteo Rizzato*** [2], ***Dr. Joachim J. Bugert*** [3], ***Dr. Ruth Brack-Werner*** [1]

1. *Institute of Virology, Helmholtz Zentrum München,* ***2.*** *Institute of Virology, Technical University of Munich,* ***3.*** *Bundeswehr Institute of Microbiology*

With low vaccination rates in endemic countries, tick-borne encephalitis virus (TBEV) poses a risk to the population who, upon infection, can develop severe complications, like encephalitis. Access to the central nervous system (CNS) by neurotropic viruses can be achieved via the blood-brain barrier (BBB), a critical cellular unit protecting the brain parenchyma from toxins and pathogens in circulation. While the impermeable characteristics of the BBB are critical for brain protection, they are also obstacles for the treatment of brain infections with antivirals. Our objective is to establish *in vitro* BBB models that would serve as tools to identify BBB-permeable antivirals and to investigate the effect of TBEV infection on the BBB. We developed a transwell insert BBB model with human brain microvascular endothelial cells (HBMEC) and pericytes (HBMVP). Due to the versatility of the insert model, we could co-culture the barrier cells with various target cell lines, including human neural stem cell line HNSC.100-differentiated astrocytes. Immunofluorescence staining of HBMEC (CD31) and HBMVP (NG2) markers provided visual confirmation of barrier formation on the insert membrane. Fluorometric assays, revealed the HBMEC-HBMVP co-culture decreased monolayer permeability in comparison to HBMEC monocultures, highlighting the importance of cellular crosstalk in BBB tightness. To validate our model as a screening tool for BBB-permeable antivirals, we tested antiretrovirals with varying CNS-Penetration-Effectiveness (CPE) scores. Preliminary data reveals our BBB model permits the passage of nevirapine (high CPE), but not saquinavir (low CPE) demonstrating that our *in vitro* model mimics *in vivo* BBB selectivity. Initial *in vitro* BBB infection experiments reveal TBEV crosses a co-culture of HBMEC and HBMVP with no increase in permeability. Utilization of our *in vitro* models may not only help in expediting the development of therapeutics against neurotropic viruses but may also provide insight into the TBEV-induced cellular responses responsible for promoting encephalitis.

Calnexin/Fabp5 Regulation of CD200 Controls T-Cell Trafficking Through the Blood–Brain Barrier and CNS Crosstalk

Wednesday, 1st October - 12:00: (Auditorium 2) - Oral

Dr. Myriam Pujol [1], ***Mrs. Alison Robinson*** [1], ***Dr. Tautvydas Paskevicius*** [1], ***Dr. Paul Eggleton*** [2], ***Dr. Alex Ferecskó*** [3], ***Dr. Nick Gutowski*** [3], ***Dr. Janet Holley*** [3], ***Dr. Miranda Smallwood*** [3], ***Dr. Jia Newcombe*** [3], ***Dr. Luis Agellon*** [4], ***Dr. Marek Michalak*** [1]

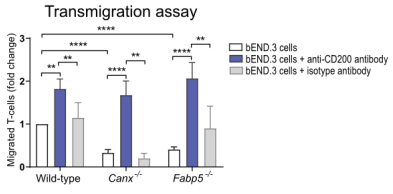
1. *University of Alberta,* ***2.*** *Revolo Biotherapeutics, University of Exeter,* ***3.*** *University of Exeter,* ***4.*** *McGill University*

We showed previously that the C-terminal domain of calnexin [Canx; endoplasmic reticulum (ER) protein chaperone] and the fatty acid binding protein 5 [Fabp5; cytoplasmic lipid chaperone] form a complex that favors T-cell trans-migration across the blood brain barrier (BBB). The absence of Fabp5 or Canx protects mice from Experimental Autoimmune Encephalomyelitis (EAE) and prevents T-cell infiltration of the central nervous system (CNS).

To investigate molecular events associated with Canx and Fabp5 deficiency in the BBB, we used gene editing techniques to generate *Fabp5*$^{-/-}$ or *Canx*$^{-/-}$ brain endothelial bEND.3 cells. Both cell lines showed significantly higher abundance of the regulatory protein CD200. Increased CD200 on bEND.3 cells mediated decreased T-cell/endothelial adhesion and decreased T-cell trans-endothelial migration (Fig. 1). Both *Fabp5*$^{-/-}$ or *Canx*$^{-/-}$ bEND.3 cells efficiently polarized allogeneic splenocytes towards a regulatory phenotype and promoted the Foxp3^{+} Treg cell subset in a CD200 dependent manner.

Flow cytometry analysis of mouse tissue samples revealed signs of increased activation of the CD200-CD200R1 axis in the CNS and periphery of *Canx*$^{-/-}$ mice. We found a higher CD200 abundance on brain endothelial cells (Fig. 2A, B) and increased percentage of Foxp3^{+}CD200R1^{+} microglia on CNS samples from *Canx*$^{-/-}$ mice (Fig.2C). Additionally, we found higher serum soluble CD200 and increased percentage of Tregs within CD4^{+} T-cell splenocytes of *Canx*$^{-/-}$ mice. These findings indicate that brain endothelial CD200 mediates immunological mechanisms that decrease T cell trans-endothelial permeability and promote a neuroprotective environment. We also showed that CD200 is inversely regulated by the Canx/Fabp5 protein complex. Targeting this protein complex could be promising for future treatment of neuro-inflammatory diseases.

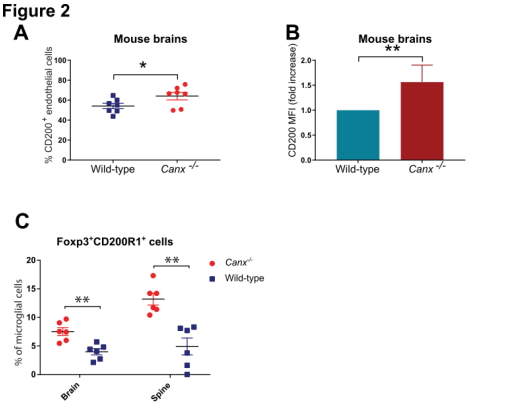
Figure 1



CD200 dependent T-cell trans-endothelial migration. Activated T-cells migrated through a monolayer of wild-type, *Canx*$^{-/-}$ or *Fabp5*$^{-/-}$ bEND.3 cells pre-treated with isotype or anti-CD200 blocking antibodies.

Figure1 2025.png

Figure 2



Increased activity of the CD200-CD200R1 axis in *Canx*$^{-/-}$ mice. Flow cytometry analysis of tissue samples from wild-type or *Canx*$^{-/-}$ mice. **A.** Percentage of CD200^{+} brain endothelial cells (CD31^{+}) from enzymatically digested brains. **B**. CD200 Mean fluorescence intensity of brain endothelial cells (CD31^{+}) from mouse brains **C**. Percentage of Foxp3^{+}CD200R1^{+} cells within microglia (CD45^{+}CD11b^{+}TMEM119^{+}) from enzymatically digested brains and spines.

Figure2 2025.png

IL-34 empowers regulatory T cells with novel non-canonical function to safeguard brain barrier integrity in neuroinflammation

Wednesday, 1st October - 12:15: (Auditorium 2) - Oral

Ms. Janne Verreycken [1], Dr. Lien Van Hoecke [2], Dr. Junhua Xie [2], Ms. Lore Van Acker [2], Ms. Elien Van Wonterghem [2], Mr. Jonas Castelein [2], Mrs. Griet Van Imschoot [2], Dr. Marlies Burgelman [2], Ms. Sarah Vanherle [3], Prof. Ilse Dewachter [3], Dr. Paulien Baeten [1], Prof. Roosmarijn Vandenbroucke [2], Prof. Bieke Broux [1]

1. *University MS Center, Department of Immunology and Infection, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium,* ***2.*** *Barriers in Inflammation Lab, VIB Center for Inflammation Research, Ghent, Belgium,* ***3.*** *Department of Neuroscience, Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium*

In pursuing strategies to address autoimmunity, regulatory T cells (Tregs) have garnered increasing attention in recent years. Beyond their established role in immunoregulation, Tregs have emerged as significant contributors in the response to brain trauma and the restoration of damaged brain tissue in neuroinflammatory diseases such as multiple sclerosis (MS) and Alzheimer's disease (AD). Here, we hypothesized that Tregs possess a previously undescribed, non-canonical function by preserving the integrity of brain barriers.

By using several depletion and transfer experiments, we have established that Tregs are crucial for maintaining blood-brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier (BCSFB) integrity *in vivo*. Additionally, we have identified the cytokine IL-34 as a pivotal factor in this newfound role of Tregs. Mechanistically, we found that IL-34 influences the expression and localization of the tight junction protein ZO-1 in BBB endothelial cells and choroid plexus epithelial cells, reinforcing the structural integrity of the brain barriers. Considering the established phenomenon of compromised brain barriers in neuroinflammatory conditions like MS, we also observed reduced IL-34 expression in Tregs derived from people with relapsing-remitting MS (RR-MS). Intriguingly, our study unveils the potential of IL-34 therapy in restoring the integrity of brain barriers in the EAE and APP^{NL-G-F} model, mimicking these neuroinflammatory disorders.

These discoveries illuminate the intricate interplay between Tregs, IL-34, and the maintenance of brain barrier integrity, opening up novel avenues for therapeutic interventions aimed at alleviating brain barrier dysfunction in the context of MS, AD and other neuroimmunological disorders.

The Blood-Brain Barrier as an Immunological Window: Brain Endothelial Cell Antigen Presentation Shapes T cell Fate

Wednesday, 1st October - 12:30: (Auditorium 2) - Oral

Ms. Sophia Nelson [1], ***Ms. Mina Negahban*** [2], ***Dr. Yuichi Chayama*** [3], ***Dr. Madigan Reid*** [2], ***Ms. Amanda Apolonio*** [2], ***Mr. Andrew Yang*** [2]

1. *Biomedical Sciences graduate program, University of California, San Francisco, CA, USA and Gladstone Institutes, Gladstone Institute of Neurological Disease, San Francisco, CA, USA,* ***2.*** *Gladstone Institutes, Gladstone Institute of Neurological Disease, San Francisco, CA, USA,* ***3.*** *Gladstone Institutes, Gladstone Institute of Neurological Disease, San Francisco, CA, USA and Daiichi Sankyo Co., Chuo-ku, Tokyo, Japan*

Immune surveillance within the central nervous system (CNS) is essential for health but operates under unique constraints, including limited immune cell access and the absence of classical lymphatic drainage or resident professional antigen-presenting cells (APCs). How the CNS maintains tolerance in health and disease, particularly of peripheral T cells, remains a critical knowledge gap. The blood-brain barrier (BBB)—an extensive interface formed by specialized brain endothelial cells (BECs)—is uniquely positioned between the brain and the circulating immune system, yet has been underappreciated as an immunological interface. Here, we provide some of the first evidence that BECs are capable of cross-presenting exogenous antigens to CD8+ T cells to actively promote tolerance. We hypothesize that BECs acquire CNS-derived antigens and cross-present them to the circulation to educate and tolerize circulating T cells. Transcriptomic and proteomic analysis finds that BECs express all machinery necessary for antigen cross-presentation, including genes thought specific to dendritic cells (DCs). Despite minimal expression of classical costimulatory molecules, BEC cross-presentation triggers robust, dose-dependent proliferation of cognate CD8+ T cells. Intriguingly, despite similar levels of T-cell receptor engagement and stronger initial activation than DC-educated counterparts, BEC-educated CD8+ T cells do not fully differentiate into effector cells. Instead, BEC-educated CD8+ T cells show reduced proinflammatory cytokines such as IFNg and adopt a central-memory phenotype, indicating BEC antigen presentation may play a role in formation and maintenance of CNS memory T cells. Leveraging genetic and adeno-associated virus (AAV)-mediated approaches, we perturbed BEC cross-presentation *in vivo* to clarify its role in shaping the CNS T cell repertoire and determine functional impacts of BEC-educated T cells in neuroinflammation and aging. In parallel, we used mass spectrometry to profile the BEC immunopeptidome across health and aging. This work positions BECs as central players in CNS immunity: by supporting memory differentiation of CD8+ T cells without inducing strong effector functions, BECs may play a previously unrecognized role in facilitating peripheral T cell education and sustaining CNS immune homeostasis. With unique exposure to brain and blood, BEC-mediated antigen presentation may serve as a promising therapeutic pathway to modulate peripheral T cells in neuroinflammation and neurodegeneration.

Gallus gallus embryo strikes back: molecular and functional insights from the developing Gallus gallus Blood-Brain Barrier

Wednesday, 1st October - 12:00: (Sala Blava 2) - Oral

Mr. Jesús Juárez-Balarezo [1], ***Ms. María Jesús Garrido Muñoz*** [1], ***Mr. Benjamin Reuse-Benavente*** [1], ***Dr. Ignacio Casanova-Maldonado*** [1], ***Dr. Verónica Palma*** [1]

1. *Universidad de Chile*

The cerebrovascular endothelium displays specific properties that distinguish brain blood vessels from the ones present in other tissues. Known as blood-brain barrier (BBB) it is characterized to be a highly selective semipermeable frontier which properties are attributed to the presence of specific transporters and tight junctions. These blood vessels are immersed in a specific microenvironment which establishment occurs during developmental stages. During brain development cues are provided importantly by neural stem/progenitor cells and glial progenitors. Signalling pathways, as canonical WNT, have been recognized as fundamental for the BBB development in different vertebrate models. Among these organisms, avian emerge as an interesting and widely used models for vascular research. Most avian BBB studies date back to the 1970s–1990s and focus primarily on electron microscopy and dye exclusion assays, with little insight into the molecular mechanisms driving barrier formation. Notably, no studies to date have integrated transcriptional, protein-level, and functional analyses of BBB development in avians embryo.

Here, we studied BBB development in *Gallus gallus* optical tectum (OT), reported as the first region in acquire a functional BBB. Through RT-qPCR of brain tissue in stages between HH32 and HH44, we found that gene expression patterns for genes as *ABCB1* and *PLVAP* are similar to patterns reported for mammals. Also, through immunofluorescence techniques we describe tight junction proteins (CLDN5 and ZO-1) presence in the OT blood vessels. With a quantitative Evans Blue permeability assay, we found an important decay in the permeability between HH36 and HH38 stages. Using fluorescent tracers and labelling for blood vessels coupled to tissue clearing techniques and lightsheet microscopy, we described in a high detailed manner the differences during the studied developmental stages. Finally, using WNT signalling disruptive molecules we assessed its involvement in chick embryo BBB development, as it's been explored for other organisms, reinforcing its conserved role in vertebrate BBB ontogeny.

Our findings re-establish the *Gallus gallus* embryo as a tractable model for studying BBB. By integrating molecular, structural, and functional data, this study provides the first comprehensive characterization of BBB development in chick embryo and highlights its potential for comparative and mechanistic neurovascular research.

Identification and Characterization of a Translational Mouse Model for Blood-Brain-Barrier Leakage in Small Vessel Disease

Wednesday, 1st October - 12:15: (Sala Blava 2) - Oral

Ms. Ruxue Jia [1], ***Dr. Gemma Solé-Guardia*** [2], ***Ms. Vivienne Verweij*** [1], ***Ms. Jessica M Snabel*** [3], ***Mr. Bram Geenen*** [1], ***Mr. Robert Kleemann*** [3], ***Dr. Anil M Tuladhar*** [4], ***Prof. Amanda Kiliaan*** [5], ***Dr. Maximilian Wiesmann*** [5]

1. *Medical Imaging, Anatomy. Research Institute for Medical Innovation, Radboudumc, Donders Institute for Brain, Cognition & Behavior, Preclinical Imaging Center, Netherlands,* ***2.*** *Dept. of Medical Imaging, Anatomy, Research Institute for Medical Innovation, Radboud university medical center, Donders Institute for Brain, Cognition and Behavior, center for medical neuroscience, PRIME, Radboud Alzheimer Center,* ***3.*** *Metabolic Health Research, The Netherlands Organization for Applied Scientific Research (TNO), Leiden, Netherlands,* ***4.*** *department of Neurology, Radboudumc,* ***5.*** *Department of Medical Imaging, Anatomy, Radboudumc*

Background

Blood-brain-barrier (BBB) dysfunction is an important pathophysiological feature in cerebral small vessel disease (cSVD). In order to develop treatments against cSVD, it is of utmost importance to unravel underlying pathological mechanisms leading to BBB dysfunction and further pathologies linked to cSVD. Therefore, availability of translational mouse models in which BBB leakage in SVD can be studied are indispensable.

Aim

We aimed to identify and characterize a mouse model that reliably and translationally recapitulates BBB impairment observed in cSVD and shares similar risk factors as human.

Methods

Transgenic leptin receptor-deficient (db/db) mice and LDL receptor knockout Leiden ($LDLr^{-/-}$.Leiden) mouse were selected. Db/db mice are prone to become obese on a chow diet, while LDLr-/-.Leiden mice become obese when fed a high-fat diet (HFD) or HFD plus high-cholesterol diet (HFD+C), and both groups of mice are accompanied by human cSVD risk factors such as hypertension. Moreover, $LDLr^{-/-}$.Leiden mice develop human-like hypercholesterolemia when fed with HFD. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) was used to evaluate the dynamic distribution of gadolinium in the brain to investigate BBB impairment, combined with NaFl measurements to support these findings. Arterial spin labeling was used to assess cerebral blood flow (CBF). Immunohistochemistry and qPCR were conducted to study tight junction integrity (ZO-1 and claudin-5), endothelial and vascular health (GLUT-1) and neuroinflammation (IBA-1 and GFAP). Plasma analyses were performed to detect dyslipidemia and vascular inflammation (VCAM-1, ICAM-1, selectin) among all experimental groups.

Results

As expected, all experimental groups of mice showed an obese phenotype but only $LDLr^{-/-}$.Leiden mice exhibited dyslipidemia and a human-like lipoprotein profile. $LDLr^{-/-}$.Leiden mice on HFD or on HFD+C also revealed BBB impairment (lower ZO-1 and Claudin-5 amount) determined by DCE-MRI and NaFl measurements, with endothelial and vascular dysfunction (lower GLUT-1 and CBF). In contrast, db/db mice only showed endothelial dysfunction (decreased GLUT-1 amount), but no BBB leakage. Moreover, not in the db/db group but only in both $LDLr^{-/-}$.Leiden diet groups, an increased neuroinflammation was also observed.

Conclusions

Our results suggest that $LDLr^{-/-}$.Leiden mice on HFD or on HFD+C, and not db/db mice, are suitable translational models for studying BBB dysfunction in cSVD.

In vivo proximity labeling identifies novel brain endothelial cell transfer receptors

Wednesday, 1st October - 12:30: (Sala Blava 2) - Oral

Ms. Alexis Schneider [1], ***Ms. Fionna Huang*** [2], ***Mr. Sahith Doddipalli*** [2], ***Dr. Haoyue Zhou*** [2], ***Ms. Amanda Apolonio*** [2], ***Dr. Duc Duong*** [3], ***Ms. Bella Ding*** [2], ***Ms. Zimo Zhang*** [2], ***Ms. Runa Cheng*** [2], ***Ms. Mina Negahban*** [2], ***Dr. Kenneth Hu*** [4], ***Dr. Nicholas Seyfried*** [3], ***Dr. Jason Cyster*** [5], ***Mr. Andrew Yang*** [2]

1. *Biomedical Sciences graduate program, University of California, San Francisco, CA, USA and Gladstone Institutes, Gladstone Institute of Neurological Disease, San Francisco, CA, USA,* ***2.*** *Gladstone Institutes, Gladstone Institute of Neurological Disease, San Francisco, CA, USA,* ***3.*** *Emory University,* ***4.*** *MD Anderson,* ***5.*** *University of California, San Francisco, San Francisco, CA, USA*

Cell surface proteins underlie cellular function and intercellular communication within complex multicellular tissues. However, no general method exists for profiling cell surface or extracellular proteins in vivo in a cell-type-specific manner. Proximity labeling (PL) enzymes, such as APEX2 and TurboID, have enabled protein network discovery in vitro, but are limited by toxicity and efficacy for profiling the in vivo extracellular compartment. Here, we have used rational engineering and directed evolution to create TORCH (Tyrosinase Oxidizer for Rapid Chemical Highlighting), an oxygen-catalyzed PL enzyme that functions in the in vivo extracellular space. TORCH can be genetically encoded and expressed on mouse cell surfaces via AAV or adoptive transfer; systemic delivery of biotin phenol (BP) yields rapid and localized labeling of TORCH-expressing cell surfaces in vivo. TORCH-expressing cells can also transfer BP to the surfaces of interacting immune and stromal cells, enabling study of how immune cells interact with brain barriers with age and disease. We apply TORCH to characterize the luminal surface of brain endothelial cells (BECs), a critical site of communication between the brain and the periphery. TORCH-mediated labeling and biotin-enrichment mass spectrometry uncover novel BEC surface proteins that are involved in protein transport across the BBB. Within these proteins, we identify novel targets for CNS drug delivery. These findings establish TORCH as a versatile tool for characterizing in vivo cell “surface-omes” and cell-cell interactions.

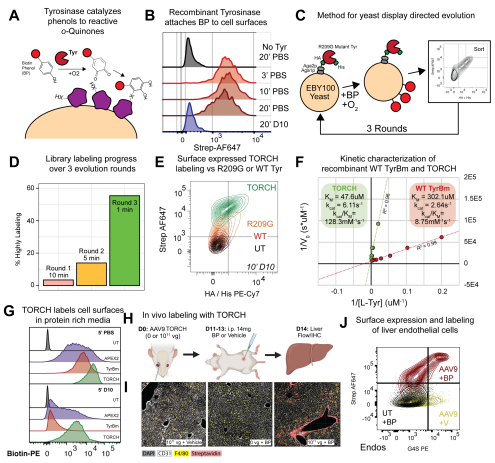


Figure 1. TORCH is a highly efficient in vivo proximity labeler. A) Schematic of oxygen-catalyzed Tyrosinase mechanism and spontaneous attachment of reactive o-quinones to protein residues. B) Surface streptavidin signal of HEK293T cells mixed with 40nM mushroom Tyrosinase and 50uM biotin phenol (BP) for 3', 10', or 20' in PBS or D10. C) Schematic of yeast display directed evolution of TyrBm R209G. D) Percentage of yeast library in upper quartile of Strep+ and HA/His+ region (relative to no BP) after designated labeling times in D10, 50uM BP. E) Flow cytometry surface streptavidin labeling and HA/His tag expression signatures of HEK293T cells transfected with WT TyrBm, R209G TyrBm, and TORCH after 10' in D10, 50uM BP. F) Lineweaver Burke plot and kinetic parameters of recombinant WT and TORCH reaction on Tyrosinase and spontaneous pigment formation. G) Flow cytometry surface biotin signal of HEK293T cells expressing surface bound APEX2, WT TyrBm or TORCH after 5' in PBS or D10 with 50uM BP. H) Schematic for in vivo AAV9 labeling experiment. I) Control and TORCH labeled liver sections stained with DAPI, CD31, F4/80 (macrophages), and Streptavidin. J) Flow cytometry results for liver endothelial cells TORCH expression (G4S) and Biotin surface signal after in vivo labelling.

Figure 1 torch is a highly efficient in vivo proximity labeler.png

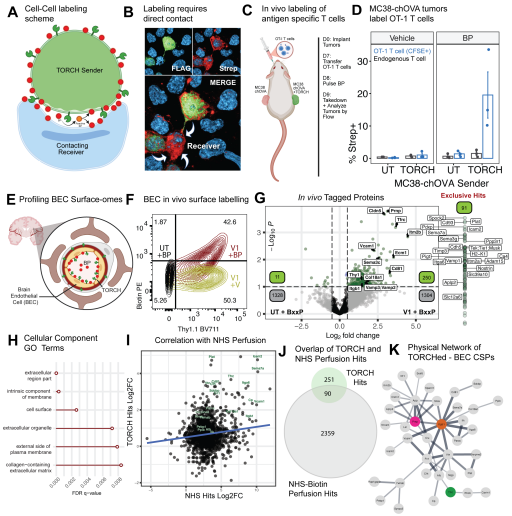


Figure 2. TORCH sender cells can label contacting cells and cell surface proteomes *in vivo*. A) Schematic. B) IF image of TORCH FLAG+ HEK293T cells labelling FLAG- receiver cells upon contact. C) Tumor infiltrating T cell labelling experiment schematic. D) Surface streptavidin level of OT-1 CFSE+ T cells (blue) vs WT T cells (black) in UT or TORCH tumors after a single Vehicle or 7mg BP injection. E) Visual of brain endothelial cell TORCH surfaceome labeling. F) Thy1.1 transduction marker and surface biotinylation of brain endothelial cells after 5E12 vg of V1 AAV and 3x7mg BP Pulses. G) Mass spec of biotin enriched proteins in V1 AAV + Biotin-xx-Phenol (BxxP) pulsed mice vs UT + BxxP mice. H) GO term enrichment of biotin tagged proteins. I) Correlation and J) venn diagram of TORCH tagged proteins vs NHS-biotin perfusion tagged proteins. K) STRING physical network of TORCH tagged proteins.

Figure 2 torch sender cells can label contacting cells and cell surfaceomes in vivo.png

Tools to decode astrocyte and vascular cell-specific proteomes throughout the vascular tree

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Noelia Pérez Ramos [1], ***Ms. Austeja Ciulkinyte*** [2], ***Dr. Steven Hill*** [3], ***Dr. Blanca Díaz Castro*** [3]

1. *UK Dementia Research Institute, University of Edinburgh,* ***2.*** *UK Dementia Research Institute, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh EH16 4SB, UK.,* ***3.*** *University of Edinburgh*

Background: The brain is supplied by a heterogenous vascular system and surrounded by perivascular cells that not only provide the brain with the necessary nutrients but also protect it from blood product infiltration. The majority of the vasculature throughout the brain is wrapped by astrocyte endfeet which allows them to act as the interface of communication between the vasculature and other cells of the central nervous system. While there is evidence that different segments of the vascular tree display distinct functions, an in-depth characterization of the brain endothelial cells and the enwrapping astrocyte endfoot proteome is lacking.
Methods: To elucidate the proteomes of astrocyte endfoot and brain endothelial cells in distinct vascular segments, we have developed 1) mouse lines that use a proximity biotin-ligase method to tag proteins associated to the plasma membrane of brain endothelial cells or astrocytes in combination with 2) a method to isolate brain vasculature of distinct sizes with the attached astrocyte endfeet.
Results: We have confirmed specific target and total coverage of tagged proteins in brain endothelial cells and the astrocyte plasma membrane in these mouse models using immunofluorescence. In addition, we confirmed the efficacy of our method to enrich arterioles and venules and capillaries. Currently, our studies are focusing on using these mice to identify the molecular pathways involved in vasculature-astrocyte interactions throughout the vascular tree using mass spectrometry.
Conclusion: Together, these new tools will enable us to study the dynamic vasculature-astrocyte endfoot interaction throughout the vascular tree.

Designing Murine Protein-Based Drugs Capable of Crossing the Blood-Brain Barrier Using Knobs-into-Holes

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Mr. Gustaf Hederoth [1], ***Dr. Nicole G. Metzendorf*** [1], ***Dr. Greta Hultqvist*** [1]

1. *Uppsala University*

Protein-based therapeutics offer significant potential for treating neurodegenerative diseases, but their effectiveness is limited by poor blood-brain barrier (BBB) permeability. One promising strategy to enhance brain uptake is receptor-mediated transcytosis via the transferrin receptor (TfR), which can be achieved by engineering bispecific antibodies to engage both the TfR and a therapeutic target simultaneously.

However, conventional symmetric bispecific antibodies bind bivalently to the TfR, which reduces brain uptake at therapeutic doses due to receptor crosslinking. This challenge can be overcome through Knobs-into-Holes (KiH) engineering, a strategy that enables the assembly of asymmetric bispecific antibodies with monovalent TfR binding by promoting heterodimerization of two distinct heavy chains.

While KiH technology has been widely used in human antibodies, its application to murine antibodies has only recently become feasible. Here, we demonstrate the expression, purification, and validation of murine KiH bispecific antibodies with monovalent TfR binding, enabling more efficient BBB transcytosis in mouse models. This advancement represents a step toward next-generation therapeutics with improved brain delivery for preclinical testing in murine models of neurodegenerative diseases, including Alzheimer's and Parkinson's disease.

Proximity to GL261 mouse glioblastoma tumors changes: pericyte - scRNA gene expression, - intracellular communication and - sub cluster distribution

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Dr. Robert Carlsson [1], ***Dr. Carolina Buizza*** [1], ***Dr. Gesine Paul-Visse*** [1]

1. *Translational Neurology Group, Department of Clinical Science, Faculty of Medicine, Lund University, Lund, Sweden*

Glioblastoma (GBM) is the most aggressive and deadly of human brain tumors. Despite surgical resection and chemotherapeutic intervention, the mean patient survival time less than 16 months. One of the main features of GBM is its invasive growth, with GBM cells migrating in the perivascular space of the brain far from the solid tumor edge. Regional cellular responses to GBM proximity, e.g. by pericytes, might be beneficial or counteract GBM proliferation, invasiveness and migration and its tumor microenvironment. We here seek to define the transcriptome of pericytes in different regional compartments of mouse orthotopic GL261 transplants. Using scRNAseq from macro-dissected non-tumor, border or tumor brain regions of mCherry- labeled GL261 transplants we define pericyte subclusters of mouse GBM pathology. More specifically cellular distribution, pericyte subcluster distribution and communication between pericyte subclusters and immune cells of the afore mentioned regions are compared. We show that proximity between pericytes and the GBM tumor influence the pericyte and pericyte subcluster transcriptome and that the pericyte compartment changes in its composition by proximity to the tumour. This potentially leads to a reduced capacity of immune regulation and immune evasion as consequence of GBM vessel co-option.

The maternal gut microbiota shapes neurovascular development in mice

Wednesday, 1st October - 13:45: (Patio Area) - Poster

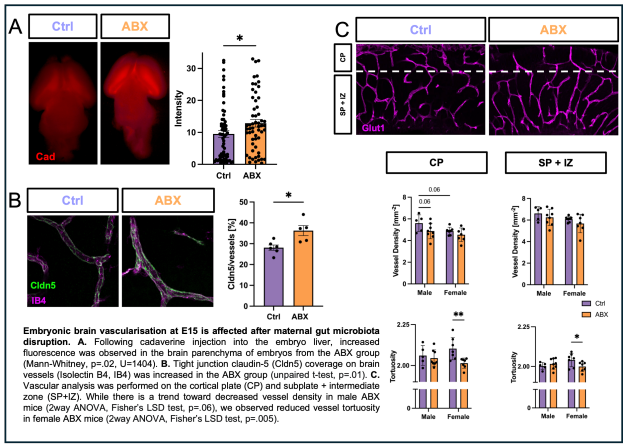
Mr. Alexandre J. C. Cergneux [1], ***Dr. Valentine Turpin*** [1], ***Ms. Jennifer Morael*** [1], ***Dr. Lorena Morales*** [1], ***Mr. Hugo J. Blair*** [1], ***Dr. Emily G. Knox*** [1], ***Dr. Jennifer Shearer*** [1], ***Prof. John F. Cryan*** [1], ***Dr. Maria R. Aburto*** [1]

1. *University College Cork, APC Microbiome Ireland*

Brain vasculature is essential for supplying oxygen and nutrients to every brain cell, orchestrating neuroglial-vascular development, and forming the blood-brain barrier (BBB). During the prenatal period, the vascular and nervous systems develop in parallel, such that disruptions in one system can impair the coordinated development of the other. While emerging evidence links perinatal perturbations of the maternal gut microbiota to abnormal brain function and behaviour in offspring and broader alterations in neurodevelopmental trajectories, its role in developmental angiogenesis and neurovascular cell types remains largely unexplored.

In this study, we investigated the effects of the maternal gut microbiota modulation during gestation on neurovascular development in the embryonic cortex of mice. Pregnant dams were treated with a broad-spectrum antibiotic cocktail one week before and throughout pregnancy to deplete the gut microbiota, and neurovascular features were analysed in embryos at embryonic day 15. Disruption of the maternal microbiota led to increased functional permeability of the embryonic brain barrier. This was associated with expanded both tight junction and pericyte coverage in the cortical plate of the male embryos. In females, maternal microbiota depletion reduced cerebral vessel tortuosity, whereas vessel density in males showed a trend toward reduction, despite no difference in branching points. Finally, the density and number of filopodia of endothelial tip cells remained unaffected.

Together, our findings provide novel insights into the role of maternal gut microbiota in promoting healthy brain vascularisation and BBB function during foetal development. These results open new avenues for understanding the complex interactions between the maternal microbiota and early brain development, with potential implications for neurodevelopmental health in offspring.



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Shedding light on molecular mechanisms of transcytosis with 4D imaging and machine learning-based analysis

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Stavroula - Valia Margaritaki [1], ***Dr. Franziska Schöppe*** [2], ***Dr. Thor Christian Møller*** [2], ***Prof. Nikos Hatzakis*** [1]

1. *Department of Chemistry, University of Copenhagen, Denmark,* ***2.*** *Therapeutics Discovery & Accelerated Execution, Novo Nordisk A/S, 2760 Måløv, Denmark*

This research investigates the molecular mechanisms of transcytosis, with a specific focus on antibody fragments, including Fabs and VHHs, as they navigate cellular barriers such as the blood-brain barrier (BBB). Employing state-of-the-art four-dimensional (4D) imaging techniques and machine learning-driven analytical frameworks, the project aims to understand the interplay between binding kinetics, pH sensitivity, and trafficking efficiency. Endothelial cell models derived from both murine and human sources serve as platforms for these investigations. Advanced imaging methodologies combined with computational analyses, enable a detailed analysis of intracellular pathways. This knowledge is crucial for optimizing therapeutic antibody designs, ultimately enhancing their efficacy in neurodegenerative disorder treatments and refining BBB transcytosis assay methodologies.

iPSC-derived blood brain barrier models for investigating the role of the BBB in Alzheimer's disease

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Laura Lilieholm-Røngren [1], ***Dr. Kenneth Thirstrup*** [1], ***Prof. Birger Brodin*** [2], ***Dr. Lasse Saaby*** [2]

1. *Bioneer A/S,* ***2.*** *Department of Pharmacy, University of Copenhagen, Denmark*

Laura Lilieholm-Røngren[1,2], Kenneth Thirstrup[2], Birger Brodin[1], Lasse Saaby[1,2]

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The blood-brain-barrier (BBB) is not only a major challenge in the delivery of drug compounds to the brain parenchyma but is also involved in the pathophysiology of neurodegenerative disorders, like Alzheimer's disease (AD). The strongest identified genetic risk factor for AD is the APOE4 gene variant, where patients homozygous for this variant have the highest risk of developing AD in contrast to carriers of the other variants of the gene (APOE2 and APOE3). The APOE gene encodes apolipoprotein E, which main function is to mediate lipid transport. Emerging evidence suggest that both AD patients and carriers of the APOE4 gene exhibit increased BBB permeability. However, the exact mechanism by which the APOE4 variant affects the BBB and how a dysfunctional BBB contribute to the AD pathophysiology remains to be elucidated.

Therefore, in this project, we wanted to investigate if the APOE4 genotype has an effect on the barrier properties of iPSC derived brain microvascular endothelial-like cells (BMEC-like cells). Since astrocytes secrete the ApoE protein, we also examined whether co-culturing the BMEC-like cells with iPSC derived astrocytes homozygous for the APOE4 variant influences the barrier integrity.

In the present study we investigated the following iPSC lines: background iPSC line (BIONi010-C) from a healthy donor with the APOE 3/4 variant, gene-edited to hold the APOE 4/4 variant (BIONi010-C-54), and an iPSC line derived from an AD patient (UKBi011-A) with the APOE 4/4 variant.

The astrocytes were differentiated using an established protocol (K.S. Dittlau et al. 2024) and were used for experiments at 4 weeks of maturation. For the co-cultures, astrocytes and BMEC-like cells were combined on day 8 of the BMEC-protocol. For both mono- and co-cultures the BMEC-like cells were used for measurement of trans-endothelial electrical resistance (TEER) and bi-directional transport experiments on day 10. Furthermore, the cultures were characterized by immunocytochemistry to investigate the expression of key astrocyte and endothelial markers.

Pericyte Role in Type-2 Diabetes and Cognition

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Dr. Shubhangini Tiwari [1], Mr. Carlos Noriega Polo [1], Dr. Osama Elabi [1], Dr. João M.N. Duarte [2], Dr. Gesine Paul-Visse [1]

1. *Translational Neurology Group, Department of Clinical Science, Faculty of Medicine, Lund University, Lund, Sweden,* ***2.*** *Wallenberg Centre for Molecular Medicine, Lund University, Lund, Sweden*

Background

Vascular alterations such as blood-brain barrier (BBB) leakage, pericyte loss, aberrant angiogenesis and associated neuroinflammation are cerebral microvascular complications linked with type-2 diabetes that contribute to cognitive decline and vascular dementia. Pericyte modulation, specifically knockout of Regulator of G-protein signaling 5 (RGS5), a protein expressed in activated brain pericytes, has in other pathological conditions lead to increased perivascular pericyte coverage, improved endothelial cell survival and restored BBB integrity. Whether pericyte modulation can achieve vascular protection also in type-2 diabetes, and if this vascular modulation can prevent the memory decline despite the presence of a diabetic state is not known.

Methods

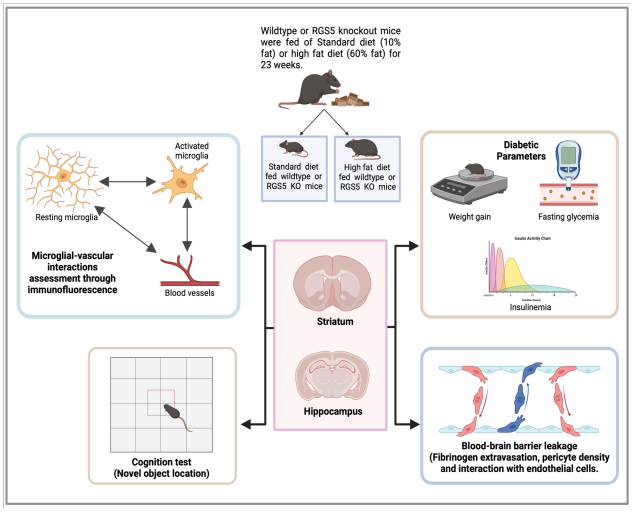
Here we utilized a RGS5 knock-out/knock-in mouse strain in a C57bl/6 background (RGS5 KO) and wildtype mice (WT). For 23 weeks, RGS5 KO and WT mice were fed either a standard diet (SD) or a high-fat diet (HFD) to induce type-2 diabetes. Diabetic phenotype was monitored by weight gain, fasting glycemia, insulinaemia, and glucose tolerance. Using immunohistochemistry and confocal microscopy, we examined BBB leakage in the striatum and hippocampus and assessed vascular pathology by analyzing vessel density and branching points, pericyte density and pericyte coverage around blood vessels. Further, we evaluated microglial activation and in particular the interaction between microglia and capillaries in striatal and hippocampal brain regions. Cognitive impairment was assessed using the novel object location test.

Results

HFD-fed mice displayed an increase in blood glucose levels and insulin resistance independent of genotype. In WT mice, type-2 diabetes caused BBB leakage, immature angiogenesis and, especially in the striatum, altered pericyte density. Those pathological changes were absent in HFD-fed RGS5 KO mice. Knockout of RGS5 also prevented microglial activation and increased the interaction between resting microglia and blood vessels in the striatum of HFD-fed mice, but not in the hippocampus. HFD led to cognitive impairment in WT mice, whereas loss of RGS5 prevented memory decline in diabetic mice.

Conclusion

The data suggests that modulating RGS5 in brain pericytes can protect the BBB, maintain microvascular homeostasis and prevent type-2 diabetes-induced memory impairment highlighting vascular changes as a pathology to target independently of metabolic interventions when preventing cognitive decline in diabetes.



Picture 1.png

Screening of blood-brain barrier permeability of drug candidates using 3D-spheroids and UPLC-MS/MS quantification

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Cindy Bay [1], ***Ms. Meike Kästing*** [1], ***Mr. Eric Mühlberg*** [2], ***Dr. Gzona Bajraktari-Sylejmani*** [1], ***Dr. Jürgen Burhenne*** [1], ***Dr. Max Sauter*** [1], ***Prof. Johanna Weiss*** [1]

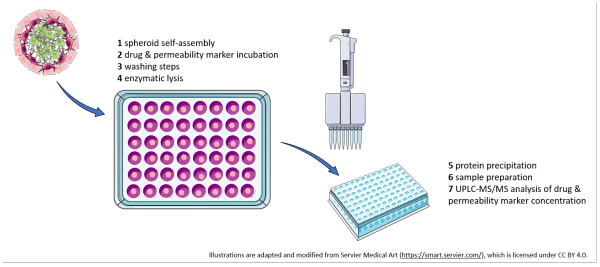
1. *1) Heidelberg University, Medical Faculty Heidelberg/University Hospital Heidelberg, Internal Medicine IX - Department of Clinical Pharmacology and Pharmacoepidemiology, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany,* ***2.*** *2) Heidelberg University, Institute for Pharmacy and Molecular Biotechnology, Department of Pharmaceutical Technology, Im Neuenheimer Feld 329, 69120 Heidelberg, Germany*

Background: The blood-brain barrier (BBB) is a highly efficient barrier shielding the central nervous system (CNS) from most drugs. BBB-permeability is a crucial question during drug development, either for the development of therapeutics for CNS-disorders or to avoid side effects. While there is a large number of BBB-models, they are either limited by their physiological significance or read-out accessibility. Therefore, we evaluated and optimized a 3D-BBB-spheroid model for its validity and applicability for an efficient and translational relevant screening of therapeutic drug candidates using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) assays.

Methods: 3D-BBB-spheroids were generated from primary human astrocytes and pericytes in combination with immortalized brain capillary endothelial cells. Correct cell localization was evaluated with CellTracker™ probes imaged by confocal fluorescence microscopy. Spheroids were incubated with a variety of drugs and concentrations were determined using UPLC-MS/MS on single-spheroid level. To ensure compatibility with these UPLC-MS/MS quantifications, a new D-amino acid-based dipeptide permeability marker was developed to measure the paracellular integrity of each spheroid and validated by opening of tight and adherens junctions with different compounds.

Results: We developed a simple and fast experimental setup enabling a screening in 96-well format for the UPLC-MS/MS analysis of intra-spheroidal drug concentrations. The resulting spheroid-to-incubation-solution-ratios can then be correlated to known brain-to-blood-ratios to evaluate the translational significance of this model. The simultaneous use of our dipeptide marker enables the exclusion of spheroids with compromised integrity on a single-spheroid level. Surprisingly, the opening of the cell-cell-connections did result in a decrease in intra-spheroid dipeptide-concentration, which was attributed to an increased wash-out. This was cross-validated with a hydrophilic fluorescent marker.

Conclusion: We established a workflow to analyze intra-spheroidal concentrations in single spheroids in a 96-well format with UPLC-MS/MS. The opening of tight junctions in our 3D-BBB-model results in a decreased permeability marker concentration, which enables the direct exclusion of compromised spheroids for downstream analysis. With this setup, the translational relevance of 3D-BBB-spheroid permeability experiments can be evaluated.



Graphical abstract bbb screening.png

Studying LRP1 endocytic trafficking pathways in Alzheimer's disease

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Giulia Maria Porro [1], ***Prof. Giuseppe Battaglia*** [2]

1. *IBEC,* ***2.*** *Institute for Bioengineering of Catalonia & University of Barcelona*

The blood-brain barrier (BBB) is a highly selective barrier composed by specialised brain endothelial cells (BECs) lining the brain vasculature. The BBB prevents the entry of toxins and pathogens into the brain while tightly regulating the transport and signalling of macromolecules. Among its key regulators is the low-density lipoprotein receptor-related protein 1 (LRP1), mainly expressed by BECs, an essential transporter mediating the transcytosis of various ligands, including amyloid-ß (Aβ) peptide, whose accumulation is a hallmark of Alzheimer's disease (AD). Reduced LRP1 expression and dysregulated endocytic trafficking at the BBB have been implicated in impaired Aβ clearance and subsequent neurodegeneration. However, the endocytic mechanisms governing LRP1 fate remain incompletely understood, particularly under inflammatory conditions that mimic AD pathology.
The project aims to elucidate the endosomal trafficking pathways regulating LRP1 surface expression in human brain microvascular endothelial cells (HBMECs), under both healthy and pathological conditions.
To track both LRP1 endocytosis and recycling, we employ complementary approaches involving cell surface labelling methods coupled with an ELISA-based detection system. This strategy allows quantitative monitoring of LRP1internalisation and its recycling back to the membrane over time. The same methodology is applied under inflammatory conditions, upon Aβ monomers, oligomers, and fibrils treatment, to mimic the AD pathology.
In parallel, microscopy-based techniques such as proximity ligation assay (PLA) are implemented to visualise the spatial association of LRP1 with Rab GTPases, specifically Rab5, Rab7, and Rab11, marking early, late, and recycling endosomes, respectively. This provides a meaningful insight into the LRP1 intracellular trafficking route. These data are further supported by the analysis of inflammatory markers.
Altogether, this study contributes to defining the molecular dynamics of LRP1 trafficking at the BBB and offers new insights into how neuroinflammation may disrupt receptor homeostasis in AD. The findings could pave the way for targeted therapeutic strategies and future clinical applications for patient-tailored therapy.

Vascular FLRT2 regulates venous-mediated angiogenic expansion and CNS barriergenesis

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Dr. Cecília Llaó Cid [1], ***Dr. Blanca Peguera*** [1], ***Dr. Piotr Kobialka*** [1], ***Ms. Linda Decker*** [1], ***Ms. Johanna Vogenstahl*** [1], ***Dr. Nensi Alivodej*** [1], ***Dr. Swati Srivastava*** [1], ***Dr. Jin Jing*** [1], ***Dr. Bettina Kirchmaier*** [1], ***Ms. Carmen Milla*** [1], ***Ms. Hannah Schlierbach*** [2], ***Prof. Anne Schaenzer*** [2], ***Prof. Till Acker*** [2], ***Dr. Marta Segarra*** [1], ***Prof. Amparo Acker-Palmer*** [1]

1. *1Buchmann Institute for Molecular Life Sciences (BMLS), Institute of Cell Biology and Neuroscience, Goethe University Frankfurt, Max-von-Laue-Str. 15, D-60438, Frankfurt am Main, Germany,* ***2.*** *Institute of Neuropathology, Justus Liebig University Giessen, D-35392 Giessen, Germany.*

Veins have emerged as the origin of all other endothelial cell subtypes needed to expand vascular networks during developmental and pathological neoangiogenesis. Here, we uncover the role of the angioneurin Fibronectin Leucine Rich Transmembrane protein (FLRT) 2 in central nervous system (CNS) vascular development in the mouse. Early postnatal FLRT2 deletion reveals specific defects in retinal veins, impacting endothelial cell proliferation, sprouting and polarity that result in reduced tip cells at the vascular front. FLRT2 interacts with VE-cadherin and together with the endocytic adaptor protein Numb contribute to the modulation of adherens junction morphology in both retina and cerebral cortex *in vivo*. Utilizing expansion microscopy, we visualize the altered dynamic distribution of VE-cadherin in tissue of FLRT2 endothelial mutants. Importantly, FLRT2 in cortical vessels regulates the crosstalk between adherens and tight junctions, influencing blood-brain barrier development. Our findings position FLRT2 as a vein-specific regulator of CNS vascular development.

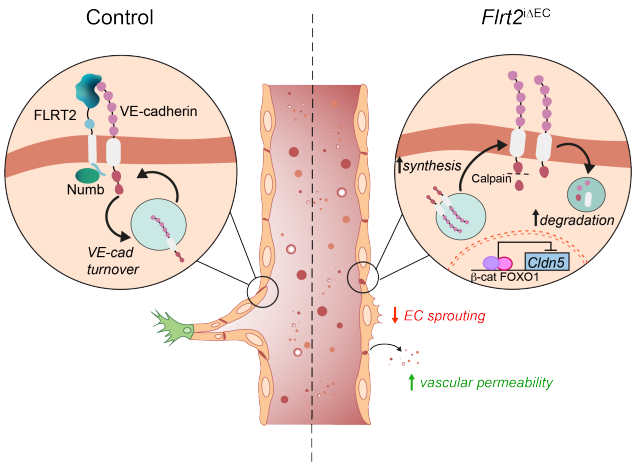


Figure 8.png

Assessment of Capillary Cerebral Amyloid Angiopathy in Human Brain Tissue and APOD and Aβ peptides as potential CAA subtype markers

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Carla Hernández Utrilla [1], ***Mr. Jelle Siers*** [1], ***Ms. Sanne Vermorgen*** [2], ***Dr. Benno Küsters*** [3], ***Dr. Floris H.B.M. Schreuder*** [1], ***Dr. H. Bea Kuiperij*** [1], ***Prof. Catharina J.M. Klijn*** [1], ***Prof. Marcel M. Verbeek*** [1]

1. *Donders Institute for Brain, Cognition and Behaviour, Centre for Cognitive Neuroimaging, Radboud University Nijmegen, PO Box 9101, 6500, HB, Nijmegen,* ***2.*** *Department of Pathology, Cancer Center Amsterdam, Amsterdam UMC location Vrije Universiteit Amsterdam, Amsterdam, The Netherlands,* ***3.*** *Department of Pathology, Research Institute for Medical Innovation, Radboud University Medical Center*

Introduction

Neuropathological examination of brain tissue reveals two cerebral amyloid angiopathy (CAA) subtypes - capillary (capCAA) and arteriolar (artCAA) - distinguished by the size of affected vessels. It is assumed that capCAA is associated with blood-brain barrier (BBB) disruption. We aimed to characterize capCAA and explore its associations with key variables, while investigating subtype-specific biomarkers.

Methods

Two blinded raters assessed capCAA presence in ten gray matter regions (40X magnification) of Aβ-stained temporal lobe sections in Controls, CAA non-hemorrhagic (CAA-NH) and CAA hemorrhagic (CAA-ICH) cases (n=83) using an established method (Thal et al., 2002). Adjacent sections were stained for endothelial cell marker CD34, with expression quantified via Fiji/ImageJ. CapCAA confirmation in 12 cases involved tracking Aβ/CD34 capillaries and co-immunofluorescence targeting Aβ, CD34, and smooth muscle actin (SMA). A replication of the established method in the full sections in an expanded cohort is underway (n=102), including staining for apolipoprotein D (APOD) and Aβ peptides (Aβ37-Aβ40, Aβ42, Aβ43) to explore their association with capCAA.

Results

Prevalence of capCAA was low across all groups (10.8-18.2%, Figure 1A) with no significant differences ($X^2(2)=.725$, p=.0696), though prevalence increased with CAA grade (Figure 1B). CapCAA was not linked to APOE e4 (p=.506), plaque burden (p=.059), CERAD scores (p=.260) or dementia diagnosis (p=.205). CD34 expression was comparable across control and CAA groups (p=.31), with 4/12 cases showing Aβ/CD34 positive capillaries (Figure 1C). Triple co-immunofluorescence confirmed capCAA in 8/12 cases. Interestingly, we identified clusters of capCAA ('florid capCAA') in 3/10 (30%) CAA cases (Figure 1D) during full-section assessments. Preliminary results showed that expression of APOD (Figure 1E) and all Aβ peptides was increased in CAA-NH and CAA-ICH than in control cases.

Discussion

CapCAA prevalence in the temporal lobe is low in both CAA and control groups, consistent with the literature. Region selection may have limited our ability to identify capCAA, thereby reducing the power to make associations with other variables. Full-section capCAA evaluation led to the identification of capCAA clusters. Triple immunofluorescence (Aβ+, CD34+, SMA-) identifies capCAA more efficiently than Aβ/CD34 positive capillaries. APOD and Aβ-peptide markers showed high CAA specificity and are under study for subtype associations.

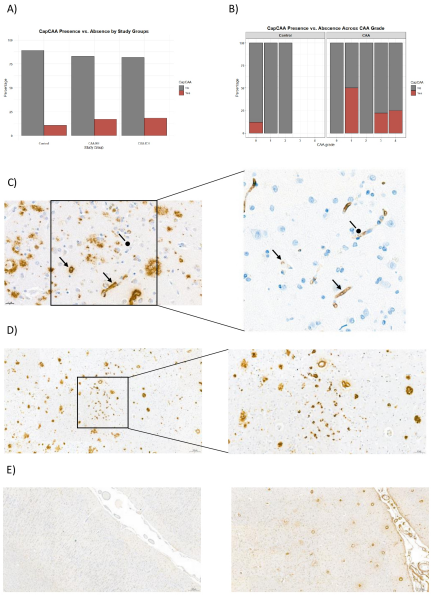


Figure 1: Prevalence of CAA in human brain temporal lobe tissue (n=83) in ten selected regions at 40X magnification taken in zig-zag manner across gray matter showed **A)** low capCAA prevalence (red bars) across Control, CAA-NH, and CAA-ICH groups, and **B)** a few control had incidental affected capillaries whereas CAA cases tend to have more capCAA with increasing CAA grade. **C)** Left image Aβ staining (40X): affected capillaries (black arrows) have Aβ within their walls while unaffected capillaries are Aβ devoid (black dotted arrow with circle end) and are all surrounded by plaques. Right image; higher magnification of CD34 staining of the same case as left image: capillaries immunoreactive to CD34 antibody in the adjacent Aβ-stained section confirm capCAA presence (black arrows) and show negative Aβ capillaries (black dotted arrow with circle end). **D)** Florid capCAA was identified in 30% of CAA assessed cases, interestingly all these were CAA-NH, on the left Aβ staining (10X) showing florid capCAA and, on the right, zoomed-in image (20X). **E)** APOD immunohistochemistry revealed specificity for CAA (right image) and absent staining in controls (left). CAA-NH; CAA non-hemorrhagic, CAA-ICH: CAA with intracerebral hemorrhage.

Figure 1.jpg

Advancing perfused BBB-on-chip technologies for throughput preclinical development

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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The human blood–brain barrier (BBB) is a highly specialized, dynamic multicellular interface that stringently regulates molecular and cellular trafficking between the systemic blood circulation and the central nervous system (CNS). Despite progress in the development of *in vitro* human BBB models, replicating the full physiological and pathophysiological complexity of the native barrier remains a significant challenge. Therefore, the utility of such models in both basic neuroscience research and preclinical evaluation of CNS-targeted therapeutics is limited.

We have previously presented an *in vitro* BBB-on-chip platform that mimics BBB structure and its dynamic reorganization in response to insults [1]. The model incorporates a human cerebral endothelial cell line, primary pericytes, and astrocytes arranged in a three-dimensional configuration. Physiological shear stress is generated through pump-free, gravity-driven perfusion. However, initial versions of the model used polydimethylsiloxane (PDMS), a material known to absorb small hydrophobic molecules, which limits compatibility with pharmacological screening.

To overcome this limitation, we have recently redesigned the platform using only hard inert plastic materials, providing compatibility with a broader range of drug and vector screening applications. The system has been optimized for workflows with larger throughput by accommodating up to 32 independent test units per standard well plate. Each unit features a vascular and brain compartment, separated by a porous membrane.

To further enhance the model's physiological relevance and applicability for disease modeling, we have evaluated the incorporation of human induced pluripotent stem cell (hiPSC)-derived endothelial cells and compared their barrier properties and polarization dynamics against traditional endothelial cell lines.

This next-generation BBB-on-chip model offers a scalable and biologically relevant platform for CNS drug testing and disease modeling and holds promise as a predictive tool in early-phase therapeutic development.

References

[1] Wei *et al.*, *Adv. Sci.* 2023, 10, 2205752

The role of Drebrin in blood-brain barrier functioning

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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Brain functionality relies substantially on extensive communication between the neuronal cells and the vascular system. Accordingly, the blood-brain barrier (BBB) is a remarkable example of the closely regulated interaction between cells of the central nervous system (CNS) and endothelial cells (ECs). It represents a selective physiological barrier between circulating blood and the brain parenchyma. While many BBB components have already been extensively studied, the role of the BBB cytoskeleton and its association with the extracellular environment remains rather unexplored. The actin-binding protein Drebrin (DBN) is highly abundant in neurons. Previous research by our group demonstrates that DBN is not only expressed in neurons but also in astrocytes after brain injury, whereas deletion of DBN in astrocytes leads to defective scar formation and escalating neurodegeneration (Schiweck et al., 2021, Kreis et al., 2019). Mechanistically, we show that DBN loss affects the trafficking of adhesion molecules that are involved in astrogliosis, such as surface receptor β1-Integrin. Based on these findings, we hypothesized that deletion of DBN in astrocytes might also affect BBB integrity since β1-Integrin is reported to link the astrocytic endfeet to the extracellular matrix (ECM) in mouse brains (Venkatesan et al., 2015). Our immunohistochemical analyses revealed DBN protein in the brain vasculature and the astrocyte endfeet in the uninjured brain. Permeability assays demonstrate BBB leakage and altered vessel morphology in non-injured adult DBN knockout mice. These defects are accompanied by increased numbers in GFAP+ astrocyte and IBA1+ microglia reactivity indicating an elevated astro- and microgliosis in cortices of DBN mice. Moreover, cytokine levels are also increased substantially in DBN-deficient mouse brains. Finally, the perturbed localization of the tight junction adaptor protein Zonula-Occludens 1 (ZO-1) in DBN knockout brains underline the important role of DBN in facilitating the structural integrity of the brain vasculature. In summary, we could demonstrate a functional role of DBN in regulating BBB integrity. In our ongoing analyses, we study the molecular mechanisms of DBN functions at the BBB. One of our main focus is hereby to determine the onset and progression of DBN dependent neurovascular defects during postnatal development and aging.

Somatostatin Therapy, Neprilysin Activation, and Amyloid Beta Reduction: A Novel Approach for Alzheimer's Treatment

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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Introduction: Neprilysin is the primary enzyme responsible for the degradation of amyloid beta (Aβ), with its levels regulated by the hormone somatostatin (SST).
Methods: We have developed a novel treatment mechanism for Alzheimer's disease (AD) by combining SST with a blood-brain barrier (BBB) transporter and a Fc fragment to extend its half-life. This treatment was tested in a murine AD model overexpressing amyloid precursor protein (APP) with the Arctic mutation in Aβ (APP_{ArcSwe}).
Results: Our findings demonstrate a significant increase in neprilysin levels, which correlates with a reduction in various forms of Aβ, including membrane-bound and intracellular Aβ aggregates, as well as Aβ42 in insoluble aggregates.
Discussion: These results suggest that neprilysin can effectively degrade Aβ with the Arctic mutation. Additionally, this treatment strategy successfully reduces both oligomeric and larger Aβ, aggregates, a challenge for other therapeutic approaches. This novel strategy holds promise as a potential therapeutic approach for AD.

Optimizing size exclusion chromatography enrichment of cerebrospinal fluid extracellular vesicles for mass-spectrometry detection of blood-brain-barrier components

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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Introduction:

Blood-brain barrier (BBB) dysfunction is commonly associated with neurodegenerative disorders, including Alzheimer's disease and cerebral amyloid angiopathy. Currently available fluid biomarkers reflecting BBB (dys)function cannot differentiate between the blood-cerebrospinal fluid barrier and the BBB. Proteins associated with extracellular vesicles (EVs) may offer a solution to this limitation as EVs may be traced back to their cellular origin potentially serving as a more specific biomarker. In this explorative study, we aim to optimise the isolation of EVs from cerebrospinal fluid (CSF) and performed a preliminary analysis to detect BBB enriched proteins in these EVs.

Methods:

Sepharose CL6B size exclusion chromatography (SEC) was used to enrich EVs from 0.5mL CSF. The particle yield was assessed with nanoparticle tracking analysis and protein concentration with µBCA. Unlabelled mass-spectrometry was performed to identify and compare the protein content of CSF with three CSF-SEC fractions. Proteins were marked (1) if present in the vesiclepedia top100 vesicle-detected-proteins, or (2) as BBB-relevant if the encoding genes were enriched in brain endothelial cells or pericytes and not expressed by neuronal or choroid plexus cells (based on the human protein atlas).

Results:

Fraction (fr) 4 contained the highest number of EVs ($4.4x10^8$ particles/mL, Table 1). In contrast, the adjacent fractions contained fewer particles/mL with more femtogram protein/particles, indicating lower particle purity (Table 1). In fr4 were 441 proteins identified, of which 49.4% was enriched or unique compared to the crude CSF, 58.5% over fr3, and 9.1% over fr5 (Table 2). In fr4, 36 EV-proteins were detected with 20 enriched relative to CSF. However, the other fractions contained a greater number of both detected and enriched EV-proteins (Table 2). Similarly, the other fractions revealed more, and more enriched BBB-related proteins compared to CSF and fr4 (Table 3).

Discussion:

Whereas CSF-SEC fr4 contained the highest number and purest of EVs as compared to adjacent fractions, this was not reflected in the mass-spectrometry protein identification results. Fr4 had fewer enriched/unique proteins with a functional link to EVs compared to other fractions. This discrepancy suggests that the enrichment method requires further optimisation to enhance the detection of EV-specific and potentially BBB-related proteins.

Table 1; particle concentration and purity				
	CSF	Fraction 3	Fraction 4	Fraction 5
Particle concentration (particles/mL)	N.A.	$8.4x10^6$ *	$4.4x10^8$	$1.7x10^8$ *
Particle purity (fg protein/particle)	N.A.	36.8 *	2.04 *	38.8 *
Table 2; # proteins identified with mass-spectrometry and # EV-related proteins				
# of total proteins detected	1019	755	441	1504
# proteins enriched in fraction x compared to CSF	N.A.	661	218	1383
# in fraction 4 identified proteins that are enriched compared to CSF, fraction 3, and 5	218	258	N.A.	40
% of fraction 4 identified proteins that are enriched compared to CSF, fraction 3, and 5	49.4	58.5	N.A.	9.1
# EVtop100 proteins detected	44	73	36	69
# EV proteins enriched in fraction x compared to CSF	N.A.	66	20	64
% of enriched EV-proteins relative to identified proteins in that fraction	N.A.	8.7	4.5	4.3
# EVtop100 proteins enriched in fraction 4 compared to CSF, fraction 3 or 5	20	4	N.A.	1
Table 3; number and gene names of BBB-enriched genes detected with mass-spectrometry				
# BBB proteins (221 genes were selected from the HPA and 34 unique proteins were detected either in CSF and/or the fractions)	10	7	3	23
Gene names of detected BBB proteins and **enriched** compared to CSF	A2M CDH5 ESAM HEG1 GIMAP8 ICAM2 MMRN2 QDPR TCN2 VWF	A2M **CDA** **EEF2** **HSD17B4** **MYH9** **MYO1C** **SLC2A1**	**A2M†** **EEF2†** **HSD17B4†**	**A2M** **ATP10A** **CD248** **CDA** **CDH5** **EEF2** ESAM **HEG1** **HSD17B4** **ICAM2** **ITGA1** **ITGB1** **MMRN2** **MYH9** **NOTCH3** **PECAM1** **PODXL** **QDPR** **ROBO4** **SLC2A1** **ST6GALNAC1** **TCN2** **VWF**
# BBB proteins enriched in fractions compared to CSF	N.A.	6	3	22

*Table 1, 2 & 3. * at or below the lower reliable detection range. † these proteins were enriched in fraction 4 over both CSF and fraction 3. # number of. Abbreviations; N.A. not applicable, CSF cerebrospinal fluid, EV extracellular vesicle, BBB blood brain barrier, HPA Human protein atlas. **Bold** written genes are enriched in the SEC-fraction over CSF.*

As table1 till 3.png

Development of an antibody-based radioligand against GFAP

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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Aims

Neuroinflammation, driven by activated microglia and reactive astrocytes, contribute to Alzheimer's disease (AD) progression. *In vivo* imaging of this process could enhance diagnosis and support anti-inflammatory therapy development. Positron emission tomography (PET) offers a promising approach, but current radioligands for glial markers are limited by high background signals and genetic variability. This project aims to develop a bispecific antibody-based radioligand targeting glial fibrillary acidic protein (GFAP), a well-established marker of reactive astrocytes. To enable efficient delivery to the brain, the antibody is engineered with a transferrin receptor (TfR)-binding domain that facilitates transcytosis across the blood-brain barrier. The resulting PET radioligand will be used to visualize reactive astrocytes in genetically modified mouse models of AD.

Method

The bispecific GFAP-scFab-8D3 antibody was expressed in Expi293 cells and evaluated *in vitro* using immunohistochemistry. For *ex vivo* studies, the antibody was radiolabeled with iodine-125 and administered to genetically engineered mouse models exhibiting AD pathology, and age-matched wild-type (WT) controls, at a dose of 0.5 mg/kg. To assess brain uptake, WT mice were euthanized 4 hours post-injection (p.i.). Additionally, both $APP^{NL\text{-}G\text{-}F}$ and age-matched WT mice were euthanized 72 hours p.i. to evaluate brain retention, i.e., binding to GFAP after elimination of unbound antibody. *Ex vivo* brain retention of the radiolabeled antibody was quantified using a gamma counter.

Results

The brain uptake at 4 hours post-injection (p.i.) was 0.145 ± 0.003 SUV (n = 3), consistent with previous findings from our group. At 72 h p.i., there was no significant difference between $APP^{NL\text{-}G\text{-}F}$, however, a trend towards higher levels in $APP^{NL\text{-}G\text{-}F}$ mice. The standardized uptake value ratio (SUVR), calculated as the concentration ratio of cerebrum to cerebellum SUV, was significantly elevated in $APP^{NL\text{-}G\text{-}F}$ mice (~1.5) relative to wild-type controls (~1.0) at 72 h p.i.

Conclusions

There was a trend toward increased brain retention of [^{125}I]GFAP-scFab-8D3 in $APP^{NL\text{-}G\text{-}F}$ mice at 72 h p.i., however, the results did not reach statistical significance. To strengthen the reliability of these findings, larger group sizes are necessary. Notably, a significant difference in SUVR was observed between the mouse models, indicating greater antibody binding in the AD models compared to controls.

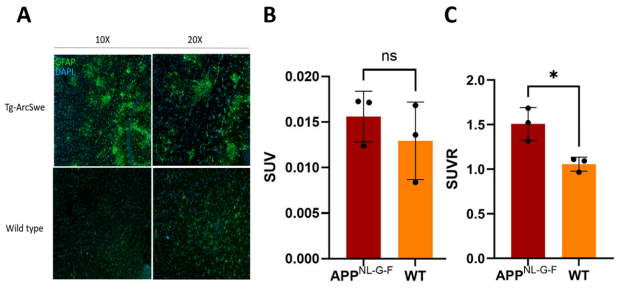

Figure 1. A) Immunohistochemical staining of wild-type and Tg-ArcSwe mouse brain sections stained with anti-GFAP antibody and DAPI. B) Brain uptake of GFAP-scFab-8D3 at 72 h post-injection in WT mice and APP^{NL-G-F} mice. C) Standardized uptake value ratio (SUVR) between cerebrum and cerebellum for GFAP-scFab-8D3 at 72 h post-injection.

Figure 1 abstract b4 .png

Investigating microglia-microvascular interactions in white matter in a mouse model of VCID using awake 2-photon microscopy

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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Chronic cerebral hypoperfusion is one of the principal causes for white matter (WM) dysfunction, which in turn predicts the development of vascular cognitive impairment and dementia (VCID). While the mechanisms linking these are poorly understood, past studies from our lab and others have shown that key mechanisms damaging white matter include disruption of the neuro-glio-vascular unit including altered microglia homeostasis, blood brain barrier breakdown and chronic microvascular inflammation. Previously, we provided compelling evidence that microglial state (abundance and function) influences WM abnormalities via interactions with endothelial cells. We now determined whether microglial states respond and contribute to reduced microvascular cerebral blood flow in a spatially-dependent manner.

To provide functional insight to microglia-microvascular interactions, subcortical in vivo two-photon microscopy was undertaken in awake CSF1R-eGFP mice ("MacGreen"). Alterations were studied in relation to progression of WM damage in a mouse model of VCID (induced by bilateral carotid artery stenosis, BCAS). To modify microglial function, a cohort of mice was treated with a CSF1R inhibitor, GW2580, that we previously found alleviated WM damage and microglial proliferation post-BCAS.

In these experiments, we first show repeated longitudinal measurements of microglial abundance and function as well as measurements of red blood cell velocity in discrete WM capillaries in the same spatial location. In line with previous studies, preliminary data indicate decreased red blood cell (RBC) velocity and increased microglia presence following BCAS surgery, while functional measures of microglial population are ongoing. Second, to this the effect initial data indicate that treatment with GW2580 may alleviate effects of chronic cerebral hypoperfusion in both RBC velocity and microglial measures.

This study adds to the growing evidence that microglia state has important functional consequences on vascular function and how regulators of microglia abundance and function may promote WM pathomechanisms relevant to VCID

A fully integrated multi-sensing blood-brain barrier-on-a-chip platform

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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Organ-on-a-chip (OOC) technologies are rapidly emerging as powerful tools in the field of biomedicine, providing an effective alternative to animal testing while preserving the physiological complexity of human biological systems. These platforms allow for precise regulation of fluidic environments through integrated perfusion systems and can incorporate sensors for real-time monitoring of physiological parameters.

Building upon this foundation, a novel, fully integrated blood-brain barrier-on-a-chip (BBB-oC) platform with multi-sensing capabilities is currently under development, representing a significant advancement in *in vitro* BBB modelling. This platform offers precise environmental control, real-time multiparametric monitoring, and high experimental reproducibility. The system features a single-use microfluidic chip with multiple replicates, each consisting of two vertically aligned channels separated by a porous polyethylene terephthalate (PET) membrane. This configuration supports the co-culture of key BBB-relevant cell types: a vascular channel lined with Human Brain Microvascular Endothelial Cells (HBMECs), and a neural compartment containing Human Brain Pericytes and Astrocytes, in direct contact with the endothelial layer through the membrane. This arrangement closely mimics the native architecture of the human BBB, facilitating physiologically accurate interactions between cell types. Physiological relevance is further enhanced through the integration of micropumps that enable independent, precisely controlled flow in each channel, effectively replicating physiological shear stress. The chip is housed within a modular carrier connected to a custom incubator that tightly regulates temperature and gas composition, maintaining optimal conditions for cell viability and function. A robust multi-sensing unit is incorporated into the system, enabling continuous, non-invasive monitoring of critical metabolic and functional parameters, including glucose, lactate, glutamate, glutamine, pH, temperature and trans-epithelial electrical resistance (TEER). This comprehensive sensing capability allows for dynamic assessment of barrier integrity and cellular responses under physiologically relevant conditions.

Altogether, this BBB-on-a-chip platform represents a scalable, cost-effective, and ethically aligned solution for biomedical research laboratories and contract research organizations, supporting the development of more predictive, human-relevant drug discovery and testing strategies.

Induction of subtle blood-brain barrier dysfunction using preclinical diagnostic ultrasound combined with microbubbles

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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The blood–brain barrier (BBB) plays a critical role in maintaining brain homeostasis by tightly regulating molecular transport. However, its integrity is often compromised with aging and in neurodegenerative diseases, contributing to disease pathology. Studying the biological consequences of BBB dysfunction independent of concomitant pathology remains challenging, largely due to the absence of reliable and inducible animal models that avoid unintended side effects such as osmotic effects, neuroinflammation, or vascular damage. In this study, we evaluated the use of Power Doppler ultrasound (PDUS) combined with microbubbles to induce widespread, bilateral BBB opening in the mouse brain. Mice received intravenous infusions of SonoMAC microbubbles during transcranial PDUS application. BBB permeability was assessed via Evans Blue dye extravasation and immunofluorescence analysis of extravasated immunoglobulins. Vessel integrity was evaluated at the ultrastructural level using transmission electron microscopy (TEM). PDUS combined with microbubbles successfully induced widespread BBB opening, as evidenced by diffuse Evans Blue staining and immunoglobulin extravasation in coronal sections. Immunoglobulin leakage was detected in all analyzed brain regions, with lower levels in white matter, likely reflecting its lower vascular density. Leakage appeared to primarily originate from capillaries while TEM analysis revealed no overt vascular damage. These findings support PDUS with microbubbles as a non-destructive, reproducible method to model widespread BBB dysfunction. This approach offers an in vivo platform to study BBB-related pathophysiological processes such as impaired clearance, protein aggregation, and neurotoxicity, as well as for investigation of therapeutic delivery to the brain parenchyma.

Ferritin light chain induces dysfunction of blood-brain barrier in Tg2576 AD mouse.

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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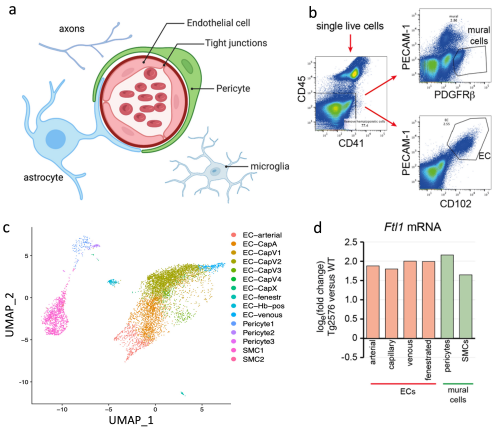
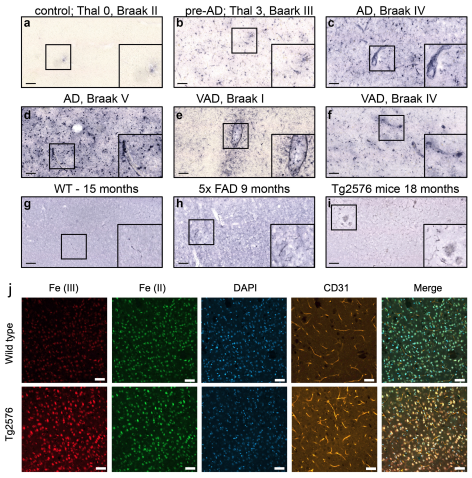
Alzheimer's disease (AD), the most common type of dementia, is a neurodegenerative disorder characterized by the progressive neuronal loss and decline in cognitive function. However, the mechanisms underlying the onset and development of AD still remain unknown. Neurovascular dysfunction is increasingly regarded as one of the main factors driving the progression of AD. The reasons responsible for the deterioration of neurovasculature are unclear, especially at the early stage of AD. Using single-cell transcriptomics, we identified the upregulation of ferritin light chain (FTL) across various subtypes of cerebral endothelial cells (ECs) in 9-month-old Tg2576 AD mouse model instead of 18-month-old Tg2576 mice. Visualizing Fe^{2+} and Fe^{3+} ions by DNAzyme-based fluorescent turn-on sensors, we revealed the enrichment of Fe^{2+} and Fe^{3+} ions in Tg2576 mice. Transendothelial electrical resistance (TEER) measurements showed the increased resistentce of human-induced pluripotent stem cell-derived brain microvascular endothelial cells (BMECs) after the reduction of FTL expression by shRNA. Bulk RNA sequencing data revealed that FTL knockdown in BMECs increased the expression of tight junction proteins, such as Claudin and Occludin. Also, TEER results showed that FTL overexpression (OE) led to the decreased resistance in BMECs and bEND.3 (brain capillary endothelial cell line). RNA sequencing data suggested that FTL OE significantly reduced the transcriptional expression of tight junction proteins. Our results suggest that upregulation of FTL in endothelial cell induces increased permeability of ECs in the brain, promoting the progression of AD in Tg2576 mice. Therefore, FTL may be a possible target for the therapy of AD.

1. Overview of BBB and scRNA-seq of vascular cells in Tg2576 mice



(a) The composition of blood-brain barrier (BBB) includes endothelial cells (ECs), mural cells and astrocytes. (b) FACS strategy for enrichment of ECs and mural cells from the adult mouse brain. (c) scRNA-seq analyses of neural vascular cells from Tg2576 mice. (d) Changes of *Ftl1* mRNA in ECs and mural cells from Tg2576 mice versus wild-type littermate.

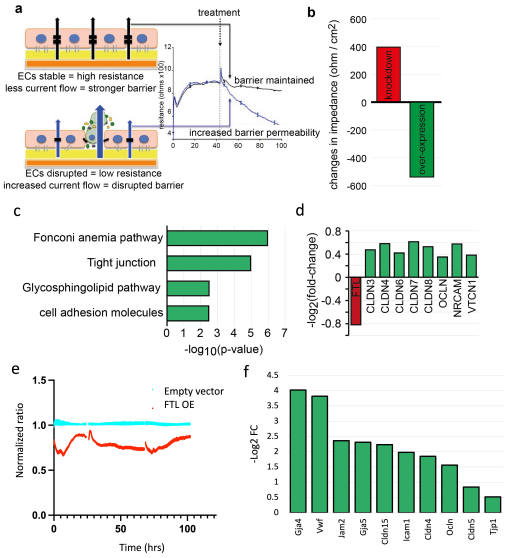
2. FTL protein levels in the vasculature of human and mouse brains and iron levels in mouse brains



(a) FTL stainings in a healthy human brain, as well as (b-d) human brains with increasing severity of AD and (VAD, e-f) vascular dementia. (g-i) FTL stainings in wild type (WT), 5x FAD and Tg2576 mouse models. (j) Analyses of iron levels using DNAzyme-based fluorescent probes

Fig1 and fig2.jpg

3. FTL impact the vascular barrier function by regulating the tight junction proteins



a. Overview of ECIS technique for TEER measurement. b. Quantification of integrity in BMECs following knockdown (red) or over-expression (green) of FTL. c. RNA-seq analyses of significantly upregulated pathways following FTL KD in BMECs. d. Significantly changed tight junction proteins in BMECs with FTL KD. e. TEER of bEND.3 cells transducted with empty vector and FTL overexpression vector. f. RNA-seq analyses of tight junction proteins in bEND.3 cells with FTL OE. N=3 per group.

Fig.3.jpg

Development of an uptake and release assay for early in vitro screening of BrainshuttleTM candidates.

Wednesday, 1st October - 13:45: (Patio Area) - Poster

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The blood-brain barrier (BBB) poses a major challenge for delivering biologics, such as antibodies, from the bloodstream into the brain due to its highly selective and restrictive nature. To overcome this, strategies focusing on receptor-mediated transcytosis have been developed, targeting specific receptors at the BBB to improve drug delivery to the brain. Early identification of biologics with favorable brain uptake and pharmacokinetic properties using *in vitro* assays is crucial for streamlining drug development and enhancing clinical success.

In this study, we present a straightforward Uptake and Release Assay (URA) designed to evaluate target mediated-transcytosis and -recycling efficiency of large molecule candidates based on their cellular release scores. The URA involves a simple pulse-chase assay using MDCK cells overexpressing the target receptor, followed by an enzyme-linked immunosorbent assay (ELISA).

Using the URA, we assessed the cellular release rates of large molecules and compared these results with *in vivo* pharmacokinetic data from a human transgenic mouse model. We also compared the URA to a more complex and established *in vitro* human transcytosis assay (Simonneau et al., 2021) and found that the URA offers a greater dynamic range, allowing for effective ranking of large molecule candidates.

In summary, we have developed a novel *in vitro* assay characterized by its simplicity, ease of use, and cost-effectiveness. This assay shows significant potential for evaluating the release efficiency of Brainshuttle^{TM} candidates, thereby aiding in the selection and optimization of lead candidates.

Additionally, the development of this new assay has been instrumental in refining our *in vitro* screening strategy by enabling a balanced use of both simple and complex systems based on candidate properties, ultimately enhancing screening efficiency and identifying promising candidates.

Simonneau et al., 2021. Investigating receptor-mediated antibody transcytosis using blood-brain barrier organoidarrays. Fluids Barriers CNS, 18(1), 43.

Impact of cocaine and alcohol consumption on blood-brain barrier: from cells to animal studies.

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Lucía Garrido Matilla [1], ***Mr. Carlos Vera Fernández*** [1], ***Ms. Eliane Swely Sanches*** [2], ***Mrs. Daniela M. Simões*** [3], ***Dr. Alberto Marcos Bermejo*** [1], ***Prof. Emilio Ambrosio Flores*** [1], ***Prof. Ana Paula Silva*** [3]

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Drug addiction is a chronic relapsing disorder characterized by compulsive drug-seeking behaviour and neurobiological adaptations. Cocaine use disorder is a significant public health problem with a prevalence of approximately 74% of cases, being a substance frequently used with alcohol. This combination is particularly concerning, as it alters cocaine metabolism, prolongs its effects, and disrupts physiological homeostasis, potentially exacerbating addiction-related outcomes.

In addition to the well-documented neurobiological changes underlying substance use, recent evidence shows that both cocaine and alcohol can induce a neuroinflammatory process that is known to highly impact blood-brain barrier (BBB). However, how combined cocaine and alcohol exposure affects the BBB is still not well understood. In this context, the pro-inflammatory cytokine IL-17A emerges as a molecule of particular interest, as previous studies have linked IL-17A to BBB disruption. Moreover, our group has reported elevated IL-17A levels in rats displaying reinstatement of cocaine-seeking behaviour, suggesting a potential role in both barrier integrity and relapse vulnerability.

Thus, in this study we investigated the BBB alterations induced by the combined administration of cocaine and alcohol, along with the possible involvement of IL-17A signalling. We employed a dual approach: (1) a rat model of cocaine and alcohol-seeking incubation (2) an *in vitro* human BBB model composed of a monolayer of brain endothelial cells (hCMEC/D3; Figure 1).

Regarding animal studies, the analysis of the rat hippocampus and striatum revealed sex-dependent reduction in claudin-5 and VE-cadherin levels following cocaine and alcohol self-administration and withdrawal. Additionally, increased expression of IL-17 receptors, particularly IL-17 receptor C, was also observed. *In vitro*, both the co-exposure and the cocaine and alcohol alone reduced transendothelial electrical resistance, indicating impaired barrier integrity. The BBB disruption was accompanied by a decrease in claudin-5 levels. Additionally, a positive trend was observed in the expression of IL-17 receptor C expression.

These preliminary results suggest that cocaine and alcohol co-exposure induce a sex-dimorphic pattern, and disrupt the BBB, likely by altering intercellular junctions and IL-17A signalling. More studies are currently undergoing to clarify in more detail the impact of cocaine and alcohol on BBB dysfunction

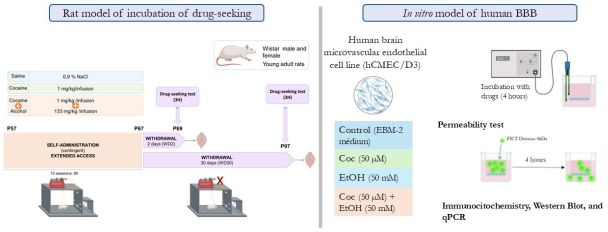
Rat model of incubation of drug-seeking
In vitro model of human BBB
Wistar male and female
Young adult rats
Saline
0,9 % NaCl
Cocaine
1 mg/kg/infusion
Cocaine
Alcohol
1 mg/kg /infusion
133 mg/kg /infusion
Drug-seeking test (5H)
Drug-seeking test (6H)
P57
P67
P69
P97
SELF-ADMINISTRATION
(contingent)
EXTENDED ACCESS
WITHDRAWAL
2 days (WD2)
WITHDRAWAL
Human brain
microvascular endothelial
cell line (hCMEC/D3)
Control (EBM-2 médium)
Coc (50 µM)
EtOH (50 mM)
Coc (50 µM) + EtOH (50 mM)
Incubation with drugs (4 hours)
Permeability test
4 hours
Immunocitochemistry, Western Blot, and qPCR

Figure 1.jpg

Interleukin-1 signaling in the blood-brain barrier influences the behavioral response to chronic social stress: investigating potential downstream mediators

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Dr. Eva Schramm [1], Ms. Ilaria Palagi [1], Mr. Arsenij Ustjanzew [2], Dr. Federico Marini [2], Dr. Matthias Klein [3], Prof. Ari Waisman [1]

1. *Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg University, Mainz,* ***2.*** *Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), University Medical Center of the Johannes Gutenberg University, Mainz,* ***3.*** *Institute of Immunology, University Medical Center of the Johannes Gutenberg University, Mainz*

Chronic stress is a major risk factor for depression and other psychiatric disorders, leading to systemic immune activation and neuroinflammation, both of which contribute to the development of depressive symptoms. The blood-brain barrier (BBB) maintains central nervous system (CNS) homeostasis by regulating the exchange of molecules and immune cells between the blood and CNS. At the forefront of this barrier, BBB endothelial cells (BECs) respond to peripheral signals, such as pro-inflammatory cytokines, and transmit these signals to the CNS during stress, contributing to pathological behavioral changes. However, the precise effects of chronic stress on BBB function and the potential role of BBB components in establishing depression-like behaviors remain unclear.

Interleukin-1 (IL-1), a key pro-inflammatory cytokine, is critically involved in the development of stress-induced behavioral changes. Its receptor, IL-1R1, is primarily expressed by BECs in the CNS but is restricted to a small subset of these cells under steady-state conditions. Notably, chronic social defeat (CSD) stress in mice up-regulates IL-1R1 expression on BECs, potentially driving BBB activation. While systemic endothelial IL-1R1 deletion alleviates stress-induced behavioral symptoms, whether IL-1 signaling specifically in BECs mediates these effects remains unclear. To address this, we investigated the contribution of IL-1 signaling in BECs to depression-like behavior during CSD. Our findings revealed that IL-1 signaling in BECs is critical for mediating stress-induced behavioral changes, as conditional deletion of IL-1R1 in BECs increased stress resilience and ameliorated behavioral symptoms following CSD. Importantly, IL-1 signaling in BECs influenced behavior independently of peripheral immune cell infiltration or neuroinflammation, suggesting alternative pathways.

To further investigate the downstream mediators of IL-1 signaling in BECs, we examined the stress-responsive gene *Hmox1*, encoding heme oxygenase-1 (HO-1). IL-1 signaling downregulates HO-1 in BECs, contributing to experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis. Given the antioxidant, anti-inflammatory, and cytoprotective properties of HO-1 reaction products, we hypothesized that upregulation of HO-1 in BECs could enhance stress resilience. However, transgenic mice with conditional overexpression of human HO-1 subjected to CSD exhibited behavioral and immunological profiles similar to those of controls, suggesting that additional downstream effectors driven by IL-1 signaling are critical for modulating stress resilience.

Impact of stress-induced inflammation on blood-brain barrier transcytosis

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Mrs. Adeline Collignon [1], ***Mrs. Fernanda Neutzling Kaufmann*** [1], ***Mrs. Alice Cadoret*** [1], ***Ms. Luisa Bandeira Binder*** [1], ***Dr. Laurence Dion-Albert*** [1], ***Dr. Manon Lebel*** [1], ***Mr. Antoine Ollier*** [1], ***Dr. Flavie Lavoie-Cardinal*** [2], ***Dr. Caroline Ménard*** [1]

1. *CERVO brain research center, Université Laval,* ***2.*** *Université Laval*

Major Depressive Disorder (MDD) affects nearly 400 million people worldwide. This heterogenous illness can be triggered by environmental factors, such as stress. Unfortunately, available treatments are targeting neuronal dysfunction and are ineffective for 30-50% of individuals with MDD. Treatment resistance is associated with an exacerbated immune response, with high levels of circulating proinflammatory cytokines. Stress-induced inflammation in the brain and subsequent mood alterations may be induced by the infiltration of peripheral immune mediators into the central nervous system. Indeed, the blood-brain barrier (BBB) is altered following chronic social defeat stress (CSDS), a mouse model of depression, and in the brain of MDD patients. Loss of BBB integrity contributes to maladaptive stress responses and depression in mice and humans, but the detailed molecular mechanisms remain to be elucidated.

Caveolae-mediated transcytosis is a key transport property at the BBB and caveolae are central to passage of plasma macromolecules. It involves the assembly of caveolae-associated proteins, mainly Caveolin-1, to form a vesicle transiting through endothelial cells. We investigated the role of caveolae in the effects of stress on BBB integrity. Our preliminary data highlight an increase of caveolae-related gene expression after CSDS in male. To gain mechanistic insights and evaluate how stress-induced inflammation modulates BBB transcytosis and cell signaling, I subjected mouse brain endothelial cells to pro-inflammatory cytokines or to serum collected from CSDS-mice *in vitro.* Our results highlighted dynamic caveolae-related genes and cells junctions' properties modulations due to inflammation. *In vivo* viral manipulations of Caveolin1 expression, an essential protein for transcytosis conduction, are ongoing to determine transcytosis modulations involvement in chronic social stress. Additionally, longitudinal anatomical and functional magnetic resonance imaging (MRI) scans in mice, prior and at various time points after stress exposure, are ongoing to assess the long-term effects of stress on BBB integrity and brain cell activity. To our knowledge, only a handful of studies have explored how stress affects BBB or transport systems properties. Investigating BBB transcytosis modulations following stress and inflammation will contribute to a better understanding of molecular mechanism underlying MDD pathology and unravel innovative therapeutic avenues.

Viral tools to alter Blood-brain barrier gene expression and decipher its role in memory and cognition

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Mrs. Alice Cadoret [1], ***Dr. Laurence Dion-Albert*** [1], ***Ms. Luisa Bandeira Binder*** [1], ***Ms. Adeline Collignon*** [1], ***Ms. Laura Menegatti Bevilacqua*** [1], ***Dr. Manon Lebel*** [1], ***Dr. Jessica Deslauriers*** [2], ***Dr. Caroline Ménard*** [1]

1. *CERVO brain research center, Université Laval,* ***2.*** *Centre de recherche du CHU de Québec Université Laval, Québec, QC, Canada*

Experiences linked to emotions impact memory consolidation and associated brain neuronal circuits. Posttraumatic stress disorder and major depressive disorder are examples of mental disorders where memory deficits are observed. We recently highlighted that stress induces blood-brain barrier (BBB) alterations in a sex and brain region specific manner in mice and human depression. BBB cells can secrete growth factors and overexpress some markers to allow behavioral responses in stressful and cognitive situations. However, little is known about the relationship between emotional valence, memory encoding and BBB function.

In this study, we looked at the effects of negative emotional valence through an aversive memory experience: fear conditioning (FearC). Male and female mice went through acquisition (auditory cue and footshocks) on day 1, context, cue and recall tests the following 3 days. We observed sex differences in behavioral and biological variables: females froze more compared to males. In transcriptomes, fibroblast growth factor 2 (*Fgf2*), which regulates BBB integrity, was upregulated in ventral hippocampus (HC) for both males and females mice receiving footshocks. FGF2 protein in footshocks mice seems to come from astrocytes in ventral HC in proximity with blood vessels. We then took opportunities of viral tools to manipulate BBB integrity via *Fgf2* and tight junction *Cldn5*. We injected AAV-shRNA-*Fgf2* in HC to see how its downregulation from astrocytes could affect behavior. We confirmed lower FGF2 signal in the HC. Also, we injected AAV-shRNA-cldn5 in dorsal HC, which lower CLDN5 levels compared to mice that received control AAV. Then, we injected a cohort with this AAV and ran memory tests (Novel object recognition, Y-maze and FearC) few weeks after injection. AAV-Cldn5 mice showed less exploration with objects and spatial arms and impairment in FearC extinction.

In summary, mice that had a negative memory experience showed BBB changes, different between male and female animals. Fgf2 could be an important link between memory dysfunction and vascular barrier impairment. This BBB modulator and Cldn5 tight junction might be key molecules of memory formation in HC. This gives us additional information on the role of the BBB integrity in memory formation.

Evaluating Sex as a Biological Variable in In Vitro Blood–Brain Barrier Models: Insights from Primary Mouse Brain Endothelial Cells

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Maria Thaysen [1], ***Ms. Line Toft Ørum*** [1], ***Ms. Alberte Bay Villekjær Pedersen*** [1], ***Dr. Nana Svane*** [1], ***Ms. Signe Emilie Dannulat Frazier*** [1], ***Dr. Lasse Saaby*** [1], ***Prof. Birger Brodin*** [1], ***Dr. Mie Kristensen*** [1]

1. *Department of Pharmacy, University of Copenhagen, Denmark*

Introduction

Preclinical and clinical research has historically exhibited a significant sex bias, with a predominance of studies conducted in male subjects (1, 2). This imbalance has had serious implications, including withdrawal of several drugs due to the emergence of severe, sex-specific adverse effects (3). Despite efforts by funding agencies and publishers to address sex bias in both preclinical and clinical research, it remains a persistent issue (4). This issue is also evident in research on the blood-brain barrier (BBB); despite growing recognition of sex-dependent differences in BBB function among males and females (5). Notably, most studies investigating these differences have been conducted in *in vivo*, leaving it unclear whether sex-dependent characteristics are retained in *in vitro* models of the BBB (5). To address this knowledge gap, we therefore sought to investigate if sex-dependent differences are present in a primary mouse BBB model.

Method

Brain capillary endothelial cells (BCECs) were isolated from cortices of sexually mature 8-weeks-old male and female C57Bl/6 mice. Endothelial cells were cultured on Transwell inserts for 5 days, prior to use. Expression of selected BBB markers was investigated using qPCR, western blotting and immunocytochemistry. Barrier tightness was assessed with transendothelial electrical resistance (TEER) measurements, and P-glycoprotein function was evaluated in bidirectional (digoxin) transport studies.

Results and perspective

mRNA expression levels of the tight junction proteins, claudin-5, occludin and ZO-1 was similar in BCEC's from male and female mice, respectively. Immunostaining of monolayers also indicated that there were no differences in localization and expression levels of tight junction proteins. However, BCEC monolayers from male mice (median: 127.3 Ω*cm^2) did display significantly higher TEER values as compared to values from BCEC monolayers from females (median: 109.4 Ω*cm^2). Finally, immunostaining revealed a trend towards a higher level of transferrin receptor staining in female BCECs compared to male BCECs, but this was not observed at mRNA level. The present study is to our knowledge the first study demonstrating sex differences in a BBB model setup. Whether the observed differences in barrier tightness can be translated to the *in vivo* situation is the target of future studies.

Stem cells-derived extracellular vesicles demonstrate promising angiogenic properties

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Mr. Vojtěch Šprincl [1]***, Ms. Uliana Kodnevski*** [2]***, Ms. Kristýna Šintáková*** [1]***, Dr. Nataliya Romanyuk*** [3]

1. *Institute of Experimental Medicine CAS, Czech Republic; Second faculty of Medicine, Charles University, Czech Republic,* ***2.*** *Faculty of Science, Charles University,* ***3.*** *Institute of Experimental Medicine CAS, Czech Republic*

Acute ischemic stroke remains a leading cause of mortality and disability worldwide. Ischemia initiates a cascade of complex pathophysiological events, further aggravated by oxidative stress during reperfusion, ultimately resulting in disruption of the blood-brain barrier (BBB). Angiogenesis is increasingly recognized as a critical component of neuroregeneration post-reperfusion. Stem cells, known for their regenerative potential, exert many of their protective effects through paracrine signaling. Among these, extracellular vesicles (EVs) have emerged as key mediators.

In our study, EVs were isolated from the cultured media of various stem cell types using sucrose cushion ultracentrifugation. Western blot analysis confirmed the presence of characteristic EV markers, including Alix, TSG101, and CD9, and demonstrated sample purity through the absence of the endoplasmic reticulum marker calnexin. The average diameter of the isolated EVs was 85 ± 40 nm.

To assess the angiogenic potential of EVs, we conducted scratch assays and tube formation assays using primary endothelial cells. EV treatment enhanced wound closure and significantly increased tube density, total tube length, and the number of junctions. Additionally, the uptake of fluorescently labeled EVs by endothelial cells was observed.

We developed a static in vitro BBB model using immortalized human brain endothelial cells co-cultured with astrocytes. Expression of tight junction proteins Claudin-5, Occludin, ZO-1, and VE-Cadherin was confirmed via western blot and immunocytochemistry. Model integrity was evaluated by transendothelial electrical resistance measurements and lucifer yellow diffusion assays. An oxygen-glucose deprivation/reperfusion (OGD/R) injury model was successfully established.

Future studies will investigate the potential of stem cell-derived EVs to preserve or restore BBB integrity following OGD/R injury. This will be assessed by evaluating both the physical permeability of the endothelial cell layer in the established model and the stability of their tight junctions.

Supported by

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Improved iPSC-based model of human brain endothelial to investigate brain drug delivery and disease modelling

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Dr. Martina Pigoni [1], ***Dr. Luisa Bell*** [1], ***Dr. Roberto Villaseñor Solorio*** [1]

1. *Roche Pharma Research and Early Development (pRED), Roche Innovation Center Basel, Basel, Switzerland*

The blood-brain barrier (BBB) is crucial for maintaining brain function. Dysfunction or loss of BBB integrity is an early indicator of several neurodegenerative diseases. However, existing human in vitro models lack the complexity of the BBB, limiting the development of brain-targeting therapies.

We developed a reliable in vitro human iPSC-derived BBB model to enhance the identity and functionality of brain endothelial cells. Using an optimized protocol, we generated high-quality induced brain endothelial cells (iCE-BECs), characterized at molecular and functional levels. Multi-omic profiling and functional transport assays showed that iCE-BECs possess a brain endothelial gene signature and demonstrate receptor-mediated transcytosis of a clinically validated BrainshuttleTM antibody targeting the transferrin receptor.

To showcase the model's applicability, we assessed the effect of apolipoprotein E4 (ApoE4), a genetic risk factor for Alzheimer's disease, on intracellular transport in brain endothelial cells. ApoE4-engineered iCE-BECs displayed altered early endosome organization, increased transferrin receptor expression, and reduced cytoplasmic iron.

The development of this BBB model with improved brain endothelial cell identity will enhance target discovery efforts and facilitate modeling pathological processes in neurodegenerative diseases at the BBB.

Modeling Oxidative Stress and Neuroinflammation on a Vessel-on-a-Chip

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Sheila Sousa Gomes Fortes [1], ***Dr. Ana Raquel P. Santa Maria*** [2], ***Dr. Fruzsina Walter*** [3], ***Ms. Judit P. Vigh*** [3], ***Ms. Anna E. Kocsis*** [3], ***Ms. Nóra Kucsápszky*** [3], ***Dr. Ana Martins*** [3], ***Dr. Krisztina Nagy*** [3], ***Dr. Péter Galajda*** [3], ***Dr. Mária Deli*** [3], ***Dr. Emanuel Carrilho*** [1]

1. *Universidade de São Paulo,* ***2.*** *Wyss Institute for Biologically Inspired Engineering,* ***3.*** *HUN-REN Biological Research Centre*

Neuroinflammation plays a crucial role in the development and progression of various neurodegenerative diseases. Therapeutic strategies aimed at protecting physiological functions of brain endothelial cells are being explored as potential treatments for these conditions. It is necessary to have experimental models that accurately represent the effects of neuroinflammation, as existing models are often not sufficiently representative of human physiology and the specific challenges of central nervous system diseases with a vascular pathology. Considering the need for a more accurate simulation strategy of the selective properties and limitations of the blood-brain barrier (BBB), we established a BBB-on-a-chip model, which consists of a co-culture of human stem cell-based brain endothelial cells and brain pericytes, to study how BBB permeability is altered under pathological conditions and to discover protective compounds to counteract BBB injury. Our preliminary results showed that the thrombin-fibrinogen interaction-based hydrogel self-assembled model holds great potential for use in preclinical studies, as it was possible to mimic BBB properties in vitro. Brain endothelial cells formed an established vascular network visualized by PECAM staining along with a positive signal for α-smooth muscle actin for pericytes. Modeling BBB injury with pro-inflammatory cytokines to promote neuroinflammation was successful with a lower calcein AM signal and higher positivity for propidium iodide. This setup will be used to test novel ways to improve BBB functions under neuroinflammation. In the future we aim to identify and optimize new therapeutic compounds before testing them on animal or human models.

Blood-Brain-Barrier permeability and Glycocalyx shedding in patients at Clinical High Risk of developing psychosis: a longitudinal cohort study.

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Dr. Helle Andersen [1], ***Mr. Ulrich Lindberg*** [2], ***Mr. Brian DellaValle*** [3], ***Mrs. Daban K.A. Sulaiman*** [1], ***Mrs. Kristina B. Gundersen*** [4], ***Mrs. Anne Sofie Dahl*** [1], ***Mrs. Sofie Amalie Sørensen*** [1], ***Mrs. Anna Feveile*** [1], ***Ms. Robabeh H. Tavangar*** [2], ***Mrs. Karina Segers*** [2], ***Mr. Mikkel E. Sørensen*** [1], ***Prof. Merete Nordentoft*** [5], ***Ms. Karen S. Ambrosen*** [1], ***Ms. Tina D. Kristensen*** [1], ***Prof. Louise Glenthøj*** [4], ***Prof. Henrik B.W. Larsson*** [2], ***Prof. Bjørn Ebdrup*** [1]

1. *Center for Neuropsychiatric Schizophrenia Research (CNSR), Mental Health Center, Glostrup, Copen-hagen University Hospital – Mental Health Services CPH, Copenhagen, Denmark,* ***2.*** *Functional Imaging Unit, Department of Clinical Physiology and Nuclear Medicine, Rigshospitalet Glostrup, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.,* ***3.*** *GLX Analytix ApS, Copenhagen, Denmark.,* ***4.*** *VIRTU Research Group, Mental Health Center Copenhagen, Copenhagen University Hospital, Mental Health Services CPH, Copenhagen, Denmark.,* ***5.*** *Copenhagen Research Centre for Mental Health (CORE), Copenhagen University Hospital, Copenha-gen, Denmark.*

Abstract:

Introduction: The etiology of psychosis remains unclear, but accumulating evidence suggests that increased blood-brain barrier (BBB) permeability may contribute to the pathophysiology[1]. Recent studies employing dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) have provided in vivo evidence of increased permeability in patients with schizophrenia spectrum disorders [2,3]. The glycocalyx (GLX), a critical structural component of the BBB, plays a key role in maintaining vascular integrity[4,5]. Previously, we demonstrated that patients with first-episode psychosis exhibited elevated circulating glycocalyx components compared to healthy controls (HC) [6], and machine learning models could distinguish patients from controls with a mean accuracy of 81%. Furthermore, there was a non-linear association between GLX shedding and symptom severity, highlighting their potential as an immuno-neuropsychiatric biomarker. However, these early disease markers have yet to be investigated in a cohort in clinical high-risk (CHR) of developing psychosis.

Objective: We will investigate the relationship between GLX integrity, BBB permeability and symptom severity in a CHR cohort and matched HCs. We aim to: (I) examine BBB permeability differences groupwise and longitudinally, (II) characterize GLX shedding patterns in blood across groups and time, and (III) assess correlations between GLX measurements, BBB permeability and symptom severity within each group.

Methods: In an ongoing observational study, we examine a cohort of CHR individuals and matched HCs. Transition to psychosis and symptom severity are monitored every 6 months in a 2-year follow-up period. Clinical assessments include psychometric evaluations, cognitive and functional measures. Biological assessments include baseline and follow-up DCE-MRI scans (with Gadolinium) and blood samples (including inflammatory and GLX markers).

Results: As of April 2025, 36 CHR and 12 HC have completed baselines assessments, with follow-up assessments undergoing. Two have completed 24-month follow-up. Two CHR participants have transitioned to psychosis. Data analysis is currently in progress.

Discussion: Our study will investigate the role of BBB dysfunction and GLX integrity in putative stages of psychosis by integrating DCE-MRI and molecular biomarkers over time. Identifying early disease markers could improve treatment stratification and facilitate targeted interventions.

The role of blood-brain interactions in the development of Huntington's disease-related neuropathology

Wednesday, 1st October - 13:45: (Patio Area) - Poster

Ms. Flávia Natale Alves Martins Borba [1], ***Dr. Thyago Cardim-Pires*** [2], ***Ms. Martine Saint-Pierre*** [2], ***Dr. Hélèna Denis*** [3], ***Mr. Victor Fourcassié*** [4], ***Dr. Arnaud Droit*** [4], ***Dr. Sylvie Breton*** [5], ***Dr. Francesca Cicchetti*** [2], ***Dr. Aurélie de Rus Jacquet*** [6]

1. *Axe neurosciences, Centre de recherche du CHU de Québec; Département de Psychiatrie & Neurosciences, Faculté de Médecine, Université Laval, Québec, QC, Canada,* ***2.*** *Centre de recherche du CHU de Québec Université Laval, Québec, QC, Canada,* ***3.*** *Centre de recherche du CHU de Québec -Université Laval, Québec, QC, Canada;,* ***4.*** *Proteomics Platform, CHU de Québec -Université Laval Research Center, Québec, QC, Canada,* ***5.*** *Département de Reproduction, santé de la mère et de l'enfant, Université Laval, Québec, QC, Canada,* ***6.*** *Centre de recherche du CHU de Québec - Université Laval, Québec, QC, Canada*

Huntington's disease (HD) is a neurodegenerative disorder caused by a genetic mutation that produces a mutant Huntingtin protein (mHTT), prone to misfolding and aggregation. These aggregates accumulate in various organs, including the brain, where it causes neural degeneration in the striatum and cortex, leading to progressive motor, cognitive, and behavioral decline. The mutant protein is also detected in cerebrospinal fluid, plasma, and extracellular matrix, potentially amplifying the disease phenotype. Interestingly, studies suggest that healthy blood has rejuvenating effects on aging tissues, and a subset of plasma molecules may cross the blood-brain barrier (BBB) and benefit individuals with HD. We **hypothesize** that healthy plasma has a distinct protein composition compared to HD plasma and contains protective factors that could improve HD-related phenotypes. Our project aims to explore blood-brain interactions that may attenuate HD pathology and identify plasma components that cross the BBB and mediate beneficial effects. To identify the potential benefits of healthy plasma, we developed a 3D BBB model generated using human induced pluripotent stem cells (iPSC) produced from control donors and people with HD. We differentiated iPSCs into endothelial cells, astrocytes, and neurons. Our models are treated with plasma from healthy donors and HD patients, as well as candidate recombinant proteins, to validate the potential benefits of specific molecules. To this end, we used immunofluorescence-based analyses to evaluate the effect of plasma treatment on neuron morphology and performed barrier integrity measures. We also labeled plasma proteins with biotin to track their cellular localization and assess their ability to cross the BBB. Our preliminary results indicate that healthy plasma improves BBB integrity and reduces neurotoxicity in HD models. Recombinant proteins exhibited protective effects against HD plasma-induced neurodegeneration. A proteomics analysis identified a subset of proteins found at lower levels in HD plasma compared to control, and we selected the best protein candidates among these hits to treat the BBB model. Notably, these proteins improved the health and morphology of HD neurons, highlighting a possible neuroprotective role for these plasmatic factors. In conclusion, this project has the potential to shed light on novel mechanisms of blood-brain interactions in health and HD.

Gate2Brain blood-brain barrier shuttle peptides: From discovery to applications and going beyond small molecules.

Wednesday, 1st October - 14:45: (Auditorium 1) - Invited Speaker

Dr. Meritxell Teixidó [1]

1. *Gate2Brain*

Dr. Meritxell Teixidó[1]

[1]Gate2Brain S.L., Barcelona, Spain.

Presenting author email address: meritxell.teixido@gate2brain.com

Gate2Brain shuttle peptides represent salvage for new or previously rejected CNS drug candidates by providing a way to cross the blood-brain barrier (BBB).

Gate2Brain technology consist on a toolbox of peptides able to cross the BBB and carry compounds covalently attached (including small molecules, peptides, proteins, antibodies, plasmids, siRNA or mRNA loaded nanoparticles, etc…) that cannot cross this barrier unaided. They have proofed to carry these cargoes in vitro and in vivo. These peptide shuttles use the existing transport mechanisms at the BBB without affecting the normal functioning of these mechanisms and preserving brain homeostasis.

By improving the delivery of therapeutic candidate to the CNS, we will ensure immediate impact in many CNS diseases patients. In addition, in a broader perspective, Gate2Brain technology may help to repurpose existing therapies previously rejected because of difficulty to reach the brain, accelerating the translation towards clinical development. Gate2Brain will also result in the application of lower concentrations of therapeutic agent, thereby significantly lowering systemic side effects and reducing the cost of the treatment.

Gate2Brain peptides combine protease resistance, capacity to carry a wide range of cargoes thanks to their versatility, low production costs, and low immunogenic risk. They provide a non-invasive, non-antigenic, permeable, stable, soluble and receptor-specific way to transport drugs across the BBB and into the CNS.

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Applications of Single Molecule Localization Microscopy (SMLM) in BBB research – discovery of unique barrier structures

Wednesday, 1st October - 15:25: (Auditorium 1) - Invited Speaker

Prof. Ayal Ben-Zvi [1]

1. *Hebrew University*

Central Nervous System (CNS) vasculature differs from vascular networks of peripheral organs by its ability to tightly control selective material exchange across capillary barriers. Capillary permeability is mostly defined by unique cellular components of the endothelium. While capillaries are extensively investigated, the barrier properties of larger vessels are understudied. Approximately 140 years have passed since the discovery of the Blood-Brain Barrier (BBB) and 55 years since its cellular components were identified. Now, by using new imaging approaches we uncover unique features of the arterial Blood-Brain Barrier (A-BBB). Recent evidence suggests that there is molecular heterogeneity among the different components of the Neurovascular Unit (NVU), particularly among endothelial cells, across different types of CNS vessels. These findings, mainly obtained from single-cell mRNA sequencing profiling, have led us to investigate the possibility of cell biological and functional heterogeneity in barrier properties and to investigate barrier properties of arterial walls. Using tracer challenges and various imaging modalities, we discovered that at the mouse cortex, the arterial barrier does not reside at the classical level of the endothelium. We found that caveolae vesicles in arteriole cells are functional transcytosis machinery components, and that a similar mechanism is evident in the human brain. Finally, through the use of Single Molecule Localization Microscopy we shed light on additional unique barrier structures. Based on our findings, we suggest shifting from a unifying view of the classical BBB to acknowledge structural and functional barrier heterogeneity.

Neuro-immune events at the blood-brain barrier in neurodegeneration

Wednesday, 1st October - 16:05: (Auditorium 1) - Invited Speaker

Prof. Helga E. de Vries [1]

1. *Department of Molecular Cell Biology and Immunology, Amsterdam UMC location Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*

TBC

3D-Printed Organ-on-Chip Models of the Human Blood-Brain Barrier: A Platform to Study BBB Function and Drug Permeability

Wednesday, 1st October - 17:15: (Auditorium 1) - Oral

Ms. ludovica montesi [1], ***Dr. Davide Lattanzi*** [2], ***Dr. Mattia Tiboni*** [3], ***Mr. Mario Hagel*** [4], ***Ms. Alice Sartini*** [1], ***Dr. Stefano Sartini*** [1], ***Dr. Tirosh Mekler*** [5], ***Prof. Netanel Korin*** [5], ***Prof. Rossana Rauti*** [1]

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The blood-brain barrier (BBB) is a key functional component of the neurovascular unit (NVU), which comprises endothelial cells, pericytes, astrocytes, and neurons. The NVU tightly regulates molecular exchange between the bloodstream and the central nervous system, maintaining brain homeostasis. Given the complexity of its anatomical structure, it's complicated to identify specific cells responsible for adverse stimuli, particularly in the context of single-cell cultures as well as the mechanisms underlying pathological conditions. Traditional in-vitro models and animal studies fail to fully recapitulate the cellular architecture and dynamic environment of the human brain. These limitations, and ethical concerns, have accelerated the search for advanced human-relevant models. Organ-on-a-chip (OoC) technology offers an advanced approach to recreate human barrier physiology under controlled conditions. Here, we present two biocompatible, stereolithography-based 3D-printed OoC platforms, capable of modelling the human BBB and investigate cell-cell interactions, barrier integrity, and transport properties.

The first device features two chambers separated by a porous membrane, with independent inlets and outlets for perfusion. Human endothelial cells and neuronal cells were co-cultured, barrier function assessed via in situ trans-endothelial electrical resistance (TEER), cell viability and function were confirmed via immunofluorescence and live calcium imaging.

The second platform consists of a single open chamber, allowing the co-culture of endothelial cells (top surface), and neurons. The chip was designed to apply controlled flow and shear stress, enabling studies of how hemodynamic forces in the vascular lumen impact BBB integrity and trigger BBB dysfunction.

Unlike PDMS-based devices, these 3D-printed models offer rapid prototyping, avoiding absorption of hydrophilic substances, and flexible sensor integration, such as TEER electrodes for real-time monitoring.

In summary, we present two modular, biocompatible BBB-on-chip platforms capable of supporting multicellular BBB cultures, real-time functional assessment, and drug screening. These tools provide a physiologically relevant alternative to animal models and represent a promising platform for investigating BBB dysfunction and CNS drug delivery.

A 3D brain microvasculature model to explore pericyte-mediated angiopoietin/Tie-2 axis alteration during cerebral malaria

Wednesday, 1st October - 17:30: (Auditorium 1) - Oral

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Introduction:

Cerebral malaria (CM) is a severe complication of *Plasmodium falciparum* malarial infection responsible for fatal blood-brain barrier (BBB) breakdown. CM pathology is associated with the cytoadhesion of *P. falciparum*-infected red blood cells (iRBCs) to the brain microvasculature, however, the mechanism behind BBB breakdown remains unknown. One host endothelial pathway of interest is the angiopoietin/Tie-2 axis, necessary for brain vasculature maturation and barrier strengthening. The angiopoietin/Tie-2 axis is composed of Tie-2, an endothelial receptor, and its corresponding ligands, barrier-protective angiopoietin-1 (Ang-1) and barrier-disruptive angiopoietin-2 (Ang-2). Decreased Ang-1, increased Ang-2 and an increased Ang-2:Ang-1 ratio in the serum are predictive markers of CM fatality. Yet, mouse models fail to fully recapitulate CM pathogenesis and current *in vitro* models lack the main source of Ang-1 secretion in the brain microvasculature, brain pericytes. Therefore, the causative factors behind angiopoietin/Tie-2 axis disruption and its significance in CM pathogenesis remain uncharacterized.

Methodology:

To investigate the mechanism of angiopoietin/Tie-2 axis dysregulation during CM, we fabricate a 3D microfluidics-based brain microvasculature model containing both human primary brain endothelial cells and pericytes, the minimal cellular components to replicate angiopoietin/Tie-2 axis signaling. By soft lithography and injection molding, we develop a 13x13 grid network of perfusable 100 µm microvessels that reproduce a wide range of physiological flow rates and wall shear stresses within a single device.

Results:

This model replicates *in vivo* brain pericyte coverage and wrapping of the endothelial vessels. Perfusion of purified iRBC egress products promote increased vascular permeability and altered pericyte ultrastructural morphology, as detected by serial block-face scanning electron microscopy. Examination of 3D microvessel supernatants by luminex assay revealed a significant decrease in Ang-1 secretion, but no changes in Ang-2 secretion, indicating that iRBC egress products directly disrupt pericyte Ang-1 secretion. Rescue by recombinant Ang-1 or AKB-9778, a therapeutic activator of Tie-2, pre-incubation partially protected against barrier breakdown, suggesting a functional role of decreased Ang-1 secretion in disease pathology.

Conclusions:

Overall, this study utilizes a 3D *in vitro* brain microvasculature model to highlight brain pericytes as novel players and therapeutic targets in angiopoietin/Tie-2 axis dysregulation during CM pathogenesis.

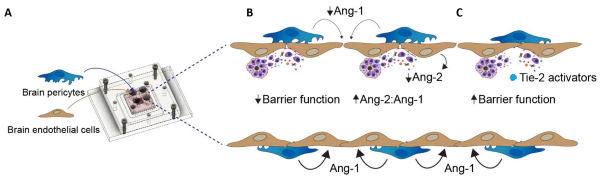

Figure 1. A) 3D brain microvasculature model fabrication. B) Disruption of brain pericyte angiopoietin-1 secretion and microvascular barrier function due to malaria egress products. C) Restoration of the angiopoietin/Tie-2 axis partially rescues barrier integrity, highlighting pericyte dysregulation as a contributor in cerebral malaria pathogenesis.

B4 2025 abstract image.jpg

A fully iPSC-derived 3D model of the human blood-brain barrier for exploring neurovascular disease mechanisms and therapeutic interventions

Wednesday, 1st October - 17:45: (Auditorium 1) - Oral

Dr. Judit Gonzalez-Gallego [1], Dr. Katalin Völgyi [1], Dr. Stephan Müller [2], Ms. Sophie Antesberger [3], Dr. Mihail Todorov [4], Dr. Rainer Malik [3], Ms. Rita Grimalt-Mirada [3], Ms. Carolina Cardoso Gonçalves [5], Dr. Martina Schifferer [6], Mr. Georg Kislinger [7], Dr. Isabell Weisheit [5], Ms. Barbara Lindner [5], Mr. Dennis Crusius [5], Dr. Joseph Kroeger [5], Dr. Mil Borri [5], Prof. Ali Ertürk [8], Prof. Mark Nelson [9], Prof. Thomas Misgeld [6], Prof. Stefan Lichtenthaler [2], Prof. Martin Dichgans [3], Prof. Dominik Paquet [10]

1. *Institute for Stroke and Dementia Research (ISD) University Hospital LMU Munich, Munich, Germany,* ***2.*** *German Center for Neurodegenerative Diseases (DZNE) Munich, Germany,* ***3.*** *Institute for Stroke and Dementia Research (ISD) University Hospital, LMU Munich, Munich, Germany,* ***4.*** *Institute for Stroke and Dementia Research (ISD) University Hospital LMU Munich, Munich, Germany, Institute for Tissue Engineering and Regenerative Medicine (iTERM), Helmholtz Zentrum München, Neuherberg, Germany,* ***5.*** *Institute for Stroke and Dementia Research (ISD), University Hospital, LMU Munich, Munich, Germany,* ***6.*** *German Center for Neurodegenerative Diseases (DZNE) Munich, Munich, Germany,* ***7.*** *Institute of Neuronal Cell Biology, Technical University of Munich, Munich, Germany,* ***8.*** *Institute for Stroke and Dementia Research (ISD) University Hospital LMU Munich, Germany, Institute for Tissue Engineering and Regenerative Medicine (iTERM), Helmholtz Zentrum München, Neuherberg, Germany,* ***9.*** *Department of Pharmacology, University of Vermont, Burlington, Vermont,* ***10.*** *Institute for Stroke and Dementia Research (ISD), University Hospital LMU Munich, Munich, Germany*

Integrity of the blood-brain barrier (BBB) is critical for brain homeostasis, and its malfunction contributes to neurovascular and neurodegenerative disorders. So far, mechanistic studies on BBB function have been mostly conducted in rodent and non-physiological *in vitro* models, which recapitulate some disease features, but have limited translatability to humans and pose challenges for drug discovery. Here we report on a fully human iPSC-derived, microfluidic 3D BBB model consisting of endothelial cells (EC), mural cells, and astrocytes. Our model expresses typical cell fate markers, forms a barrier in vessel-like tubes, and enables perfusion, including with human blood. We optimized iPSC differentiations and validated cellular fates by comparison to published datasets and extensive benchmarking vs. primary cells with proteomic profiles provided in an online database. To demonstrate suitability for translational research, we applied the model to investigate deficiency of FOXF2, a major risk gene for cerebral small vessel disease. Deletion of *FOXF2* in EC induced key features of BBB dysfunction, including compromised cell junction integrity and enhanced caveolae formation. Proteomic analysis further revealed dysregulation of endocytosis and cell junction pathways. Disease features phenocopied those seen in mice with endothelial cell-specific Foxf2 deficiency, validating the relevance of our *in vitro* model to investigate *in vivo* phenotypes of neurovascular disease. Moreover, lipid-nanoparticle-based treatment with *Foxf2* mRNA rescued BBB deficits in our *FOXF2* KO model, demonstrating its potential for drug development.

A fully human pluripotent stem cell-derived blood-brain barrier model advances treatment strategies for NMDAR autoimmune encephalitis and validates the therapeutic potential of neurotropic adeno-associated viruses

Wednesday, 1st October - 17:15: (Auditorium 2) - Poster

Dr. Angelo Iannielli [1], ***Dr. Serena Giannelli*** [1], ***Dr. Mirko Luoni*** [1], ***Prof. Josep Canals*** [2], ***Dr. Vania Broccoli*** [1]

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The blood-brain barrier (BBB) is a highly functionalized vascular interface which regulates the exchange of substances between the neural parenchyma and its periphery. BBB leakage, leading to its uncontrolled permeability, is increasingly recognized to facilitate the onset of neuropathologies and aggravate their clinical progression. In vitro models of the BBB have rapidly evolved into elaborated structures that mimic its spatial architecture and multicellular nature. However, their cellular components are currently highly heterogeneous in origin and maturation state. Here, we have developed novel procedures to establish reproducible and scalable sources of endothelial, mural and astroglial cells generating a fully human pluripotent stem cell (hPSC)-derived BBB model, termed thBBBA. hPSC-derived BBB cell types are readily assembled into thBBBAs that develop mature functional properties with high barrier impermeability. Mature thBBBAs can also be generated by frozen hPSC-derived cell samples, providing a simple and scalable off-the-shelf system for general use. thBBBAs were instrumental in identifying the critical pathological role of an IL-6 autocrine source in disrupting thBBBA integrity, increasing its permeability to NMDAR antibodies from autoimmune encephalitis patients, and revealing the therapeutic effects of tocilizumab in this disease. Additionally, we have shown that thBBBAs are an invaluable system for evaluating the clinical potential of novel engineered AAV neurotropic capsids, previously selected in animal models or in vitro systems.

The contribution of leukocytes to cerebral malaria vascular pathogenesis in a 3D blood-brain barrier model

Wednesday, 1st October - 17:30: (Auditorium 2) - Oral

Mrs. Alina Batzilla [1], ***Mr. Fumio Nakaki*** [1], ***Dr. Livia Piatti*** [1], ***Mr. Daniel Schraivogel*** [2], ***Dr. Silvia Sanz Sender*** [1], ***Mrs. Pia Dernick*** [1], ***Mrs. Mireia Altadill Balsells*** [3], ***Mrs. Hannah Fleckenstein*** [1], ***Mrs. Jon Bezney*** [4], ***Mr. Andreas Gschwind*** [4], ***Mr. Miguel Lopez Botet*** [3], ***Mrs. Lars Steinmetz*** [2], ***Mr. James Sharpe*** [1], ***Mrs. Gemma Moncunill*** [5], ***Dr. Maria Bernabeu*** [1]

1. *European Molecular Biology Laboratory (EMBL, Barcelona),* ***2.*** *European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Heidelberg,* ***3.*** *Pompeu Fabra University, Department of Medicine and Life Sciences, Barcelona,* ***4.*** *Department of Genetics, Stanford University School of Medicine, Stanford,* ***5.*** *ISGlobal, Barcelona*

Cerebral Malaria (CM) is a severe neurovascular complication of *Plasmodium falciparum* infections, in children characterized by brain swelling due to blood-brain barrier (BBB) disruption. Postmortem samples provide evidence of immune cells accumulating in the microvasculature of CM patients, suggesting a role of proinflammatory immune response in the pathogenesis of the disease. However, it remains unclear whether leukocyte activation and accumulation are primary contributors to BBB breakdown or merely a consequence of BBB damage. To investigate the dynamics and consequences of leukocyte interactions with the BBB, we stimulated peripheral blood mononuclear cells from healthy European/US donors with *P. falciparum* (*Pf*-PBMC) and perfused them through an engineered 3D-BBB model composed of primary human endothelial cells, pericytes, and astrocytes. Single-cell RNA sequencing (scRNAseq) analysis confirmed the activation of T-cells, monocytes and NK cells and revealed an increased binding of *Pf*-PBMC to resting brain endothelial cells. This increased binding was mostly driven by CD8 and innate T-cells and associated with activation and conformational change of the binding receptor LFA1, quantified by FACS. The binding of *Pf*-PBMC led to an increased expression of inflammatory transcripts in all the cells that compose the BBB model, validated by NFκB nuclear translocation and ICAM1 upregulation in immunofluorescence assays. Furthermore, scRNAseq and immunofluorescence results showed a dysregulation of endothelial processes suggestive of barrier disruption, including cytoskeletal rearrangement, adherens junction disruption, and apoptosis. Permeability measurements using both 2D real-time impedance assays and fluorescent dextran perfusion in the 3D-BBB model confirmed that *Pf*-PBMC cause vascular disruption resulting in an increased permeability. Blocking of endothelial-leukocyte interactions with an anti-ICAM1 monoclonal antibody resulted in decreased vascular barrier disruption by *Pf*-PBMC in the 3D-BBB model. Taken together, our results showed that leukocytes, upon *P. falciparum*-stimulation, cause vascular disruption in a 3D-BBB model. This disruption occurred in absence of *P. falciparum*-infected red blood cell sequestration and could be inhibited by blocking leukocyte binding, suggesting that an activated innate immune response is sufficient to elicit BBB disruption.

Investigating the role of human miRNAs in the disruption of blood-brain barrier integrity during Plasmodium falciparum infection

Wednesday, 1st October - 17:45: (Auditorium 2) - Oral

Dr. Nahla Metwally [1], ***Mrs. Marlena Kemper*** [1], ***Mr. Viktor Michaelis*** [1], ***Ms. Pilar Martinez Tauler*** [1], ***Ms. Nadja Goetz*** [1], ***Prof. Iris Bruchhaus*** [1]

1. *Bernhard-Nocht-Institute for tropical medicine*

Plasmodium falciparum (*P. falciparum*), the malaria parasite species associated with the highest death rate, is known to cause severe complications that seem to differ within the age range of patients. Pregnant women and children under 5 years of age are the most likely to suffer from severe malaria, with the latter accounting for 67% of all malaria deaths worldwide. Cerebral malaria, most common in children aged 2-16, leads to a dramatic loss of blood-brain barrier (BBB) integrity, which can cause brain edema, hypoxia, and an elevation in intracranial pressure, ultimately leading to coma and death.

It is unknown whether microRNAs play a specific role in the complications of severe malaria, although microRNAs control 60% of the genes expressed in the human body. This project focuses on unraveling the alteration in miRNA profiles under P. falciparum infection in a dynamic in vitro model of the human BBB.

Therapeutic miRNA is an emerging medical field. This study investigates miRNAs' roles in *P. falciparum* infection complications and aims to identify strategies to reverse miRNA levels in severe malaria. Using miRNAs as a therapeutic tool offers two advantages: they are natural molecules processed by the body's systems, and they target multiple genes in a pathway, ensuring a broad yet specific response.

In our data, we reported microRNA candidates expressed explicitly in brain ECs, and their secreted extracellular vesicles (EVs). We showed that shear stress plays a role in switching variable signaling pathways in brain ECs, such as IL-8 signaling and tight junctions. Specific miRNAs we found differentially expressed in the ECs seem to control these pathways. Incubation with ring-stage infected red blood cells (iRBCs) results in activation of endocytic pathways in brain ECs. These miRNAs were significantly altered after 8 hours of coincubation with ring-stage iRBCs at a shear stress of 1.5 dyne/cm2.

Comparative proteomics of brain parenchyma and vasculature in mouse and human ageing

Wednesday, 1st October - 17:15: (Sala Blava 2) - Oral

Ms. Austeja Ciulkinyte [1], ***Dr. Steven Hill*** [1], ***Dr. Bethany Geary*** [2], ***Dr. Blanca Díaz Castro*** [1]

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Ageing is the strongest risk factor for various neurodegenerative diseases. In particular, the brain vasculature plays an important role during pre-clinical stages of many neurodegenerative conditions, such as Alzheimer's disease and vascular dementia. Despite numerous efforts, the exact molecular contributions of ageing towards the manifestation of neurodegenerative disease remain incompletely understood.

Mouse models are widely used in neurodegeneration and ageing research. However, to ensure the translational potential of mouse models is maximised, there is a need to identify what aspects of ageing are shared between mouse and human. Previous attempts to evaluate the extent of molecular similarities in mouse and human ageing have returned conflicting results. Additionally, no direct species comparisons of the ageing brain at the proteome level have been performed.

To address this, we have performed a proteomic analysis of cortical brain tissue and isolated vasculature from young and aged mouse and human samples (Figure 1a). We identified proteins whose expression changes significantly with advanced age in both species (Figure 1b-e) and asked which molecular functions they participate in (Figure 1f). This data allows us to compare cross-species similarity at both individual protein and functional pathway levels.

Next, we correlated the direction of change of individual proteins (not shown) and protein pathways (Figure 1g) to study how well ageing effects are conserved across species in cortical brain tissue and brain vasculature. Additionally, we also correlated proteomic changes between cortical brain tissue and brain vasculature within the same species (not shown). This data enables the study of ageing effects in different tissue types from the same species, and in comparing the same tissue type from different species.

Overall, our findings are an important step towards understanding the translatability of using mouse models in ageing research. Preliminary findings suggest that although general functional pathways are similarly perturbed in mouse and human ageing, these may be driven by different mechanisms, which is an important consideration for future studies.

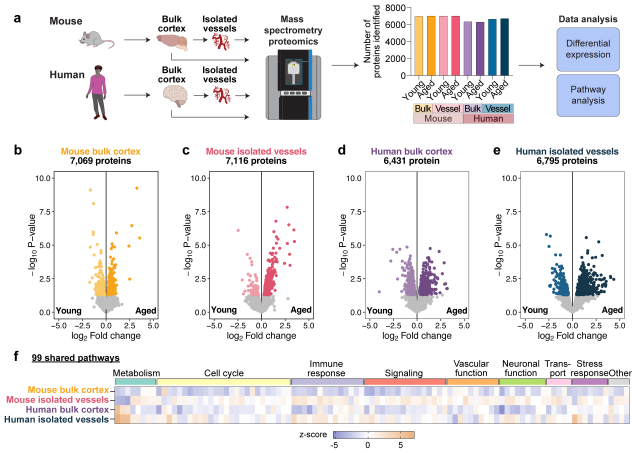

Figure1 ac comparative proteomics.png

Endocytic Turnover of Cell-Membrane Proteins as a Driver of Blood-Brain Barrier Specialization and Dysfunction

Wednesday, 1st October - 17:30: (Sala Blava 2) - Oral

Ms. Alba Tomas Sitjes [1], ***Dr. Gianluca Arauz Garofalo*** [2], ***Ms. Valentina Schastlivaia*** [1], ***Prof. Giuseppe Battaglia*** [3], ***Dr. Daniel Gonzalez Carter*** [3]

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Introduction: The blood-brain barrier (BBB) exerts essential functions for proper brain activity, such as nutrient transport, signal transduction, immune cell transmigration and pathogen restriction. While these functions are known to be regulated by the identity and abundance of cell-surface proteins present on brain endothelial cells (EC), a parameter which remains underexplored is how protein endocytic turnover rate (ETOR)—the rate of protein internalization from the cell membrane— governs BBB physiology.

Objectives: High-throughput proteomics were employed to quantify the cell-surface abundance of individual proteins in isolated primary rodent EC across time to generate ETOR maps for a large array of proteins. ETOR maps are compared between peripheral (liver and lung) and brain EC under healthy and inflammatory conditions to examine the role of ETOR on BBB specialization and dysfunction. Furthermore, leveraging published proteomic data of age-related protein changes *in vivo*, we examine correlations between ETOR and protein dynamics in aging brain endothelial cells. Finally, we incorporate protein features (including ETOR, molecular weight, cell localization, abundance dynamics) into multi-dimensional vector representations to generate mathematical predictive models of age-related protein changes at the BBB.

Results: Analysis was carried out on 1,496 proteins shared between all three phenotypes (liver, lung and brain EC). Protein abundance was strongly correlated both between peripheral EC (liver vs. lung, r^2 = 0.96), and between peripheral and brain EC (liver/lung vs. brain, r^2= 0.60/ 0.59, respectively). However, while ETOR was significantly correlated between peripheral EC (r^2 = 0.57), ETOR correlated poorly between peripheral and brain EC (liver/lung vs. brain, r^2= 0.14/ 0.07, respectively), despite similarities in cell-membrane composition (i.e. abundance) between all phenotypes. Furthermore, inflammation shifted the ETOR profile of BEC towards a peripheral EC profile. Interestingly, a strong correlation with *in vivo* protein aging dynamics was seen only for ETOR in BEC.

Discussion: These findings demonstrate that distinct endocytic dynamics of cell-membrane proteins drive the phenotypic specialization of the BBB and may contribute to inflammatory dysfunction and changes in protein abundance in the brain vasculature during aging. Hence, ETOR dysregulation may underly patho-physiological mechanisms of the BBB currently underappreciated.

Modulating Blood-brain barrier low-density lipoprotein receptor-related proteins (LRP) receptors using multivalent drugs.

Wednesday, 1st October - 17:45: (Sala Blava 2) - Oral

Mr. Marco Basile [1], ***Dr. Catia Lopes*** [2], ***Mr. Nicola Manicardi*** [1], ***Dr. Valentino Barbieri*** [1], ***Dr. Matilde Ghibaudi*** [1], ***Dr. Vanina Cosenza*** [1], ***Prof. Lorena Ruiz Pérez*** [1], ***Prof. Giuseppe Battaglia*** [2]

1. *Institute for Bioengineering of Catalonia (IBEC),* ***2.*** *Institute for Bioengineering of Catalonia & University of Barcelona*

Neurodegenerative diseases, such as Alzheimer's disease (AD), represent a major health challenge, with limited therapeutic options available primarily due to the difficulty of delivering treatments effectively across the blood-brain barrier (BBB). One hallmark of Alzheimer's disease (AD) pathology is the accumulation of amyloid-beta (Aβ) peptides in the brain parenchyma, driven in part by impaired BBB clearance mechanisms. The low-density lipoprotein receptor-related proteins (LRP) receptors, particularly LRP1 and LRP8, play crucial roles in the receptor-mediated transcytosis and clearance of Aβ peptides across the BBB. Hence, enhancing receptor-mediated transport represents a promising therapeutic strategy.

In this study, we utilized biodegradable and biocompatible poly(ethylene glycol)-poly(lactic acid) (PEG–PLA) micelles to investigate their potential in promoting brain barrier (BBB) transcytosis of Aβ peptides. We specifically explored the concept of multivalent super-selectivity, a phenomenon whereby nanoparticle avidity significantly enhances specific interactions with targeted receptors on brain endothelial cells (BECs). By carefully tuning the ligand density on PEG–PLA nanoparticles, we achieved precise control over nanoparticle avidity, which is crucial for selectively promoting transcytosis over endocytosis.

Binding assays conducted in vitro demonstrated distinct binding patterns based on ligand specificity, highlighting the importance of the ligand in nanoparticle-BEC interactions. Meanwhile, permeability assays identified formulations capable of efficient transcytosis. Gene and protein expression analyses further validated the potential therapeutic effect, revealing modulation of key BBB-associated biological markers. In parallel, our investigations into receptor dynamics demonstrated differential interactions of LRP1 and LRP8 with intracellular mediators Rab5 and PACSIN2 upon exposure to Aβ40 and Aβ42 assemblies. These findings revealed novel insights into receptor-specific clearance pathways, identifying LRP8 as a promising new target for Aβ clearance.

Overall, our study demonstrates the significant potential of multivalently functionalised PEG–PLA nanoparticles to selectively enhance receptor-mediated transcytosis at the BBB. This strategy not only improves our understanding of transcytotic mechanisms but also provides a robust framework for developing effective nanotherapeutics aimed at mitigating neurodegeneration in Alzheimer's disease and other related conditions.

First Metabolomic Signature of Blood-Brain Barrier Opening Induced by Microbubble-Assisted Ultrasound

Wednesday, 1st October - 18:05: (Auditorium 1) - Oral

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Microbubble (MB)-assisted ultrasound (US) is a promising physical method to increase non-invasively, transiently, and precisely the permeability of the blood-brain barrier (BBB) to therapeutic molecules. Previous preclinical studies established the innocuity of this procedure using complementary analytical strategies including transcriptomics, histology, brain imaging, and behavioral tests. This cross-sectional study using rats aimed to investigate the metabolic processes following acoustically-mediated BBB opening in vivo using multimodal and multimatrices metabolomics approaches. After intravenous injection of MBs, the right striata were exposed to 1-MHz sinusoidal US waves at 0.6 MPa peak negative pressure with a burst length of 10 ms, for 30 s.
Then, the striata, cerebrospinal fluid (CSF), blood serum, and urine were collected during sacrifice in three experimental groups at 3 h, 2 days, and 1 week after BBB opening (BBBO) and were compared to a control group where no US was applied.
A well-established analytical workflow using nuclear magnetic resonance spectrometry and non-targeted and targeted high-performance liquid chromatography coupled to mass spectrometry were performed on biological tissues and fluids. In our experimental conditions, a reversible BBBO was observed in the striatum without physical damage or a change in rodent weight and behavior. Cerebral, peri-cerebral, and peripheral metabolomes displayed specific and sequential metabolic kinetics. The blood serum metabolome was more impacted in terms of the number of perturbated metabolisms than in the CSF, the striatum, and the urine. In addition, perturbations of arginine and arginine-related metabolisms were detected in all matrices after BBBO, suggesting activation of vasomotor processes and bioenergetic supply. The exploration of the tryptophan metabolism revealed a transient vascular inflammation and a perturbation of serotoninergic neurotransmission in the striatum. For the first time, we characterized the metabolic signature following the acoustically-mediated BBBO within the striatum and its surrounding biological compartments.

Editing a gateway across the BBB for endovascular delivery of neural progenitor cells after stroke

Wednesday, 1st October - 18:20: (Auditorium 1) - Oral

Ms. Beatriz Achón Buil [1], ***Ms. Nora Hanna Rentsch*** [1], ***Dr. Rebecca Zoe Weber*** [1], ***Ms. Chantal Nicole Bodenmann*** [1], ***Ms. Kathrin Jasmina Zürcher*** [1], ***Dr. Ruslan Rust*** [2], ***Dr. Christian Tackenberg*** [1]

1. *University of Zurich,* ***2.*** *University of Southern California*

Stem cell-based therapy has emerged as a promising strategy for treating stroke, but it still presents several challenges, such as the optimal route of administration. In a clinical setting, endovascular delivery of cells is preferred over intracerebral injection. However, stem cells must cross the blood-brain barrier (BBB) and might get trapped in peripheral organs, resulting in an insufficient number of cells reaching the lesion. To optimize their delivery, we aimed to mimic immune cells as they extravasate into the brain parenchyma under pathological conditions, including stroke. For instance, since the chemokine CXCL12 is released after stroke, we transduced human iPSC-derived neural progenitor cells (NPCs) to overexpress its receptor CXCR4 (present in leukocytes). Transduced NPCs migrated more efficiently than wild-type NPCs towards 10nM CXCL12 in a Boyden chamber assay. Although this is a promising result, it overlooks the complexity of the extravasation process across the BBB. Therefore, we carried out a CRISPR activation screen to determine which molecules enhance NPC migration towards the stroke lesion. NPCs were transduced with a catalytically inactive Cas9 fused to a transcriptional activator, along with specific guide RNAs (gRNAs) to upregulate 40 genes involved in the extravasation process. We validated the system *in vitro* via Western Blot, Boyden chamber assay, and cell-based ELISA. NPCs expressing the gRNA library were injected intra-arterially in mice on days 3 or 7 following photothrombotic stroke induction. One day post-injection, NPCs were isolated from the stroke region and processed for downstream sequencing. We further plan to combine top candidates into the same plasmid to enhance the delivery of NPCs to the ischemic lesion. We will track the distribution of NPCs via bioluminescence imaging and analyse the behaviour and motor recovery of mice subjected to experimental stroke. The generation and preclinical validation of NPCs with improved brain-homing ability will advance stem cell-based therapy for restoring lost functions after stroke.

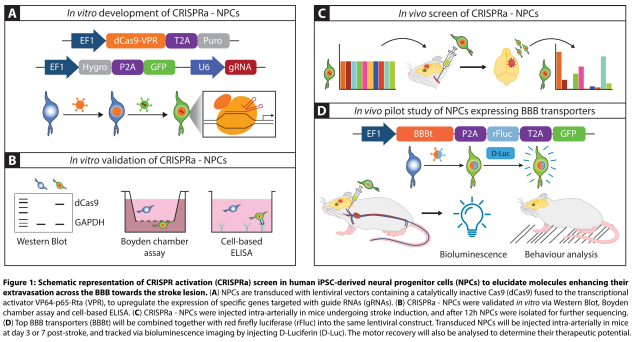


Figure 1: Schematic representation of CRISPR activation (CRISPRa) screen in human iPSC-derived neural progenitor cells (NPCs) to elucidate molecules enhancing their extravasation across the BBB towards the stroke lesion. (**A**) NPCs are transduced with lentiviral vectors containing a catalytically inactive Cas9 (dCas9) fused to the transcriptional activator VP64-p65-Rta (VPR), to upregulate the expression of specific genes targeted with guide RNAs (gRNAs). (**B**) CRISPRa - NPCs were validated *in vitro* via Western Blot, Boyden chamber assay and cell-based ELISA. (**C**) CRISPRa - NPCs were injected intra-arterially in mice undergoing stroke induction, and after 12h NPCs were isolated for further sequencing. (**D**) Top BBB transporters (BBBt) will be combined together with red firefly luciferase (rFluc) into the same lentiviral construct. Transduced NPCs will be injected intra-arterially in mice at day 3 or 7 post-stroke, and tracked via bioluminescence imaging by injecting D-Luciferin (D-Luc). The motor recovery will also be analysed to determine their therapeutic potential.

Crispra for npc migration across bbb post-stroke.jpg

ETS-factor guided iPSC-endothelial model recapitulates hallmarks of Cerebral Malaria pathogenesis

Wednesday, 1st October - 18:05: (Auditorium 2) - Oral

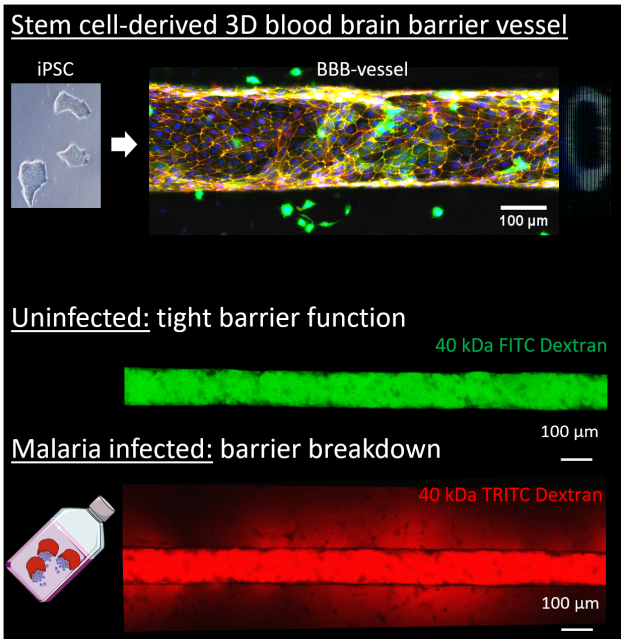
Dr. Francois Korbmacher [1], ***Dr. Hannah Fleckenstein*** [2], ***Dr. Rory Long*** [2], ***Dr. Livia Piatti*** [2], ***Dr. Silvia Sanz Sender*** [2], ***Dr. Borja Lopez Gutierrez*** [2], ***Dr. Mitsuhiro Matsuda*** [3], ***Dr. Miki Ebisuya*** [3], ***Dr. Maria Bernabeu*** [2]

1. *EMBL Barcelona,* ***2.*** *European Molecular Biology Laboratory (EMBL, Barcelona),* ***3.*** *Cluster of Excellence Physics of Life, TU Dresden*

During malaria infection, interactions between *Plasmodium falciparum* parasites and the brain endothelium can disrupt the blood-brain barrier (BBB) and cause brain swelling, hallmarks of cerebral malaria (CM), the most severe form of the disease. However, the pathophysiological mechanisms underlying parasite-vessels interaction in the brain remain poorly understood. Recent advancements in more physiological 3D *in vitro* brain endothelium models, largely driven by the use of induced pluripotent stem cells (iPSCs), have significantly improved our ability to study disease mechanisms and test potential interventions. Despite these advances, many iPSC-derived models remain immature or exhibit conflicting epithelial gene expression profiles that do not fully align with native brain endothelium, potentially limiting their utility in infection studies.

Recognizing the critical role of ETS transcription factors in endothelial identity, we developed an iPSC line with inducible expression of three ETS transcription factors (ETV2, FLI1, and ERG) to generate endothelial cells (ECs) with physiological blood-brain barrier function for malaria infection studies. Comprehensive benchmarking demonstrates that these cells exhibit significantly improved endothelial characteristics compared to conventional iPSC-derived ECs, closely mirroring brain endothelial cell properties at transcriptional, ultrastructural, and functional levels. By successfully recapitulating key pathogenic events of CM *in vitro*—such as elevated parasite binding and accumulation to endothelial cells and malaria-induced barrier dysfunction—this model enables to identify the mechanisms contributing to CM. During malaria-induced progressive barrier dysfunction, RNA-Seq analysis reveals gradual downregulation in gene expression related to tight and adherence junction formation as well as other cell structural processes, shedding light on the mechanisms underlying BBB-breakdown.

This iPSC-derived model thus represents a novel *in vitro* platform for CM infection, with enhanced barrier properties that can be leveraged for therapeutic development and personalized bio-medical research.



250425 fig1.png

Brain endothelial cells exhibit increased activation of β-catenin and decreased transcription of the Wnt/β-catenin pathway inhibitors Nkd1, Axin2, and Apcdd1 in a mouse model of Plasmodium induced blood brain barrier disruption

Wednesday, 1st October - 18:20: (Auditorium 2) - Oral

Mr. Marshall Roedel [1], ***Ms. Nia Brooks*** [1], ***Mr. Jared Andersen*** [1], ***Dr. Tracey Lamb*** [1]

1. *University of Utah*

Malaria is a serious global disease, as in 2023 there were 263 million cases and 597 thousand deaths globally. One of the most common causes of death from malaria is cerebral malaria (CM), a severe malaria outcome that entails coma, vasogenic brain edema, and death in 15-20% of cases that are treated with antimalarials. Experimental cerebral malaria (ECM) is a mouse model of CM in which C57BL/6J mice are infected with the rodent specific Plasmodium berghei ANKA (PbA) and subsequently exhibit accumulation of parasites in the brain vasculature, brain edema, downregulation of endothelial tight junction proteins, and death within six-ten days after the infection. In ECM the infiltration of cytotoxic CD8+ T-lymphocytes into the brain is necessary for death and brain edema, as is the expression of MHC class I molecules on brain endothelial cells. Furthermore, mice with dysfunctional perforin or granzyme B are completely protected from ECM. While this suggests that the blood brain barrier(BBB) in ECM may be disrupted through the killing of brain endothelial cells that are presenting Plasmodium peptide by CD8+ cytotoxic T-lymphocytes, previous studies have shown that there is very little death of brain endothelial cells in infected mice, while the downregulation of tight-junction proteins is more prominent. β-catenin activity has been shown to control the expression of tight-junction protein in brain endothelial cells, and we used immunofluorescence to investigate its activity in endothelial cells in brain sections in mice with ECM to determine its contribution to tight-junction dysregulation. We found that infected mice had increased activation of β-catenin in brain endothelial cells compared to naive (Fig 1). While β-catenin activity is usually seen to promote BBB integrity, in some contexts β-catenin localization to the nucleus results in BBB disruption. Single-cell RNA sequencing of brain endothelial cells with ECM shows a downregulation of transcription of the Wnt/β-catenin inhibitors Nkd1, Axin2, and Apcdd1 (Fig 2). These data suggest that reduced expression of β-catenin inhibitors may induce the localization of β-catenin localization to the nucleus and BBB dysfunction.

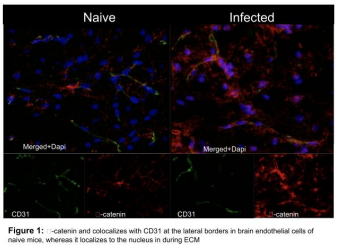


Figure 1: -catenin and colocalizes with CD31 at the lateral borders in brain endothelial cells of naive mice, whereas it localizes to the nucleus in during ECM

Figure 1.jpg

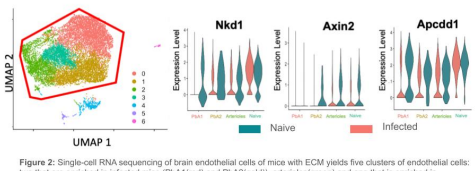


Figure 2: Single-cell RNA sequencing of brain endothelial cells of mice with ECM yields five clusters of endothelial cells: two that are enriched in infected mice (PbA1(red) and PbA2(gold)), arterioles(green) and one that is enriched in uninfected mice (Naive). Almost all endothelial cell cluster showed a decrease expression of the Wnt/ beta-catenin inhibitors *Nkd1*, *Axin2*, and *Apcdd1*.

Figure 2.jpg

Blood-Brain Barrier Integrity as a Critical Mediator of Neuroprogression in Psychiatric Disorders

Wednesday, 1st October - 18:05: (Sala Blava 2) - Oral

Dr. Gerry (Gerasimos) Konstantinou [1], ***Prof. Jerry Warsh*** [2]

1. *1. University of Toronto,* ***2.*** *University of Toronto*

Neuroprogression, the pathological trajectory of psychiatric disorders involving progressive structural and functional brain deterioration, has increasingly been recognized as a defining feature of conditions such as schizophrenia (SZ), bipolar disorder (BD), and major depressive disorder (MDD). While multifactorial in origin, growing evidence implicates blood-brain barrier (BBB) dysfunction as a critical nexus linking systemic stressors to central pathophysiology.

This review integrates evidence from molecular, imaging, genetic, and translational domains to position BBB breakdown not merely as an epiphenomenon but as a driver of psychiatric illness progression. The BBB, comprising endothelial cells, astrocytic end-feet, pericytes, and tight junction proteins (e.g., claudin-5, occludin), forms a dynamic neurovascular unit (NVU) essential for cerebral homeostasis. Disruption, mediated by oxidative stress, inflammatory cytokines, metabolic derangements, and genetic variants, increases permeability and neuroinflammation, affecting neuronal-glial function, synaptic plasticity, and neuronal resilience, promoting neuroprogressive cascades.

Clinical studies reveal elevated serum biomarkers of BBB dysfunction (e.g., claudin-5, occludin), particularly in BD and SZ, alongside reduced expression of tight junction genes in post-mortem tissue. Advanced neuroimaging (e.g., dynamic contrast-enhanced MRI, arterial spin labeling) identifies regional BBB leakage correlating with symptom severity and illness duration. Notably, a subpopulation of BD patients with insulin resistance demonstrates heightened BBB permeability, suggesting a metabolic-neurovascular link.

Therapeutically, agents such as lithium, metformin, VEGF/MMP inhibitors, and HDAC1 modulators show potential in restoring BBB integrity and attenuating neuroprogression. Gut-brain-microbiota axis modulation and antioxidant strategies also represent novel interventional directions. Furthermore, transcriptomic profiling of induced pluripotent stem cell (iPSC)-derived brain microvascular endothelial cells suggests that BBB dysfunction may arise early in development, offering new insights into disease risk and prevention.

Altogether, these findings suggest that BBB dysfunction is both a marker and a mechanism of neuroprogression in psychiatric disorders. Viewing the BBB as a dynamic, targetable interface redefines therapeutic possibilities and provides a promising avenue for precision psychiatry to alter disease course, enhance treatment response, and improve long-term outcomes.

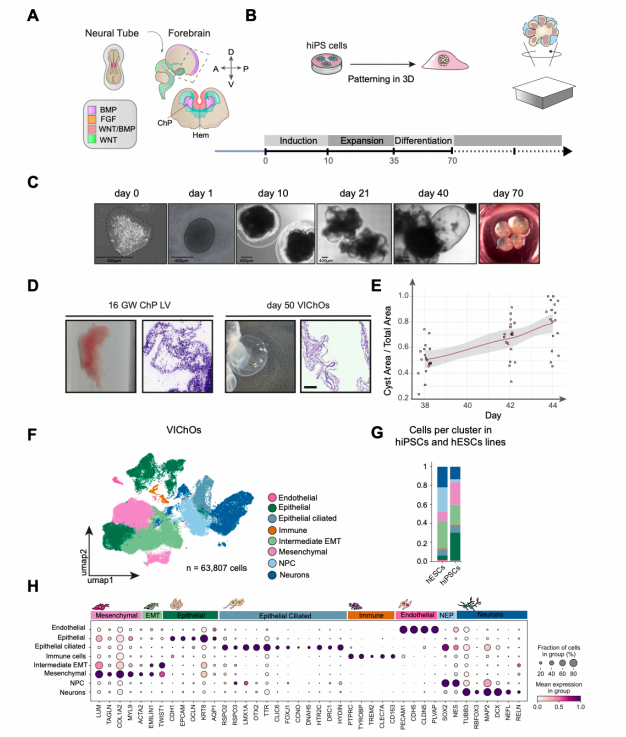
Unlocking the Guardian of the Brain: Choroid Plexus and Neurodevelopmental Insights

Wednesday, 1st October - 18:20: (Sala Blava 2) - Oral

Dr. Vanessa Aragona [1], ***Mr. Illia Simutin*** [1], ***Dr. Sara Mancinelli*** [1], ***Mr. Matteo Miotto*** [2], ***Dr. Giuseppe Martano*** [2], ***Prof. Katia Cortese*** [3], ***Prof. Simona Lodato*** [1], ***Dr. Maura Galimberti*** [4], ***Prof. Elena Cattaneo*** [5]

1. *Humanitas University, Via Rita Levi Montalcini 4, Pieve Emanuele, Milan, Italy,* ***2.*** *Neuro Center, IRCCS Humanitas Clinical and Research Center, Via Manzoni 56, Rozzano-Milan,* ***3.*** *Cellular Electron Microscopy Laboratory, DIMES, Department of Experimental Medicine, Human Anatomy, School of Medical and Pharmacological Sciences, University of Genoa, Via Antonio de Toni 14, 16132, Genoa, Italy,* ***4.*** *Laboratory of Stem Cell Biology and Pharmacology of Neurodegenerative Diseases, Department of Biosciences, University of Milan, 20122, Milan, Italy.,* ***5.*** *Laboratory of Stem Cell Biology and Pharmacology of Neurodegenerative Diseases, Department of Biosciences, University of Milan, 20122, Milan, Italy*

The choroid plexus (ChP), a vascularized structure, plays a pivotal role in cerebrospinal fluid (CSF) production and brain homeostasis by regulating ion transport, nutrient delivery, and waste clearance. Beyond these functions, the ChP acts as a sensor and modulator of environmental signals, influencing brain development and contributing to the pathophysiology of neurodevelopmental disorders. Growing evidence implicates inflammation and immune dysregulation in childhood neuropsychiatric conditions such as schizophrenia and autism, highlighting the ChP as a key amplifier of these pathological processes. Given its anatomical proximity to the subventricular zone, deciphering the mechanisms of ChP function is crucial for understanding brain maturation and exploring its potential as a therapeutic target. Animal models incompletely model human physiology, while *in vitro* systems lack key components to replicate ChP complexity. To address this, we developed VIChOs (Vascular Immune ChP Organoids) as a novel 3D human ChP model capturing the cellular heterogeneity of the ChP, extending beyond its well-known epithelial barrier function. VIChOs faithfully replicate the histological and ultrastructural features of native ChP tissue. Using single-cell RNA sequencing, we longitudinally characterized the diverse cell-type composition of VIChOs, revealing a dynamic cellular landscape regulated by subtype-specific signaling pathways. The development of VIChOs into tissue with neural, endothelial, and immune cell populations is accompanied by the dynamic secretion of a CSF-like fluid, which contains actively synthesized neurotrophic and signaling molecules. By leveraging the inherent heterogeneity of VIChOs, we conducted hypothesis-driven perturbations to dissect how microenvironmental cues influence ChP cytoarchitecture and secretory profiles. These experiments unveiled robust, context-dependent shifts in cellular organization and secretome composition, underscoring the utility of VIChOs as a platform for mechanistic studies.
Our work establishes VIChOs as a novel human ChP model that captures the tissue's full complexity. It provides a scalable, physiologically relevant system for exploring ChP biology in health, disease, and therapeutic discovery.



A) Schematic of morphogen expression in the developing human fetal nervous system. **B)** VIChOs differentiation protocol. **C)** Representative brightfield images of each protocol stage. **D)** Human fetal ChP (Nissl stained from BrainSpan Atlas of the Developing Human Brain) and H&E-stained sections of VIChOs epithelium. Scale bar, 50 µm. **E)** Smooth plot illustrating the trendline of the cyst-to-tissue area ratio throughout VIChOs development. Each dot represents an individual organoid. Statistical significance was determined using an Unpaired t-test. A significant difference was observed between day38 and day42 (p=0.0006, ***); day38 and day44 (p<0.0001, ****); day42 and day44 (p=0.04, *). **F)** UMAP of 63,807 integrated cells from VIChOs at different timepoints and derived from PGP GM23338 and H9 lines. **G)** Graph showing the percentage of cell types in the two hiPS and hES cell lines used to generate VIChOs. **H)** The dot plot shows representative selected markers used to annotate each cellular population.

Figure1.png

Regulation of the Blood-Brain Barrier in Health and Disease

Thursday, 2nd October - 09:00: (Auditorium 1) - Keynote speakers

Dr. Richard Daneman [1]

1. *University of California, San Diego*

TBC

Cerebrovascular Dynamics in Dementia: Exploring Dysfunctional Brain Cell Interactions

Thursday, 2nd October - 10:00: (Auditorium 1) - Invited Speaker

Dr. Axel Montagne [1]

1. *1. UK Dementia Research Institute, The University of Edinburgh, UK; 2. Institute for Neuroscience and Cardiovascular Research (INCR), The University of Edinburgh, UK; 3. BHF-UK DRI Centre for Vascular Dementia Research*

Cerebral small vessel disease (SVD) is a leading contributor to dementia and stroke, with pathology centred on the brain's microvasculature. Increasing evidence highlights the role of endothelial activation, pericyte dysfunction, blood–brain barrier (BBB) dysfunction, and inflammatory responses in driving disease onset and progression. Importantly, many of these vascular mechanisms are also implicated in Alzheimer's disease (AD), pointing to overlapping pathways of neurovascular dysfunction across dementias.

In our lab, we combine translational approaches across human and preclinical models to investigate these mechanisms. Using advanced imaging, we assessed age-related and disease-associated changes in pericyte and endothelial function in both mouse and human brain tissue. Circulating vascular biomarkers were evaluated in patient cohorts and correlated with MRI features of SVD. Complementary experimental models in mice, including endothelial- and pericyte-targeted manipulations, were used to probe causal relationships between vascular dysfunction, BBB integrity, and microglial responses.

Together, these investigations reveal the importance of endothelial–pericyte crosstalk in vascular vulnerability and highlight conserved mechanisms across species. This integrated strategy advances our understanding of SVD pathophysiology and its overlap with AD, identifying potential avenues for biomarker development and therapeutic intervention across neurodegenerative diseases.

Transport mechanisms of CD98hc in human brain endothelial cells

Thursday, 2nd October - 11:10: (Auditorium 1) - Oral

Dr. F. Sila Rizalar [1], ***Dr. Roberto Villaseñor Solorio*** [1]

1. *Roche Pharma Research and Early Development (pRED), Neuroscience and Rare Diseases (NRD), Roche Innovation Center Basel*

Targeting receptor-mediated transcytosis (RMT) receptors for crossing the blood-brain barrier (BBB) represents an emerging and clinically validated strategy to increase the brain permeability of biologics. The use of the transferrin receptor (TfR) to deliver antibodies to the brain is well- characterized and currently under evaluation in clinical trials. Recent studies have identified CD98hc as a promising target for antibody-driven RMT across the BBB with highly differentiated pharmacokinetics (Chew et al., 2023; Pornnoppadol et al., 2023; Zuchero et al., 2016). CD98hc is a type II single-pass transmembrane protein that constitutes the heavy chain of the heterodimeric amino acid transporter LAT-1 (Newstead, 2019). It also binds integrin-β chains and regulates downstream signaling pathways controlling cell spreading, survival and growth (Feral et al., 2005). The mechanisms that enable CD98hc to cross the BBB remain an outstanding question in the field.

Using a novel *in vitro* BBB model derived from human induced pluripotent stem cells (hiPSCs) (Bell et al., bioRxiv) we investigated the transport of CD98hc to identify the pathway(s) for internalization and transcytosis across the BBB. We used antibodies targeting CD98hc or TfR, and demonstrated that internalization dynamics of the two proteins differ. Next, we generated CRISPR/Cas9 knock-in hiPSC lines expressing fluorescently labeled endogenous CD98hc. Live- and fixed-cell imaging of brain endothelial cells differentiated from these labelled iPSCs revealed that endogenous CD98hc follows a distinct intracellular transport route compared to TfR. We will present data on how these two proteins are trafficked.

Overall, these findings uncover novel intracellular trafficking mechanisms of CD98hc, providing critical insights that could advance the development of more efficient and selective strategies for delivering therapeutics across the BBB.

Optimizing brain delivery of the neuroprotective peptide NR2B9c via D-amino acid stabilization and transferrin receptor targeting

Thursday, 2nd October - 11:25: (Auditorium 1) - Oral

Ms. Maria Thaysen [1], Dr. Henrik Franzyk [2], Prof. Bente Gammelgaard [1], Prof. Petrine Wellendorph [2], Dr. Mie Kristensen [1]

1. *Department of Pharmacy, University of Copenhagen, Denmark,* ***2.*** *Department of drug design and pharmacology, University of Copenhagen, Denmark*

Maria Thaysen[1], Henrik Franzyk[2], Bente Gammelgaard[1], Petrine Wellendorph[2], Mie Kristensen[1]

[1]Department of Pharmacy, [2]Department of Drug Design & Pharmacology, University of Copenhagen, Denmark

Correspondence: mie.kristensen@sund.ku.dk

Introduction. There is currently no pharmacological treatment to prevent brain damage following ischemic stroke, although peptides that inhibit stroke-induced neuronal death are under development. One example is NR2B9c, conjugated to the cell-penetrating peptide Tat to promote blood-brain barrier permeation and neuronal uptake [1]. However, Tat-NR2B9c (NA-1) does not achieve clinically relevant brain entry [2], likely due to poor plasma stability and broad biodistribution [3].

Aims. This study investigates whether conjugation of NR2B9c to the transferrin receptor-targeting peptide T7 [7] and stabilization with proteolytically stable D-amino acids improve brain delivery and reduce off-target accumulation.

Methods. Linear peptide constructs with Tat replaced by L- or D-T7 and a novel dimeric peptide with two D-T7 arms were synthesized. Constructs were labeled with selenomethionine for detection by ICP-MS. Peptide stability was assessed in mouse plasma, and biodistribution was evaluated in male and female C57Bl/6 mice following intravenous administration of 3 nmol/g and 1 h circulation.

Results. D-T7-NR2B9c constructs exhibited improved plasma stability compared to L-forms. All constructs were detected in brain lysates, with linear peptides achieving ~2% ID/g and dimeric peptides ~1% ID/g. In addition, dimeric constructs displayed reduced off-target accumulation compared to linear peptides and the NA-1 reference. Interestingly, brain accumulation was higher in female mice than male mice across all T7 constructs but not for the NA-1 reference.

Conclusions. Our findings demonstrate that replacing the Tat moiety of NA-1 with T7, combined with the use of D-amino acids, improves plasma stability and enables NR2B9c brain delivery. Replacing Tat with T7 and stabilization with D-amino acids improved plasma stability and enabled brain delivery. Linear constructs showed higher brain accumulation, whereas dimeric constructs reduced off-target organ uptake, suggesting improved safety profiles. Observed sex differences highlight the importance of considering biological variables in future preclinical development.

References

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[2] Hill M et al. (2020) *Lancet* 395:878–887

[3] Kristensen M et al. (2020) *Pharmaceutics* 12:1–24

[4] Siying L et al. (2021) *Mol Pharm* 18:1480–1485

New isoform-dependent targeting for improved receptor-mediated transcytosis across the blood-brain barrier

Thursday, 2nd October - 11:40: (Auditorium 1) - Oral

Dr. Marco Vigo [1], ***Ms. Marina Placci*** [1], ***Prof. Silvia Muro*** [2]

1. *Institute for Bioengineering of Catalonia & University of Barcelona,* ***2.*** *Catalan Institution for Research and Advanced Studies & Institute for Bioengineering of Catalonia*

Drug delivery to the brain is highly impaired by the blood-brain barrier (BBB). To overcome this problem, drugs and drug carriers are often targeted to endothelial receptors involved in BBB transcytosis. However, the fact that these receptors, as most body proteins, are not expressed in an exclusive configuration but as various isoforms has been long overlooked. Illustrating this paradigm, we focused on intercellular adhesion molecule 1 (ICAM-1), an endothelial receptor overexpressed in many pathologies. ICAM-1 has five extracellular domains (D1 to D5) that can be targeted, yet most prior targeting studies have used D2-specific antibody (Ab) R6.5. While this provides BBB transport in cellular models, literature suggests the presence of ICAM-1 isoforms lacking D2 (-D2 ICAM-1) *in vivo* in mice (Fig.1A). In this study, we investigated for the first time the presence of -D2 ICAM-1 isoforms in human brain endothelial cells. Quantitative RT-PCR, Western blot, and radioimmunotracing revealed the abundant presence of-D2 ICAM-1 isoforms at both mRNA and protein levels (Fig.1B,C). To study isoform-dependent targeting, we developed recombinant cells lines expressing -D2 ICAM-1 vs. full-length ICAM-1 and used them to compare the targeting efficiency of commercial anti-ICAM-1 Abs (15.2, R6.5, G-5, H4) and new Abs we developed using phage display (B4, B6, B11, C12, G2). One commercial (G-5) and two new (B4, B6) Abs were the best at targeting cells expressing-D2 ICAM-1 recombinantly (>$2x10^{12}$ sum intensity/well, 15-fold above R6.5 control). They also induced uptake by endothelial cells expressing the full pool of ICAM-1 isoforms (40-80% NPs in 1 h). These Abs were coated on polymer nanoparticles (NPs) and the resulting formulations (Fig.2A) showed good targeting specificity (>10-fold vs. IgG NPs). Using BBB cell models with validated barrier function (presence of cell junctions and lack of NP leakage), these formulations exhibited efficient transcytosis across endothelial linings, with one of them (coated with Ab B4) being the best (95% transcytosis in 24 h; Fig.2B). Therefore, this study illustrates that the identification of both receptor isoforms expressed by the BBB and targeting moieties capable of recognizing them holds great promise to improve brain targeting for drug delivery purposes.

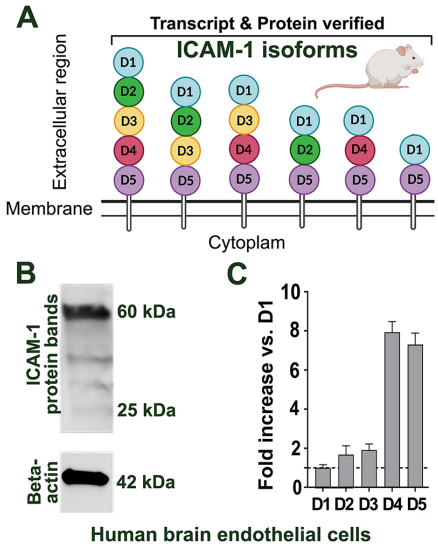

Figure 1. ICAM-1 isoforms. (A) Isoforms found in mice (doi: 10.3390/biology12050743). (B) Western blot using anti-ICAM-1 versus anti-Beta-actin on cell lysates from activated HBMECs. (C) ICAM-1 domain levels detected by qPCR, compared to D1. Data are mean ± SEM.

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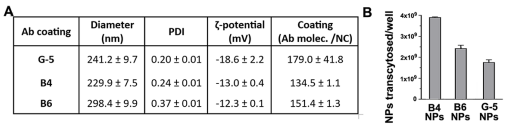

Ab coating	Diameter (nm)	PDI	ζ-potential (mV)	Coating (Ab molec. /NC)
G-5	241.2 ± 9.7	0.20 ± 0.01	-18.6 ± 2.2	179.0 ± 41.8
B4	229.9 ± 7.5	0.24 ± 0.01	-13.0 ± 0.4	134.5 ± 1.1
B6	298.4 ± 9.9	0.37 ± 0.01	-12.3 ± 0.1	151.4 ± 1.3

Figure 2. Anti-ICAM-1 NPs and their transcytosis. (A) Characterization using DLS, electrophoretic mobility, and radiotracing of model polystyrene nanoparticles (NPs) after coatedin them with new (B4, B6) or commercial (G-5) anti-ICAM-1 antibodies (Abs). (B) Trancytosis of 125I-labeled Ab-coated NPs acros confluent human brain endothelial linings growing on Transwel filters (24 h transport). Data are average ± SEM.

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From DNA Constructs to Cryo-TEM: Toward 3D Characterization of LRP1

Thursday, 2nd October - 11:10: (Auditorium 2) - Oral

Mr. Alessandro Ronzoni [1], ***Prof. Giuseppe Battaglia*** [1], ***Mr. Victor Mejias*** [1], ***Dr. Joana Fort*** [2], ***Prof. Manuel Palacin*** [2], ***Mr. Gian Marco Tuveri*** [1], ***Dr. Catia Lopes*** [1], ***Dr. Amayra Hernandez*** [1], ***Dr. Iris Batalha*** [1]

1. *Institute for Bioengineering of Catalonia & University of Barcelona,* ***2.*** *IRB Barcelona*

LRP1 (Low Density Lipoprotein Receptor-Related Protein) is a large transmembrane receptor, member of the LRP family. LRP1 helps to regulate plasma lipid levels and prevents the accumulation of cholesterol-rich particles in the vasculature. This large endocytic receptor is widely expressed in various tissues and has diverse functions. Research into the precise mechanisms through which LRP1 works is crucial for developing targeted therapeutic strategies.

LRP1 is an integral membrane protein of 600kDa; it undergoes a proteolytic processing in the trans-Golgi compartment, that generates two subunits with molecular masses of 85 and 515kDa. LRP1 protein undergoes a shedding process, during which the extracellular subunit detaches from the transmembrane one.

There are some works that characterize fragments of the protein, but none report the entire 3D structure. The absence of a publicly available structure poses significant disadvantages for drug discovery and efforts targeting this receptor.

The structure of the LRP2, a member of the LRP family closely related to LRP1, was recently solved by Beenken et al. LRP2 is a homodimer, in which the large extracellular domains fold on each other creating a globular structure. Although in literature LRP1 has always been shown as a monomer, simulations suggest that it might fold into a homodimer very similarly to LRP2.

We aim to characterize the structure of LRP1. To do so, pure, intact, and stable, LRP1 protein needs to be obtained. We generated DNA constructs with specific characteristics to enable us to express LRP1 in HEK cells and purify it.

We generated a main construct, with a twin streptag, fused to the C-terminal domain of LRP1, we started expressing the protein in HEK cells, and we started purifying in small-scale experiments. We then scaled up the process, obtaining a higher amount of protein, which enabled us to start analysis. Currently, we are analysing the results of the negative staining TEM and we are going into cryo-TEM imaging to properly characterize the structure.

Also, with a stable purified protein, we aim to perform phage display experiments to identify new ligands of LRP1, and SPR experiments to characterize binding affinity of known and new ligands.

Computational analysis of delivery targets and trafficking at the blood-brain barrier: toward the design of brain-penetrant therapeutics

Thursday, 2nd October - 11:25: (Auditorium 2) - Oral

Dr. Habib Baghirov [1]

1. *Åbo Akademi University*

Background

Receptor-mediated transcytosis (RMT) is one approach to brain delivery. Multi-organ atlases allow searching for RMT targets by comparing brain capillary endothelial cells (BCEC) with biodistribution-relevant peripheral cells. I sought to create a proteomics atlas of these cells. I also analyzed protein turnover, deeming it crucial for all targets and especially those that do not transport large ligands natively and whose use in RMT may thus rely on hijacking their recycling. I then hypothesized that interaction of BCEC-nonspecific and BBB-specific proteins may cause the former to form BCEC-specific epitopes, and sought to identify them. I also speculated that for targets with slow internalization, one could induce endocytosis by binding extracellular regions to expose possibly inactive cytosolic endocytic motifs, and sought to identify targets suitable for such triggered endocytosis and to design constructs enabling it. I finally reasoned that after intracellular dissociation, binding endosomal proteins may be the only way to enable faster-than-diffusional cargo movement, ideally abluminal, and sought to identify such proteins.

Methods

To create atlases, I processed the spectra of cell type-resolved proteomics datasets and supplemented this with scRNAseq analysis. To analyze isoforms, I used peptide-level proteomics, long-read transcriptomics, and sufficiently deep short-read transcriptomics data. To identify BCEC-specific epitopes, I structurally analyzed interactions of BCEC surface proteins. To analyze turnover and trafficking, but also identify intracellular targets that could intercept cargo in the endosome, I used in vivo proteomics data and in vitro protein-protein interactions and organelle fractionation datasets. To design endocytosis-triggering constructs, I mined targets for conserved cytosolic endocytic motifs, then built allostery maps to locate suitable extracellular regions, and finally modeled binders targeting those regions.

Results

Proteomics atlas, albeit shallow, allows target identification. Turnover data can inform target choice. Isoforms of several hits increase their BCEC specificity or, conversely, may complicate their use. Several targets are parts of heterodimers; targeting their interface could improve specificity. Structures yielding BCEC-specific epitopes show high confidence scores yet require validation. Same applies to endocytosis-triggering constructs. Analysis of protein-protein interactions and subcellular fractionation helps predict endosomal targets that could intercept constructs and, unlike surface proteins, would ideally be bound at acidic pH.

The influence of individual ECM protein coatings on the matrix secreted by endothelial cells: mechanistic studies for molecularly engineering instructive cell culture substrates

Thursday, 2nd October - 11:40: (Auditorium 2) - Oral

Dr. Gaia Serrini [1], Ms. Chiara Manni [1], Dr. Sara Silva [1], Dr. Helena Azevedo [1], Dr. Rui Pereira [1]

1. *i3s, University of Porto*

The blood-brain-barrier (BBB) is formed by an organized network of endothelial cells, pericytes and astrocytes, jointly known as neurovascular unit (NU) which is structurally sustained and biochemically guided by the basement membrane (BM) [1]. Endothelial cells (ECs) typically grow as monolayers forming a tight barrier between blood and tissues. ECs are highly reactive cells, sensing the biochemical and physical signals of the environment. Elucidating the environmental factors that influence endothelial cell functions for forming tight cellular barriers is key for establishing reliable in vitro models of the blood-brain barrier (BBB).

To uncover the biochemical factors, we cultured human endothelial cells (HUVECs) on surfaces coated with individual extracellular matrix (ECM) proteins (collagen I, IV, fibronectin, laminin, all at 50 μg/ml) for 4 hours, 1, 3 and 5 days and investigated the matrix secreted by cells (nascent proteins), adapting a labeling technique, Bioorthogonal Noncanonical Amino Acid Tagging (BONCAT [2]. By co-staining, we have also identified the effect of the different protein coatings on specific endothelial markers such as CD144, CD31, Zonula Occludens-1, claudin-5, and occludin. BONCAT technique was optimized for the culture of human endothelial cells, enabling the specific labelling and visualization of nascent proteins. Our findings revealed distinct temporal remodeling dynamics of each nascent protein, highlighting the noteworthy influence of protein costings on endothelial cell ECM remodeling over the 5 days of culture. Co-staining analysis for collagen type I, IV, fibronectin and laminin, together with the results obtained with BONCAT technique, showed a distinctive secretion and spatial pattern deposition and arrangement of nascent proteins. Nascent collagen type IV showed a network morphology with basal distribution while collagen type I, similar to fibronectin, formed loose fibrils at apically suggesting a possible role on cell polarity regulation. Combining the BONCAT technique with other assays will provide routes to monitor local nascent proteins by endothelial cells in real time offering deeper insights into the role of the BM in BBB physiology.

Acknowledgments: Work was funded by Fundação para a Ciência e a Tecnologia, I.P. (3D-BBB project, 2022.01690.PTDC) and by European Union's Horizon 2020 research and innovation program (MOBILIsE project, 951723).

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[2]-doi: 10.1038/s41596-021-00652-9

In vitro validation of PARPi-BBBpS derivatives for metastatic triple negative breast cancer

Thursday, 2nd October - 11:10: (Sala Blava 2) - Oral

Mr. Leonardo Miranda [1], ***Mr. Thomas Brunner*** [2], ***Mrs. Maria Gómez*** [3], ***Mrs. Aitana Nacher*** [3], ***Dr. Jordi Llop*** [3], ***Dr. Max Keller*** [2], ***Prof. Pierre Koch*** [2], ***Dr. Vera Neves*** [4], ***Dr. Marco Cavaco*** [4], ***Prof. Miguel Castanho*** [4]

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Triple-negative breast cancer (TNBC) is a particularly challenging subtype of breast cancer (BC) characterized by high rates of metastasis, especially to the brain. It shares many pathological features with BC that have mutations in the BRCA1/2 genes, which are crucial for the DNA damage response (DDR). Numerous proteins play roles in DDR pathways, including poly(ADP-ribose) polymerase-1 (PARP1), which is responsible for repairing DNA breaks. To date, the FDA and EMA have approved two PARP inhibitors (PARPis) for use as monotherapy: Olaparib and Talazoparib, specifically for BRCA-mutated BC. While these drugs show positive outcomes for systemic disease, their effectiveness in treating brain metastases (BM) is limited by the blood-brain barrier (BBB). To enhance drug delivery to the brain, our research group has developed BBB peptide shuttles (BBBpS) as carriers that achieve brain accumulation above 0.5% (ID/g in mice), surpassing other previously published BBBpS. In this study, we aimed to improve the brain accumulation of Olaparib by conjugating it to various BBBpS, thereby creating peptide-drug conjugates (PDCs). The initial phase of our research involved generating and validating Olaparib derivatives with linkers of varying hydrophilicity and length. After *in vitro* characterization, we selected derivative 73 due to its similar PARPi effect and biological activity against different TNBC cell lines, both with and without BRCA mutations, compared to the original Olaparib. Simultaneously, we functionalized our two most promising BBBpS (BBBpS_1 and BBBpS_2) in different regions and evaluated their BBB translocation capabilities using an *in vitro* BBB model. Based on the most favorable functionalization site and BBB translocation capacity, we chose two derivatives (BBBpS_1A and BBBpS_2A) and conjugated them to derivative 73, resulting in BBBpS_1A-73 and BBBpS_2A-73. Although both PDCs exhibited a slightly lower PARP inhibitory effect and biological activity compared to Olaparib and derivative 73, the translocation efficacy of BBBpS_1A and BBBpS_2A was maintained, in our *in vitro* model. The next steps include conducting efficacy studies in mice with BM to assess brain accumulation *in vivo* and validate the potential of this strategy for advancement into further preclinical studies.

Hyperosmotic Polymeric Nanochains for Efficient Blood-Brain Barrier Transmigration and Glioblastoma Therapy

Thursday, 2nd October - 11:30: (Sala Blava 2) - Oral

Dr. Shambhavi Pandey [1], ***Dr. Pankaj Garg*** [2], ***Prof. Jong Hoon Chung*** [2]

1. *Institut Quimic de Sarria - University Ramon Llull,* ***2.*** *Seoul National University*

The blood-brain barrier (BBB) poses a formidable challenge in treating central nervous system (CNS) pathologies, precluding approximately 98% of potential therapeutics, regardless of their intrinsic efficacy, from reaching target sites in the brain. This severe limitation necessitates the development of innovative drug delivery systems capable of efficient BBB transmigration. While the BBB selectively facilitates molecular movement through carrier-mediated transport and transcytosis (adsorptive or receptor-mediated), current clinical strategies often rely on invasive techniques like osmotic opening with hypertonic mannitol solutions. We propose a novel, non-invasive approach that integrates this osmotic principle directly into the therapeutic delivery agent. To address this critical unmet need, we engineered linearly aligned, nucleic acid-complexed polydixylitol-based polymeric nanochains (X-NCs). These X-NCs possess inherent hyperosmotic properties, which enable them to facilitate their own transmigration across the BBB/blood-tumor barrier (BTB) and subsequently navigate through the intricate, deeper regions of the brain tumor microenvironment. Beyond their osmotic advantage, the high aspect ratio of the nanochains confers shape-dependent functional benefits, allowing for enhanced payload capacity and promoting efficient cellular uptake via nuclear factor of activated T cells-5 (NFAT5)-mediated mechanism. We demonstrated the therapeutic potential of this platform by loading the nanochains with serine hydroxymethyltransferase 1 (SHMT1) siRNA. In glioblastoma xenograft brain tumor mouse models, these SHMT1 siRNA-loaded X-NCs not only successfully transmigrated the BTB, but remarkably reduced tumor size by 97%. This study unequivocally illustrates a state-of-the-art strategy where hyperosmotic nanochains, leveraging their high aspect ratio and aligned structure, accelerate therapeutic effects in aggressive brain tumors by efficiently overcoming the BBB/BTB through an NFAT5-dependent cellular uptake mechanism, paving the way for enhanced CNS drug delivery.

A Site-Specific MiniAp4–Trastuzumab Conjugate Prevents Brain Metastasis

Thursday, 2nd October - 11:50: (Sala Blava 2) - Oral

Dr. Macarena Sanchez [1], ***Dr. Benjamí Oller-Salvia*** [2], ***Dr. Mariam Masmudi-Martín*** [3], ***Dr. Meritxell Teixidó*** [4], ***Dr. Manuel Valiente*** [3], ***Prof. Ernest Giralt*** [5]

1. *Insitute of Parasitology and Biomedicine "López-Neyra",* ***2.*** *Institut Quimic de Sarria - University Ramon Llull,* ***3.*** *CNIO,* ***4.*** *Gate2Brain,* ***5.*** *IRBBarcelona*

Monoclonal antibodies (mAbs) are revolutionizing cancer therapy. However, their effectiveness in treating brain tumors or metastases is often limited by the blood-brain barrier (BBB) and the blood-tumor barrier (BTB). To overcome this challenge, researchers have explored molecules that exploit the body's natural transport pathways on brain endothelial cells, known as brain shuttles, which can facilitate the passage of large molecules and nanoparticles across the BBB. Among these, protease-resistant peptides like MiniAp-4 have shown particular promise.

We have recently described the synthesis, characterization, and evaluation of site-specific antibody conjugates—antibody-shuttle conjugates (ASCs)—using the anti-HER2 monoclonal antibody trastuzumab (Tz) linked to four MiniAp-4 molecules. These ASCs retain the original antibody's binding ability and induce cell cycle arrest. Notably, the MiniAp-4 conjugates demonstrate improved transport across an in vitro BBB model compared to unconjugated Tz and Tz linked to Angiopep-2, a brain shuttle that has progressed in clinical trials. Most importantly, in a mouse model of brain metastasis, the Tz-MiniAp-4 conjugate showed a preferential ability to cross the BBB/BTB, significantly reducing metastasis formation. This approach could be adapted to other antibodies targeting central nervous system diseases. Overall, MiniAp-4 enhances the delivery of trastuzumab to the brain, offering a promising strategy to prevent brain metastases.

Molecular Determinants Underlying Blood-Brain Barrier Targeting Peptides

Thursday, 2nd October - 12:00: (Auditorium 1) - Oral

Dr. Marco Cavaco [1], Mr. Javier Valle [2], Ms. Patrícia Fraga [1], Mr. Rúben Silva [3], Prof. João Galamba Correia [3], Prof. David Andreu [2], Prof. Miguel Castanho [1], Dr. Vera Neves [1]

1. *Gulbenkian Institute for Molecular Medicine|Faculdade de Medicina, Universidade de Lisboa,* ***2.*** *Proteomics and Protein Chemistry Unit, Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona Biomedical Research Park,* ***3.*** *Centro de Ciências e Tecnologias Nucleares and Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa*

The use of peptides to facilitate the delivery of therapeutics to the brain is gaining increasing relevance in brain pharmacology and medicinal chemistry. However, the specific properties that enable blood-brain barrier (BBB)-penetrating peptides (BBBpS) to mediate brain entry remain poorly understood. Typically, the development of such molecular tools is based on the assumption that cell-penetrating peptides (CPPs), which have demonstrated the ability to traverse cellular membranes, can also cross more complex biological barriers, such as the BBB. While CPPs have shown efficacy in transporting diverse cargoes, including proteins, nucleic acids, small molecules, and nanoparticles into cells, their success in achieving effective brain delivery remains limited. Here, we have identified the molecular determinants for brain targeting by peptides while discriminating between BBBpS and CPPs. BBBpS present high BBB translocation in vitro (~50 % at the brain side after 24-hours); while CPPs have high retention in brain endothelial cells and poor BBB translocation (~15 % at the brain side after 24-hours) [1]. The results were corroborated in vivo, where higher brain accumulation was attained for BBBpS (2-fold increase when compared with a CPP). This methodology can be used to assist in the design of peptides with potential brain penetration from amino acid residue sequences. As a proof of concept, a BBBpS, PepH3 [2], was conjugated to neurotherapeutics (e.g. peptides and biologicals) aimed to treat brain metastases of breast cancer. The results show a 4-fold increase in concentration at the brain side when PepH3 is used [3].

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2. Neves V, et al. ACS Chem Biol. 2017. DOI: 10.1021/acschembio.7b00087
3. Cavaco M, et al. Biomed Pharmacother. 2024. DOI: 10.1016/j.biopha.2024.116573

Blood-brain barrier shuttling anti-mouse CD98hc nanobodies

Thursday, 2nd October - 12:15: (Auditorium 1) - Oral

Dr. Laura Rue [1], ***Mr. Tom Jaspers*** [2], ***Mr. Pieterjan Van Maele*** [2], ***Ms. Isabelle Degors*** [3], ***Prof. Bart De Strooper*** [4], ***Prof. Maarten Dewilde*** [5]

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The blood-brain barrier (BBB) limits the therapeutic perspective for central nervous system (CNS) disorders such as Alzheimer's disease. Interestingly, monoclonal antibodies generated against receptor-mediated transcytosis receptors are able to transport therapeutic biologicals across the BBB. Nanobodies, which are the variable domain isolated from camelid heavy-chain only antibodies have also shown their potential to deliver therapeutics into the CNS. Here we aimed to expand the panel of nanobodies targeting other brain endothelial cell-surface proteins. Here, CD98hc (involved in the transport of amino acids across the BBB) was chosen, as it has been previously suggested to allow drug shuttling across the BBB. DNA immunization followed by selections on CHO cells overexpressing the target of interest resulted in specific anti-human/cynomolgus CD98hc and anti-mouse CD98hc binders with affinities in the low nanomolar range. Anti-mouse CD98hc binders were screened *in vivo* by their potential to deliver a BACE1 inhibiting antibody in the brain. Interestingly, nanobodies fused to the anti-BACE1 antibody 1A11 in a monovalent format effectively reduced $A\beta_{1\text{-}40}$ levels in the brain after intravenous delivery, indicating the efficiency of the shuttle to deliver a therapeutic moiety over the BBB. In the future we will explore whether the anti-human CD98hc nanobodies are also able to shuttle across the BBB in a CD98hc humanized mouse model.

In vivo functional genomics to study transferrin uptake at the blood-brain barrier

Thursday, 2nd October - 12:30: (Auditorium 1) - Oral

Mr. Andrew Pan [1], ***Mr. Indigo Rose*** [2], ***Dr. Martin Kampmann*** [2], ***Dr. Andrew Yang*** [3], ***Mr. Vincent Castillo*** [4]

1. *Biomedical Sciences graduate program, University of California, San Francisco, CA, USA and Gladstone Institutes, Gladstone Institute of Neurological Disease, San Francisco, CA, USA,* ***2.*** *University of California, San Francisco, San Francisco, CA, USA,* ***3.*** *Gladstone Institutes, Gladstone Institute of Neurological Disease, San Francisco, CA, USA,* ***4.*** *UCSF MSTP Program and Gladstone Institute*

The blood-brain barrier (BBB) serves as a major impediment to delivery of nearly all therapies to the brain. Recently, receptor mediated transcytosis has emerged as a “trojan horse” strategy for delivering protein therapies into the brain parenchyma and has seen clinical success in enzyme replacement therapies. This therapeutic strategy is primarily centered around designing antibody shuttles to target transferrin receptor (TFRC), which transports iron-bound holo-transferrin into the brain. However, once proteins enter the brain endothelial cells (BEC) that comprise the BBB, very little is understood about how these cargoes are sorted for delivery into the brain, recycled back to the luminal membrane, or trafficked to lysosomes for degradation. Little is understood too about the molecular regulators that govern receptor expression, which may also impact delivery of therapeutics that depend on those receptors. By understanding the molecular regulators of transport, we may uncover new targets top therapeutically enhance delivery of a wide array of therapies. We leveraged recent advances in the field including BEC-specific AAV capsids to perform the first *in vivo* CRISPR screen to uncover drivers of transferrin uptake as a model for protein transport across the BBB. We validated that knockdown of TFRC as a control reduced both TFRC surface expression on BECs and also transferrin tagged with ATTO647 uptake. We then delivered a small library of sgRNAs targeting endo-lysosomal genes and druggable genes that may be therapeutic levers to enhance protein delivery. Even at a low coverage of 400x cells per sgRNA, we were able to identify a potential novel role of Ly6 family members, canonically known to be immune markers, for transferrin uptake.

Harnessing Structural Complexity for Phenotypic Targeting of the Blood-Brain Barrier

Thursday, 2nd October - 12:00: (Auditorium 2) - Oral

Dr. Valentino Barbieri [1], ***Dr. Catia Lopes*** [2], ***Mr. Marco Basile*** [1], ***Dr. Vanina Cosenza*** [1], ***Prof. Giuseppe Battaglia*** [2]

1. *Institute for Bioengineering of Catalonia (IBEC),* ***2.*** *Institute for Bioengineering of Catalonia & University of Barcelona*

Since the introduction of the concept of superselectivity [1], targeting strategies based on weak multivalent ligand–receptor interactions have emerged as powerful alternatives to conventional drug design. Unlike high-affinity targeting, which often results in nonspecific uptake due to indiscriminate receptor binding, multivalency enables selective engagement only under specific biological conditions, offering superior selectivity and specificity. Our phenotypic targeting strategy capitalises on this principle by leveraging characteristic cellular features, such as receptor expression and membrane structure, to guide nanoparticles toward diseased tissues with high selectivity.

This approach proves particularly beneficial in pathologies affecting tissues protected by physiological barriers, where traditional ligand-based targeting often falls short. Notably, we previously demonstrated the effectiveness of our strategy for BBB crossing [2] [3] in the context of amyloid beta clearance.

By shifting the focus from isolated receptors to the broader biological context, phenotypic targeting requires sophisticated nanoparticle engineering [4]. We achieve this through supramolecular self-assembly of amphiphilic block copolymers, enabling the creation of peptide-functionalised polymeric nanoparticles with tuneable avidities, immune evasion capabilities, and fine-tuned biological interactions.

In our design, the hydrophilic corona plays a multifunctional role; beyond imparting stealth, it modulates ligand accessibility and mediates repulsive interactions with the glycocalyx, enhancing selective engagement. Furthermore, the modularity of our supramolecular platform enables us to generate asymmetric morphologies and control the spatial distribution of ligands, thereby better reflecting the heterogeneous presentation of receptors on cell surfaces.

These design elements are coupled with integrated barcoding strategies, enabling unique identification of nanoparticle formulations and facilitating high-throughput assessment of their targeting performance in vitro and in vivo. Our experimental data can then be fed into our theoretical framework to inform the rational development of next-generation nanomedicines. Ultimately, our work aims to establish design principles that enable precise, context-driven, and personalised therapeutic delivery through phenotypic targeting.

References

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In situ molecular structures and architectures of the blood brain barrier: cryoET as a tool for cerebrovascular research

Thursday, 2nd October - 12:20: (Auditorium 2) - Oral

Dr. Maddie Gilbert [1], ***Dr. René Frank*** [1]

1. *University of Leeds*

The neurovascular unit (NVU) forming the blood brain barrier (BBB) constitutes a highly specialized and complex structure that is essential for brain function. Here, we present integrated cellular and molecular architectures of the BBB within surgical brain biopsies from living human donors using cryo-fluorescence-guided cryo-electron tomography (cryoET). We collected a cryoET dataset of 3D molecular maps at nanometre-resolution of cerebral microvessels within vitrified brain tissue in a hydrated, near-native state (Fig 1). These tomographic volumes revealed the first direct in situ visualisation of proteins, macromolecules, and cellular structures that comprise the BBB within human brain tissues, including the organisation of the basement membrane, intracellular filaments, and endothelial cell transcytosis intermediates. Combined with fluorescent labelling of molecular targets and subtomogram averaging (STA), we have previously demonstrated that cryoET is a uniquely powerful technique capable of resolving the polypeptide backbone fold of proteins within intact human brain tissue (Gilbert et al, 2024). The cryoET workflows developed here are poised to reveal novel insights into NVU structure, with potential future applications, including characterising structural defects of the BBB in neurological disease.

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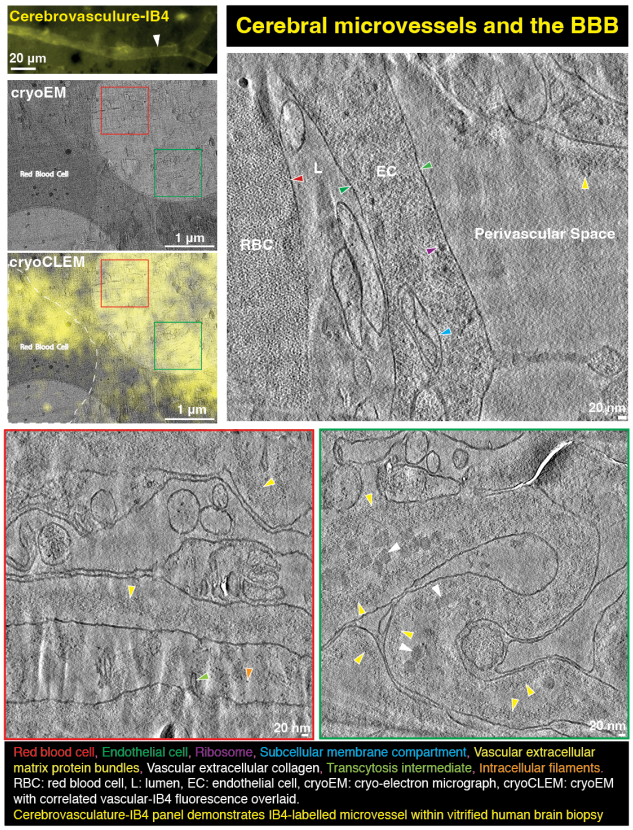

In situ architectures of the blood brain barrier-fig1.png

Decoding the Mechanics of Brain Endothelium: CAV1-Dependent Invaginations as modulators of neurovascular coupling

Thursday, 2nd October - 12:40: (Auditorium 2) - Oral

Dr. Fidel Nicolas Lolo [1], ***Dr. María Isabel Alvarez*** [2], ***Dr. Elena Tortosa*** [3], ***Dr. Carmen Nieto-Vaquero*** [1], ***Dr. Virginia García Sánchez*** [1], ***Dr. Maria Ángeles Moro*** [1], ***Dr. Manuela García López*** [3], ***Prof. Carmen Ruiz de Almodovar*** [2], ***Prof. Miguel Angel Del Pozo*** [1]

1. *CNIC (Spanish National Cardiovascular Research Centre),* ***2.*** *University of Bonn,* ***3.*** *Universidad Autonoma de Madrid (UAM)*

Endothelial cells of the brain vasculature are continuously exposed to mechanical forces that regulate essential functions including endocytosis, barrier integrity, and neurovascular coupling (NVC). Plasma membrane (PM) tension serves as a critical mechanotransductive cue, and specialized PM invaginations—such as caveolae—buffer abrupt mechanical stress in arteriolar endothelial cells, where shear forces and blood flow are high. Notably, capillary endothelial cells, which experience lower mechanical load, lack morphologically detectable caveolae yet express Caveolin-1 (CAV1), the principal structural component of caveolae. Recent findings from our lab (Lolo et al., Nat. Cell Biol. 2023) have identified a novel class of CAV1-dependent invaginations, termed *dolines*, which form independently of caveolae and respond to subtle shifts in PM tension—suggesting a new mechanoadaptive role for CAV1 in capillaries. Building on this discovery, our research aims to dissect the distribution, regulation, and functional implications of CAV1-based structures across the cerebrovascular network. We combine advanced *in vitro* systems with *in vivo* behavioral analyses to: (i) map the spatial distribution of dolines and other CAV1-dependent structures across the brain endothelium; (ii) investigate how lipid composition modulates their biogenesis and stability; (iii) determine how phosphorylation at tyrosine-14 (Y14)—a key post-translational modification of CAV1—affects membrane tension sensing and nanoparticle internalization; (iv) evaluate how neuronal activity dynamically regulates CAV1 structures during neurovascular signaling; and (v) assess the downstream behavioral consequences of modulating these mechanosensitive pathways in animal models. We will present preliminary findings elucidating the role of endothelial CAV1 in neurovascular coupling, providing new insights that may advance our understanding of complex brain functions such as memory consolidation.

Characterising the effects of stimulus modality and hypercapnia on neurovascular coupling response

Thursday, 2nd October - 12:00: (Sala Blava 2) - Oral

Dr. Jack Leacy [1], ***Ms. Kate Walsh*** [1], ***Mr. Eoin Moynihan*** [1], ***Ms. Louise Buckley*** [1], ***Prof. Ken O'Halloran*** [1]

1. *University College Cork*

Neurovascular coupling reflects the temporal and spatial relationship between regional cerebral blood flow and local neuronal activity. Research tools which are routinely used to measure the NVC response include transcranial doppler ultrasound (TCD) and functional near infrared spectroscopy (fNIRS). Both these techniques can be used to visualise the NVC mechanism in response to brain-region specific tasks. To date there is little concensus on the degree to which task selection impacts the NVC response. Whether slight alterations in task magnitude or difficutly impacts the overall NVC response has important implications for inter-study comparisons between research groups. The aim of this investigation was to characterise the effects of different visual and motor challenges on the NVC response within the occipital and motor cortices.

21 healthy individuals (9 males, 24.62 ± 5.54yrs, 73.24 ± 14.24kg) were recruited as part of this investigation, reporting to the lab on two seperate occassions. One visit employed TCD and visual challlenges to assess NVC within the occipital cortex. Visual challenges included: 1) room light, 2) flashing checkerboard, 3) reading tasks and 4) metronome ball.

The other test day utilised fNIRS and motor challenges to assess NVC within the motor and pre-motor cortices. Motor tasks included: 1) fingertapping, 2) thumb to digit exercise and 3) isometric flexion. Participants alternated between periods of task exposure and rest.

Our results found significant differences in the NVC response within both the occipital and motor cortices which were task-dependent (see figures 1/2). Our investigation highlights the need to consider stimulus choice when making inter-study comparisons in NVC literature. Moreover, our investgiation supports the requirement for a standardised battery of stimuli when conducting NVC research in healthy human participants.

In conclusion, our investigation finds that the NVC response is dependent upon the stimulus applied, refuting the idea that all visual and motor challenges are equal when conducting NVC research. This phenomenon likely reflects differences in neuronal pool engagement, and subsequent metabolic change, between challenges. Further research is required to clarify whether this observation extends to other brain regions and whether differences in neuronal activation are supported by measurements of brain activity.

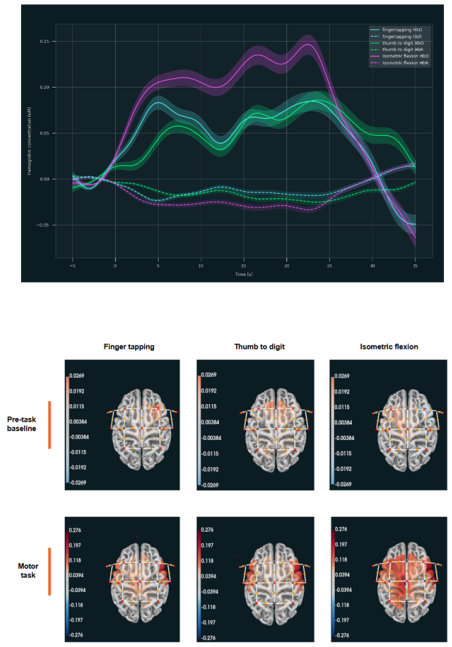

Figure 2. fNIRS NVC waveform and cerebral heat maps. A comparison of the motor task-induced response within the motor and premotor cortices is provided. An average waveform for the concentration (uM) of oxyhaemoglobin (HbO; solid line) and deoxyhaemoglobin (HbR; dashed line) can be seen above for each motor task: finger tapping (blue), thumb to digit (green), and isometric flexion (purple). This waveform includes a five-second pre-stimulus period, followed by a 27-second stimulus period. Cerebral heat maps are also provided indicating the relative change in oxyhaemoglobin (HbO) for both a two-second pre-stimulus (pre-task baseline) and 27-second stimulus period (motor task) for each challenge. Areas of increased colour intensity indicate a higher relative change in HbO. The white lines, yellow dots, and red dots overlayed represent the fNIRS channels, emitters, and detectors, respectively.

Nvc response within the motor cortices.png

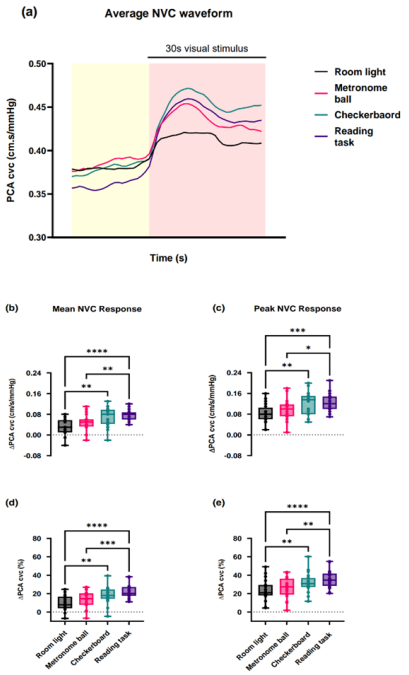

Figure 1. Mean and peak haemodynamic response. A comparison of the visually evoked haemodynamic response within the posterior cerebral artery (PCA) is provided. An averaged waveform for absolute cerebrovascular conductance (cm.s/mmHg) during each visual challenge is provided for the PCA **(a)**. The waveform includes a twenty-second period prior to visual stimulation (yellow-shaded region) followed by 30 seconds of visual stimulus (red-shaded region). The magnitude of the mean and peak visually evoked haemodynamic response is provided **(b-e)**. The absolute change in mean PCAcvc (Δcm.s/mmHg) **(a)** and the peak PCAcvc (Δcm.s/mmHg) **(b)** are provided along the top row. In comparison, the relative change in PCAcvc (Δ%) for both the mean **(d)** and the peak response **(e)** are provided along the bottom row. Data is presented in box and whisker format, showcasing individual data points, median, and interquartile range with max and min values shown. * = p<0.05, ** = p<0.01, *** = p<0.001.

Nvc response within the occipital cortex.png

Dual role of brain endothelial Gpr126 in blood-brain barrier development and ischemic stroke

Thursday, 2nd October - 12:20: (Sala Blava 2) - Oral

Dr. Monica Giannotta [1], ***Dr. Nikolaos Kakogiannos*** [2], ***Dr. Anna Agata Scalise*** [2], ***Mr. Claudio Maderna*** [2], ***Dr. Andrea Benvenuto*** [3], ***Dr. Giorgia Serena Gullotta*** [1], ***Dr. Marco Bacigaluppi*** [1], ***Prof. Elisabetta Dejana*** [2]

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The blood–brain barrier (BBB) acquires unique properties for regulation of the neuronal function during development. The genesis of the BBB coupled with angiogenesis is orchestrated by the Wnt/β-catenin signaling pathway. Aside from the importance of Wnt/β-catenin signaling, the molecular mechanisms that regulate these processes are poorly understood. Here, we identify the brain endothelial adhesion G-protein–coupled receptor Gpr126 as a novel target gene of Wnt/β-catenin signaling that is required for postnatal BBB development, and its expression is detrimental for ischemic stroke in adults. We show that Gpr126 expression is high in mouse brain endothelium during BBB formation, but decreases in the adult. Inactivation of Gpr126 in postnatal endothelial cells results in vessel enlargement and impairs acquisition of the BBB characteristics, such as increased neurovascular permeability, and reduced basement membrane protein deposition and pericyte coverage. Mechanistically, Gpr126 is required during developmental angiogenesis to promote endothelial cell migration, acting via an interaction between Lrp1 and α3β1-integrin, which couples vessel morphogenesis to BBB formation. Interestingly, in adult mice with an established BBB, the lack of Gpr126 expression in acute ischemic stroke is protective and coupled with reduced microglia activation, which contributes to an improved neurological outcome. These data identify Gpr126 as a promising therapeutic target to treat ischemic stroke.

Near Infrared Light-responsive Nanoparticles as an opportunity for brain target delivery

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Mrs. Rafaela Ferrao [1], ***Prof. Lino Ferreira*** [1], ***Ms. Susana Simoes*** [2], ***Prof. Akhilesh Rai*** [1]

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There is an urgent need for strategies capable of bypassing the blood-brain barrier (BBB) and promoting efficient brain drug delivery. Here we report polymeric nanoparticles (NPs) with interesting physicochemical characteristics suitable for future applications in brain drug delivery without triggering a pro-inflammatory response and compromising brain endothelium function and integrity. Here, we report that polydopamine nanoparticles conjugated with transferrin peptides (Tf-PDA NPs) and activated by a near-infrared (NIR) laser, were able to modulate BBB permeability, both in vitro and in vivo models. In vitro, in a continuous monolayer of mouse brain endothelial cells, NIR-laser-activated Tf-PDA NPs were able to induce alterations in BBB permeability compared with bare PDA NPs (i.e. without Tf peptide), without inducing measurable levels of cytotoxicity or affect BBB integrity. Tf-PDA NPs elicited no major acute activation of macrophages upon NIR laser exposure. In mice, the intravenous administration of Tf-PDA NPs followed by the transcranial activation with a NIR laser was able to open the BBB resulting in a higher accumulation of Tf-PDA NPs in the brain as compared to bare PDA NPs and promote local Evans Blue accumulation. Overall, Tf-PDA NPs may be used as an effective lighttriggering carrier to deliver drugs into the brain for treating multiple disorders. Overall, the conjugation of Tf peptide and the photothermal properties inherent to PDA NPs boost brain accumulation without triggering an inflammatory response.

Blood-Brain Barrier Disruption Impairs Sleep via Downregulation of Orexin Signaling

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Jessica Furtado [1], ***Dr. Marc Schneeberger*** [1]

1. *Yale University*

The Blood Brain Barrier (BBB) plays a critical role in maintaining brain homeostasis by tightly regulating the exchange of ions, nutrients, and immune cells between the circulation and the central nervous system. While sleep and the circadian rhythms are known to influence BBB function, the reverse relationship, how BBB integrity impacts sleep regulation, remains poorly understood. This question has broad clinical relevance, as disruption of the BBB is a hallmark of numerous neurological disorders, often accompanied by sleep disturbances.

To explore this link, we utilized a genetic mouse model with endothelial-specific deletion of *Unc5b*, which induces a size-selective BBB leak without gross vascular malformations. Spatial transcriptomic profiling revealed a marked downregulation of *Hcrt* (hypocretin/orexin) in the lateral hypothalamus, a region critical for sleep-wake regulation and metabolic homeostasis. Correspondingly, in vivo behavioral assays using PhenoTyper (Noldus) systems uncovered fragmented sleep-wave cycles and disrupted sleep architecture in the Unc5b mutant mice. These alterations were accompanied by anxiety-like behaviors and reduced exploratory activity across multiple tests. Metabolic phenotyping further demonstrated abnormalities in locomotor activity, feeding behavior, and energy balance.

Our findings reveal that BBB dysfunction can directly impair hypothalamic orexigenic signaling, disrupting sleep architecture and metabolic regulation. This work identifies the BBB as an active modulator of neurobehavioral states and implicates vascular dysfunction as a contributing factor in sleep-related and neuropsychiatric disorders.

Deciphering central nervous system autoimmunity in Down syndrome

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Cristina Lau [1], ***Dr. Micah Donovan*** [1], ***Dr. Angela Rachubinski*** [1], ***Dr. Matthew Galbraith*** [1], ***Dr. Kelly Sullivan*** [1], ***Dr. Joaquin Espinosa*** [1]

1. *Linda Crnic Institute for Down Syndrome*

Down syndrome (DS), caused by the triplication of chromosome 21 (trisomy 21), is the most prevalent chromosomal condition in the United States. Human chromosome 21 carries four of the six interferon receptor (IFNR) genes. This triplication results in significant immune dysregulation and chronic inflammation, characterized by elevated levels of various pro-inflammatory cytokines. These immune disturbances contribute to many co-occurring conditions in individuals with DS, including increased susceptibility to autoimmune disorders and neurological dysfunction.

Our team recently identified 25 autoantibodies that are overrepresented in people with DS, 11 of which are significantly associated with neurological phenotypes. In follow-up studies, we detected autoantibodies in the peripheral plasma of individuals with DS that specifically target myelin basic protein (MBP), a protein primarily found in the central nervous system (CNS). Together, these findings suggest that individuals with DS may experience CNS autoimmunity.

Under normal conditions, the CNS is protected by various barriers, whose integrity is largely maintained by tight junction (TJ) proteins. Barriers such as the blood-brain barrier (BBB) prevent harmful substances, immune cells, and antibodies from infiltrating the brain. However, if the BBB is compromised, harmful elements can enter the CNS. Elevated levels of pro-inflammatory cytokines, matrix metalloproteinase-1 (MMP-1), and anti-collagen IV antibodies observed in DS are known to weaken CNS barriers and may facilitate the entry of autoantibodies into the CNS, increasing the risk of CNS autoimmunity in DS.

Disruption of the BBB can be detrimental, as seen in disorders such as multiple sclerosis and Alzheimer's disease (AD), the latter being particularly prevalent in individuals with DS. Despite the critical role of the BBB in protecting the CNS, their integrity in individuals with DS remains poorly understood. Using primary human samples and mouse models of DS, we aim to deepen our understanding of the mechanisms by which *IFNR* overexpression contributes to autoimmunity and CNS barrier dysfunction in DS, in hopes of improving neurological outcomes for individuals with DS and advancing research on the effects of dysregulated inflammatory pathways on CNS barriers.

Integrating different approaches for establishing a multi scale functional validation platform for RNA-based drugs in the central nervous system (MULTIVAL)

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Chiara Aliprandi [1], ***Dr. Beatrice Simonis*** [2], ***Dr. Cecilia Bombelli*** [2], ***Dr. Francesca Ceccacci*** [2], ***Prof. Michela Matteoli*** [1], ***Dr. Eliana Lauranzano*** [1], ***Dr. Marco Rasile*** [3]

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Background: The blood–brain barrier (BBB) is a major obstacle to delivering RNA-based therapeutics to the central nervous system (CNS). Functionalized lipoplexes have emerged as promising carriers capable of exploiting adsorptive- and receptor-mediated transcytosis to traverse the BBB. Within the MULTIVAL platform - Spoke 9 at the National Center for Gene Therapy and RNA Technology (CN3) - we evaluated the transport efficiency and underlying mechanisms of DPPC–Cholesterol liposomes across an in vitro BBB model.
Methods: Different cationic DPPC–Cholesterol liposomes were functionalized and labeled with a nitrobenzoxadiazole (NBD) fluorophore. Uptake kinetics were first screened in primary murine brain microvascular endothelial cells (BMECs) at 37 °C versus 4 °C to distinguish energy-dependent endocytosis from passive diffusion. Next, we studied liposomes' transport across a BBB model by resveratrol insertion in liposomes using a static configuration, quantifying it both by HPLC and fluorimetry. Finally, potential transcytosis pathways were probed via immunofluorescence co-localization studies of clathrin and caveolin with internalized cargo.
Results: Different DPPC–Chol liposomes exhibited robust uptake by BMECs at 37 °C, with negligible internalization at 4 °C, confirming an active, endocytosis-mediated mechanism. In the static BBB platform, functionalized liposomes successfully traversed the endothelial monolayer without compromising barrier integrity.
Conclusions: Our in vitro results demonstrate that DPPC–Cholesterol–based liposomes could mediate energy-dependent transport of cargo across BBB models. This platform offers a robust preclinical screening tool to optimize lipoplex composition and functionalization for the development of CNS-targeted RNA therapeutics.

Pharmacological validation of a novel BBB-on-chip using an engineered silicon micromesh MEA chip

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Chiara Diacci [1], Dr. Mara Lucchetti [1], Dr. Inês Tomé Ribeiro [1], Dr. Carla Riera Domingo [1], Dr. Laura Rue [2], Mr. Ian Comyn [1], Dr. Eddy-Tim Verjans [1], Dr. Wiebe Vanhove [1], Dr. Thomas Hopfes [1], Dr. Thomas Servotte [1], Dr. Yoke Chin Chai [1], Dr. Daniel Vera [1], Dr. Rabea Hanifa [1], Dr. Stephane Donnay [1], Prof. Maarten Dewilde [3], Dr. Dries Braeken [1], Dr. Mar Condor [1]

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Understanding the BBB is paramount for neuroscience, drug development, and disease modeling. Conventional BBB models, utilizing animals or animal-derived components, are limited in replicating human-specific physiology. Recent advancements in human cell models, microfluidics, organ-on-a-chip technology and biosensors have enabled the development of more physiologically relevant human *in vitro* BBB models. However, existing models are limited in their scalability and still rely on endpoint and label-based methods, which hampers building robust models. Silicon nanofabrication offers an alternative to polymer-based Organ-on-Chip (OoC) platforms, enabling mass manufacturing, high reproducibility, and sensor integration.

This work presents a new micromesh MEA chip that extends to the third dimension, serving as a tissue interface. This porous membrane ensures optimal communication between the different neurovascular cells composing the BBB. To recapitulate the BBB physiological nature, primary human brain microvascular endothelial cells, astrocytes and pericytes are included in a tissue engineered manner distributed among two different compartments vertically connected via the porous membrane. Cell densities, types and key chip features were extensively explored by means of transendothelial electrical resistance (TEER) measurements to achieve physiologically relevant hierarchical cytoarchitectures. The resulting BBB model evinces the expression of BBB hallmarks including tight junction formation by the hBMVEC monolayer, as well as communication between the different neurovascular cells. Permeability assays and receptor-mediated transcytosis experiments revealed low passive diffusion rates of Dextran 70 kDa in the triculture setup compared to hBMVEC monocultures, along with enhanced active transport of Transferrin-488. Furthermore, our platform supports the parallel testing of multiple conditions and enables robust data correlation, facilitating the training of statistical models and Machine Learning (ML) to set up a model-driven optimal experimental design framework for the further optimization of the BBB. Together, these findings and the ML-driven correlation model highlight the potential of our system for advancing BBB research and transport analysis, showing a promising alternative to the state-of-the-art Transwell® model, integrating all neurovascular unit components and being compatible with TEER and other sensor data.

In conclusion, we present a novel *in vitro* model that could accelerate drug discovery, reduce animal testing, and provide a more reliable platform for evaluating drug safety and efficacy.

Enhancing neurodegenerative disease research with brain-on-a-chip technology and advanced biosensor system

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Anna Panteleeva [1], ***Dr. Sujey Palma Florez*** [1], ***Prof. Josep Samitier Martí*** [1], ***Dr. Anna Lagunas Targarona*** [1], ***Dr. Monica Mir*** [1]

1. *IBEC*

Neurodegenerative disorders (NDDs), such as Alzheimer's disease (AD), remain a critical global health challenge. Despite extensive research efforts, there is still no cure for AD with the majority of NDD drug candidates failing to reach the final phases of drug discovery. A key obstacle in drug development is the blood-brain barrier (BBB), which plays a crucial role in regulating the exchange of substances between the bloodstream and the brain. While the BBB protects the brain, its dysfunction contributes to NDDs progression and hinders drug delivery, leaving the vast number of therapeutic candidates unsuccessful in clinical trials.

Traditional animal models have provided valuable insights into NDDs and drug delivery mechanisms, but they fail to fully replicate the complexity of human neural responses. Brain-on-a-chip (BoC) technology is a promising tool, offering controlled environments to study neuronal networks. Integrating a BBB component into a BoC system significantly enhances its physiological relevance, enabling the study of complex BBB properties [1,2].

Our research advances BoC technology by combining a microfluidic device, connected to a peristatic pump, technologies such as multi-electrode array (MEA) and transepithelial/transendothelial electrical resistance (TEER), and biosensors to create a comprehensive BBB model (Figure 1). By co-culturing endothelial cells, pericytes, astrocytes and neurons, we replicate key BBB elements and neural interactions.

This setup allows real-time monitoring of BBB permeability and integrity via TEER, neural activity via MEA electrodes, and neuronal degradation using biosensors. Preliminary results demonstrate promising outcomes. This innovative approach improves the physiological relevance of BoC systems and accelerates drug development and personalized therapies for NDDs, providing a pathway towards more effective treatments.

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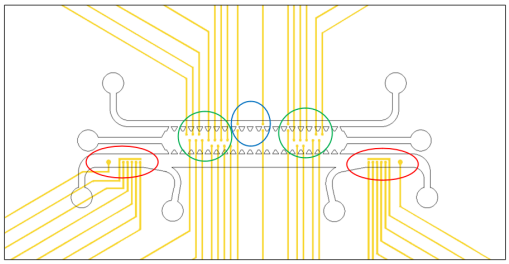

Figure 1. The blueprint of a microfluidic device and electrodes used for different purposes: biosensors for NfL detection, circled in red; MEA can be used for neuronal activity measuring, in green; TEER measures BBB permeability, in blue.

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Imaging of brain-targeted antibody shuttles to improve immunotherapies in Alzheimer's disease

Thursday, 2nd October - 13:45: (Patio Area) - Poster

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A significant advancement has been made in the treatment of Alzheimer's disease (AD) over the past five years, propelled by the success of clinical trials utilizing passive immunization strategies targeting amyloid beta aggregation, a well-established hallmark of AD. Several anti-amyloid therapies have received FDA approval; however, emerging data indicate limited efficacy, largely due to poor penetration of therapeutics into the brain and notable side effects associated with anti-amyloid monoclonal antibodies. Patients treated with these antibodies may experience vascular damage, blood-brain barrier (BBB) leakage, and other adverse effects. Additionally, the observed cognitive improvements are typically modest.

To address the suboptimal delivery of antibodies to the brain, we utilize antibodies (primarily lecanemab) engineered with brain shuttles that exhibit high affinity for endothelial cell receptors such as CD98hc and TfR1. This modification is expected to enhance the accumulation of monoclonal antibodies (mAbs) within the brain parenchyma, particularly around amyloid aggregates. We also anticipate observing reduced vascular damage, decreased microglial activation, diminished astrocyte reactivity, and less brain atrophy in response to treatment with shuttled mAbs. Two imaging techniques will be employed: two-photon and light sheet microscopy. Two-photon imaging will serve as an *in vivo*, real-time method to monitor mAb transport across the BBB. Light sheet imaging will complement two-photon microscopy by expanding the pharmacodynamic and pharmacokinetic profiling from the mouse cortex to the entire mouse brain.

Preliminary two-photon imaging data show promising results, revealing distinct patterns of antibody distribution in the mouse cerebral cortex depending on the presence of the brain shuttle. Initial light sheet imaging data demonstrate the feasibility of distinguishing vascular state, microglial activity, and amyloid plaque localization throughout the whole mouse brain.

The objective of our project is to characterize the pharmacodynamics and pharmacokinetics of monoclonal antibody brain delivery at nanoscale resolution and in real time. These results will advance our understanding of the limitations and benefits of novel immunotherapies.

Therapeutic Effects of Polyphenols on Human BBB Spheroids and Drosophila Models of Hyperhomocysteinemia

Thursday, 2nd October - 13:45: (Patio Area) - Poster

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Hyperhomocysteinemia (HHcy), characterized by elevated levels of homocysteine (HCys) in the blood, is a major risk factor for neurovascular dysfunction, contributing to mechanisms such as oxidative stress, inflammation, and disruption of the blood-brain barrier (BBB). Maintaining BBB integrity in the context of HHcy is a crucial therapeutic target. Polyphenols, bioactive dietary compounds renowned for their antioxidant and anti- inflammatory properties, have emerged as potential modulators of HCys metabolism and protectors of vascular structures. Previous studies of our group have shown that oral supplementation with polyphenol-rich extracts from *Salicornia ramosissima* significantly reduces HCys plasma levels in both healthy individuals and patients who have experienced a transient ischemic attack.

In this study, we aimed to evaluate the direct effects of *S. ramosissima* polyphenols on HCys-induced BBB damage. To achieve this, we developed a three-dimensional human BBB spheroid model that incorporates both HCys and hypoxia to mimic the neurovascular damage observed in HHcy. We assessed the release of inflammatory markers, as well as changes in the structural integrity and permeability of the BBB spheroids after treatment with *S. ramosissima* polyphenol-rich extract.

Additionally, we explored the effects of the extract in an in vivo Drosophila melanogaster model of HHcy. Supplementing Drosophila food with increasing doses of *S. ramosissima* extract significantly extended the lifespan of flies exposed to elevated homocysteine levels.

Together, these complementary in vitro and in vivo models underscore the potential of polyphenols to modulate HCys-induced vascular damage, supporting the development of targeted nutritional strategies aimed at counteracting homocysteine-related neurovascular dysfunction

3D Blood-Brain Barrier (BBB) Model for Studying Migration of VitD3DC-Tolerized T Cells In Vitro

Thursday, 2nd October - 13:45: (Patio Area) - Poster

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Background: The blood-brain barrier (BBB) is a highly selective physical barrier the blood and the neuronal microenvironment of the central nervous system (CNS). BBB dysfunction leads to increased cell infiltration into brain tissue, which is associated with numerous neurological and neurodegenerative disorders, like multiple sclerosis (MS). Understanding T cell migration across the BBB is vital for developing targeted therapies.
Objectives: (A) To establish an *in vitro* BBB system that accurately mimics the structural and functional properties of the BBB under homeostatic and MS-like pathological conditions. (B) To evaluate the migratory potential of VitD3DC-tolerized T cells using a dynamic BBB model.
Methods: This 3D *in vitro* BBB model involves a co-culture of human brain microvascular endothelial cells (HBMVEC) and human astrocytes (HA) embedded in a collagen-based hydrogel matrix. The model replicates the cellular interactions found in the BBB, allowing the study of cellular adhesion to the BBB endothelium and migration towards the CNS during pathologic conditions.
Results: The *in vitro* BBB model demonstrated high fidelity in replicating the key features of the *in vivo* BBB environment, including tight junction integrity and selective permeability. Under pathological conditions simulating neuroinflammation in MS, there was a notable disruption in barrier integrity, facilitating T cell migration. VitD3DC-tolerized T cells showed significantly reduced migration capacity across the BBB compared to non-tolerized autoreactive T cells, indicating effective tolerance induction.
Conclusion: Our 3D BBB model provides a robust platform for studying T cell migration in recruitment into the CNS during neuroinflammation in the context of multiple sclerosis. VitD3DC-induced tolerance significantly reduces the migratory capacity of autoreactive T cells, highlighting its potential therapeutic role in preventing CNS infiltration in autoimmune conditions like MS. Further studies will focus on refining this model and exploring the underlying mechanisms of T cell tolerance and migration.

Evaluating Angiopep-2 conjugation as an approach to enhance the brain delivery of a BCL-XL inhibitor

Thursday, 2nd October - 13:45: (Patio Area) - Poster

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Glioblastoma multiforme (GBM) is the most common form of brain cancer in adults, with a median survival rate of ~15 months post-diagnosis. BH3-mimetic compounds which inhibit the anti-apoptotic protein, BCL-X_L, are currently under investigation for their therapeutic potential in GBM. However, in order to be a viable treatment, these compounds must cross the blood-brain barrier (BBB) and reach sufficient concentrations within the brain to exert their anticancer activity. *In vivo* studies have shown that conjugation with the BBB-penetrating peptide Angiopep-2 (Ang-2), which targets the low-density lipoprotein receptor-related protein 1 (expressed at the BBB and upregulated in GBM cells), significantly enhanced the brain and tumour exposure of various anticancer agents, such as paclitaxel.[1] Therefore, in the present study, we investigated whether the brain uptake of a BCL-X_L inhibitor with relatively low brain penetration is increased when delivered as an Ang-2 peptide conjugate.
The BCL-X_L inhibitor was conjugated to Ang-2 at a 1:1 or 1:3 peptide-drug ratio *via* an ester linkage designed to allow cleavage of the free BCL-X_L inhibitor. C57BL/6J mice were dosed *via* intraperitoneal or intravenous injection with the inhibitor at a nominal dose of 5 mg/kg, or a molar equivalent dose of the Ang-2-BCL-X_L conjugate containing up to 5 mg/kg of inhibitor. Concentrations of BCL-X_L inhibitor and the intact conjugates were quantified in plasma and brain homogenate using liquid chromatography-tandem mass spectrometry, at designated timepoints up to 8 hours post-dose. Brain uptake was assessed according to the calculated brain-to-plasma ratio (B:P). Conjugate stability and release of free BCL-X_L inhibitor was also evaluated in isolated plasma and brain homogenate *ex vivo*.
A maximal average B:P of 0.125 ± 0.02 was determined for the inhibitor alone, indicative of low-to-moderate brain uptake. Unexpectedly, the BCL-X_L inhibitor was not detected at appreciable concentrations in brain homogenate in mice dosed with the Ang-2-BCL-X_L-inhibitor conjugates, or in brain homogenate *ex vivo*. The B:P ratios of the intact Ang-2 conjugates were indeed lower than those of inhibitor alone, suggesting that the conjugates exhibited limited BBB permeability. Studies are underway to elucidate the underlying reasons behind these outcomes.
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BBB maintenance in the uremic environment: Implications of AhR activation and permeability

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Leah Hernandez [1], ***Ms. Miriam Rosina*** [2], ***Ms. Beth Aitken*** [1], ***Dr. Elisa Sartirana*** [2], ***Ms. Asrah Al Moussaui*** [1], ***Dr. Samsul Arefin*** [1], ***Dr. Peter Barany*** [1], ***Dr. Lars Wennberg*** [1], ***Dr. Anna Witasp*** [1], ***Dr. Nina Kronqvist*** [1], ***Dr. Vincenzo Cantaluppi*** [2], ***Dr. Peter Stenvinkel*** [1], ***Dr. Karolina Kublickiene*** [1]

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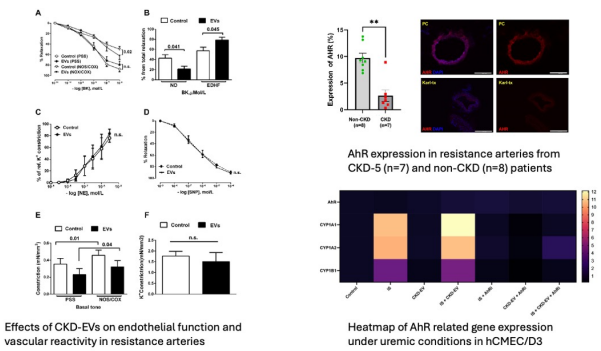
Background: Chronic kidney disease (CKD) increases cardio- and neurovascular risk, due to compromised BBB integrity. Aryl hydrocarbon receptor (AhR) is activated by uremic toxins and extracellular vesicles (EVs) from CKD patients. CKD-EVs carry uremic toxins and inflammatory mediators that potentially can contribute to BBB impairment. This study aims to investigate the effects of CKD-EVs and selected uremic toxins on the microvasculature function and structure and on human brain microvascular endothelial cells with focus on candidate genes expression and BBB function by means of permeability.

Methods: hCMEC/D3 were cultured and exposed to indoxyl sulfate (IS) and CKD-EVs alone or in combination, with or without AhR inhibitor. Gene expression of AhR and related downstream genes were measured by qPCR. AhR expression in resistance arteries from CKD patients and controls were assessed using immunohistochemistry. Subcutaneous fat was incubated with CKD-EVs and analysed for tight junction expression in visible microcirculation. Vascular function studies were conducted to evaluate endothelial function ex vivo.

Results: hCMEC/D3 exposed to IS showed increased expression of AhR downstream genes CYP1a1, CYP1a2, and CYP1b1 versus control. The induction was enhanced when IS was combined with CKD-EVs. Incubation with CKD-EVs alone did not seem to alter the gene expression. The AhR inhibitor was observed to suppress the induction of CYP genes. Ex-vivo experiments indicate that endothelial-derived factors differ between CKD-EVs-treated and non-treated groups, suggesting that CKD-EVs contribute to endothelial dysfunction by impairing nitric oxide (NO) pathway, potentially acting as uremic toxin in synergy with other toxins. AhR expression was lower in resistance arteries of CKD patients (2.6%) versus controls (9.7%). Incubation of subcutaneous fat with CKD-EVs resulted in a consistent though not significant, reduction in the expression of tight junction proteins claudin-5, occludin, and JAM-1 in the microcirculation of fat.

Conclusion: Preliminary findings show that uremic condition activates AhR signaling. CKD-EVs may contribute to endothelial dysfunction by impairing NO mediated vasodilatation. These CKD-derived EVs may act synergystically with other toxins to exacerbate vascular damage, and potentially disrupt tight junctions. Further research understand AhR and EVs-mediated mechanisms in microcirculation is needed to develop therapeutic strategies aimed at reducing cerebrovascular morbidity in CKD patients.



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Identification of new TFR1 interactors using TurboID

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Mr. Adrià Puigdemont [1], ***Dr. Goren Saenz-Pipaon*** [1], ***Dr. Eduardo Ruiz-López*** [1], ***Dr. Benjamí Oller-Salvia*** [1]

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The blood-brain barrier (BBB) acts as a highly selective permeability barrier that protects the central nervous system (CNS) from potentially harmful substances that may be circulating in the blood. However, this protective function also poses an important challenge for the delivery of many therapeutics into the brain, limiting the efficacy of treatments for neurological disorders such as brain tumours or Alzheimer's and Parkinson's diseases. Receptor-mediated transcytosis has emerged as a promising strategy to overcome this limitation by leveraging the natural transport of receptor-binding molecules into the CNS. Among the most attractive receptor targets is Transferrin Receptor 1 (TFR1), due to its abundant expression on the luminal surface of the brain endothelium and its essential role in iron transport into the brain. Still, despite the growing interest in exploiting TFR1 for drug delivery, the molecular mechanisms underlying its intracellular trafficking and transcytosis regulation remain poorly understood. In this study, we have engineered a fusion of TFR1 with TurboID, a biotin ligase, to enable proximity-dependent biotinylation of nearby proteins, allowing the identification of previously uncharacterized interactors using mass spectrometry-based proteomics. These results will help develop novel strategies to enhance TFR1-mediated delivery of therapeutics across the BBB.

Brain-on-a-chip models: novel stem cell-based microelectronic and fluidic devices with translational potential

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Judit P. Vigh [1], Ms. Anna Kocsis [1], Dr. Ana Raquel P. Santa Maria [2], Dr. Gergő Porkoláb [3], Ms. Nóra Kucsápszky [1], Ms. Emese Belai [1], Dr. Silvia Bolognin [4], Prof. Jens C. Schwamborn [4], Dr. Sándor Valkai [1], Dr. András Kincses [1], Dr. Roland Wirth [1], Ms. Anikó Szecskó [1], Dr. Mária Mészáros [1], Dr. Szilvia Veszelka [1], Dr. Melinda Pirity [1], Prof. András Dér [1], Dr. Mária Deli [1], Dr. Fruzsina Walter [1]

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Novel lab-on-a-chip and organ-on-a-chip cell culture models are essential to investigate cerebral drug delivery, pathology, and protection of tissues in disease states. Microfluidic chip devices allow more complex and physiological modelling of the blood-brain barrier (BBB) and enable the co-culture of multiple human cell types, paving the way to decrease the need for animal experiments. The latest trend in pharmaceutical drug testing is the use of human brain organoids derived from induced pluripotent stem cells (iPSC). My work summarizes our efforts to create and optimize new, dynamic BBB-brain organoid microelectronic devices by the co-culture of human brain endothelial cells, brain pericytes with human midbrain organoids of hematopoietic or iPSC origin. Our lab-on-a-chip devices enable visual observation, impedance, and permeability measurements across the brain endothelial monolayer, and also the introduction of fluid flow to mimic blood circulation. In the midbrain organoid model, healthy and Parkinson's disease patient-derived cells were introduced to the system, and the effects of the organoids on the barrier integrity were evaluated. Targeted nanoparticles penetrated the BBB and entered the brain organoids successfully. The addition of cortical organoids enabled the testing of the effects of further pathologies. Changes in gene and protein expression of brain endothelial cells with or without the organoids were also identified. This complex system can be a valuable tool for pharmaceutical testing, pathology modelling, and toxicological studies.

Funding:
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Protective effect of ecdysteroids on the blood-brain barrier

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Ana Martins [1], ***Dr. Ana Raquel P. Santa Maria*** [2], ***Ms. Judit P. Vigh*** [3], ***Dr. Máté Vagvolgyi*** [4], ***Mr. David Laczko*** [4], ***Dr. Zoltan Szabo*** [5], ***Dr. Tamas Janaky*** [5], ***Dr. Viktor Horváth*** [2], ***Dr. Fruzsina Walter*** [3], ***Dr. Mária Deli*** [3], ***Dr. Attila Hunyadi*** [4]

1. *HUN-REN Biological Research Centre, Institute of Biophysics,* ***2.*** *Wyss Institute for Biologically Inspired Engineering,* ***3.*** *HUN-REN Biological Research Centre,* ***4.*** *Institute of Pharmacognosy, University of Szeged,* ***5.*** *Department of Medical Chemistry, University of Szeged*

Ecdysteroids are present in several edible plants and food supplements. Several of these compounds are known to exert a cytoprotective effect in mammals, including humans. Inspired by this bioactivity, in the current study we investigated the potential of 20-hydroxyecdysone (20E) and its derivatives to protect the blood-brain barrier (BBB) from oxidative stress.

Brain capillary endothelial cells provide the functional basis of the blood-brain barrier. We hypothesize that, *in vivo*, dietary ecdysteroids in the blood can interact directly with brain capillary endothelial cells. During this study, we investigated how 20E and some of its derivatives can affect the viability of hCMEC/D3 brain endothelial cells and barrier tightness. We also examined the effect of the compounds in combination with tert-butyl hydroperoxide, to model oxidative stress. Permeability and TEER measurements, as well as immunohistochemistry were used to examine the integrity of the blood-brain barrier upon treatment.

We observed that several ecdysteroids had a positive effect on barrier integrity. Under inflammatory and oxidative stress conditions, 20E and calonysterone protected the barrier from damage.

Our results show that ecdysteroids have the potential to be used as protective agents against damage caused by oxidative stress to the integrity of the blood-brain barrier.

Acknowledgments: NKFI-FK 137808, BO/00224/23, UNKP-23-5-SZTE-709 (AM). NKFI-K 134704 and TKP2021-EGA-32 (AH).

Multiscale physiologically-based pharmacokinetics (PBPK) modeling for enhanced brain targeting

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Zhendong Xie [1], ***Prof. Giuseppe Battaglia*** [2], ***Prof. Xiaohe Tian*** [3], ***Prof. Lorena Ruiz Pérez*** [1]

1. *Institute for Bioengineering of Catalonia (IBEC),* ***2.*** *Institute for Bioengineering of Catalonia & University of Barcelona,* ***3.*** *Sichuan University West China Hospital*

There are very few effective treatments for CNS disease due to tightly regulated barriers that maintain its delicate environment and protect neural tissue. Nanoparticles show great potential in the new generation of therapeutics, which has attracted significant interest from researchers due to their enhanced selectivity, reduced side effects, and ability to overcome biological barriers within the body. In our study, we fabricate synthetic nanoparticles to selectively target the highly selective semipermeable blood-brain barrier (BBB). These nanoparticles are constructed using amphiphilic poly[oligo(ethylene glycol) methyl methacrylate]-poly(2-(diisopropylamino)ethyl methacrylate) (P[(OEG)10MA]20-PDPA100), with some of the polymer chains adorned with peptide ligands as targeting units. In *vitro* tests show that the number of functional ligands directly influences their binding probability to target receptors. We employ phenotypic association theory (PAT), based on the multivalence effect, to elucidate the paramount parameters of this selectivity from a thermodynamic standpoint. We also collected in vivo data and employed a physiologically based pharmacokinetic (PBPK) model to simulate nanoparticle distribution in mice and to validate the efficiency of nanoparticle targeting the BBB. Using this model, we predicted drug distribution at various dosing concentrations to determine the best BBB targeting efficiency. In summary, we present a comprehensive workflow encompassing nanoparticle design and distribution prediction to minimize ineffective trials prior to clinical testing.

Rational Discovery of PDZ3 inhibitors to increase CNS paracellular permeability

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Rory Whelan [1], ***Dr. Grainne Hargaden*** [1], ***Dr. Andrew Knox*** [1]

1. *Technological University Dublin*

Background Anchoring of the JAM-A C-terminal motif (TSSFLV) to the third PDZ domain (PDZ3) of zonula occludens-1 (ZO-1) stabilises tight junctions of the blood–brain barrier (BBB). Disrupting this protein–protein interaction (PPI) is a promising strategy for transient and controllable BBB opening.

Methods ≈1 million compounds from the SPECS screening collection were protonated, tautomerised, converted to 3D conformers, and filtered through a pharmacophore derived from the X-ray structure 3TSZ, yielding 235 hits. Docking with FRED/ChemGauss-4 (OpenEye Scientific Software) and visual inspection prioritised two candidates—BZ1 and dDCA—whose poses replicated key JAM-A contacts. Binding was quantified using a donor-quenching FRET assay constructed from sCy3-labelled ZO-1 PSG and sCy5-labelled TSSFLV. Functional activity was assessed in bEnd.3 endothelial monolayers on Transwells, with trans-endothelial electrical resistance (TEER) recorded over 24 h.

Results The assay returned a JAM-A/ZO-1 dissociation constant of 31.1 ± 12 µM at 25 °C, consistent with published values and validating the assay format. At 50 µM, BZ1 and dDCA increased the K_D to 62.7 ± 29 µM and 54.5 ± 20 µM, respectively, indicating competitive inhibition. In bEnd.3 monolayers, both compounds induced a 38 ± 7 % drop in TEER at 4 h ($p < 0.01$, $n = 3$), followed by full recovery to 112 ± 15 % of baseline at 24 h, indicating reversible junctional loosening without cytotoxicity.

Conclusions An integrated computational (VS) and biochemical (FRET/TEER) pipeline validates PDZ3 as a tractable target for modulating BBB permeability and identifies BZ1 and dDCA as promising lead scaffolds. Ongoing work will visualise real-time JAM-A relocalisation and translate the approach to high-resistance iPSC-derived brain microvascular endothelial cells.

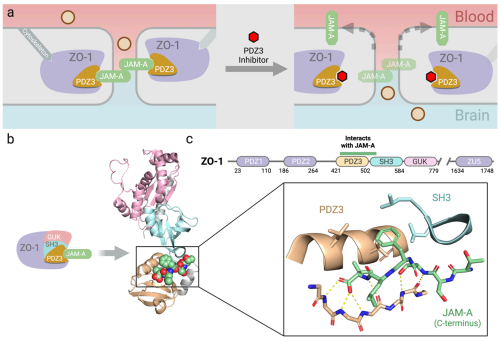


Figure 1. Development of PDZ3 inhibitors for transient blood–brain barrier opening. **a)** Schematic of the proposed mechanism: a small-molecule PDZ3 inhibitor blocks the interaction between the PDZ3 domain of ZO-1 and the C-terminal PDZ-binding motif of the tight-junction protein JAM-A, displacing JAM-A from the lateral membrane and thereby increasing paracellular permeability, **b)** X-ray crystal structure of the ZO-1 PSG fragment in complex with the JAM-A C-terminus (PDB ID: 3TSZ).

Figure 1.png

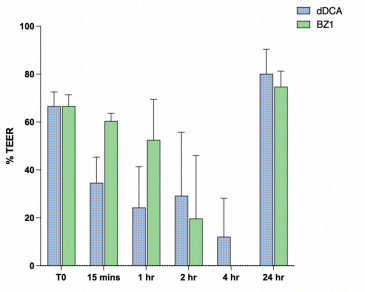


Figure 2. Transendothelial electrical resistance (TEER) of bEnd.3 monolayers following treatment with 50 µM dDCA or BZ1 over 24 hours.
TEER was measured at 0 min, 15 min, 1 h, 2 h, 4 h, and 24 h post-treatment and normalised to vehicle control (0.03 % DMSO, set as 100%). Media-only wells (cell-free inserts) were used to define 0% TEER. Data are presented as mean % TEER ± SD (N = 3 per condition).

Figure 2.png

CNS delivery of blood-brain barrier-crossing anti-Nogo-A antibodies: CNS penetration and Fc effector functions

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Sandrine Joly [1], ***Prof. Vincent Pernet*** [1]

1. *University of Bern/Bern University Hospital-Inselspital*

Background and rationale. Nogo-A is a potent inhibitor of neuronal plasticity in the central nervous system (CNS). The infusion of Nogo-A-neutralizing antibodies, such as 11C7 monoclonal antibody, in the cerebrospinal fluid (CSF) of rodents enhances neuronal plasticity after brain and spinal cord injury. However, antibody delivery in the CSF, across the blood-brain barrier (BBB), is invasive and may not be optimal.

Hypothesis and objectives. The intravenous injection (i.v.) of BBB-crossing anti-Nogo-A antibodies may be a non-invasive and efficacious approach to stimulate CNS neuron recovery after injury. To test this hypothesis, we used transferrin receptor 1 (TfR1) as a Trojan horse.

Methods. A BBB-crossing antibody, thereafter called 11C7-scFv8D3, has been engineered by recombinantly fusing the light chain of 11C7 with the single-chain variable fragment (scFv) of 8D3, an IgG specifically binding mouse TfR1. The binding properties of 11C7-scFv8D3 and 11C7 were assessed by biolayer interferometry (BLI), ELISA, Western blotting and immunofluorescence on spinal cord section. *In vivo*, the antibody levels were monitored in blood, CSF and CNS tissues of mice by ELISA after intravenous administration. Potential cytotoxic effects of 11C7-scFv8D3 on reticulocytes were evaluated with standard hematology tests. Antibody interactions with FcRn and FcgRIIB and FcgRIII were examined by BLI.

Results. Our data revealed that 11C7-scFv8D3 bound Nogo-A equally well as 11C7 (KD$\leq$1 pM). Moreover, 11C7-scFv8D3 had a similar affinity for TfR1 to that of 8D3 IgG (KD~0.4 nM). These data were corroborated by ELISA and Western blotting. Following i.v., the level of 11C7-scFv8D3 rapidly decreased in plasma compared with 11C7, but reached much higher levels than 11C7 in the brain and the spinal cord. In the blood of mice receiving 11C7-scFv8D3, the density of reticulocytes was dramatically reduced. In BLI experiments, the KD of 11C7-scFv8D3 was higher than that of 11C7 for FcRn (4X), but much weaker for FcgRIIB and FcgRIII (4-5 X).

Conclusions and discussion. 11C7-scFv8D3 penetrates into the mouse CNS by interacting via TfR1. However, grafting a scFv8D3 module at the C-term of 11C7 modifies Fc effector functions that may affect antibody clearance and cytotoxic effects in the periphery.

Development of an orthogonal receptor to transport drugs to the brain

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Burcu Yaldiz [1], ***Dr. Eduardo Ruiz-López*** [1], ***Mr. Adrià Puigdemont*** [1], ***Mr. Benjamin Max Konecny*** [1], ***Dr. Benjamí Oller-Salvia*** [1]

1. *Institut Quimic de Sarria - University Ramon Llull*

Neurological diseases such as Alzheimer's, Parkinson's, and brain tumors remain major challenges in medicine due to the limited efficacy of existing therapeutics. The blood-brain barrier (BBB), while essential for brain protection, severely restricts drug delivery. Current brain shuttle systems leveraging receptor-mediated transcytosis (RMT) offer limited transport enhancement, primarily due to the lack of receptors combining high transport efficiency, BBB-specific expression, and minimal peripheral distribution. In our project, called Creating an Orthogonal Gate to the Brain (OBGate) —funded by the European Research Council— we focus on developing synthetic receptors to be expressed at the BBB and designed to interact exclusively with a custom ligand while avoiding interactions with endogenous ligands. In this poster, we present one of our approaches, based on a natural receptors with transcytosis capacity. Receptor variants have been screened for binding efficiency and evaluated for their expression and internalization capacity. We are currently exploring a range of variants to maximize transport across endothelial cells. By minimizing off-site interactions and enabling precise molecular recognition, this approach lays the groundwork for improving the targeted delivery of therapeutics across the BBB.

iPSC-derived microvascular 3D blood-brain barrier model to study microglia driven pathogenesis in cerebral malaria

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Judit González Gallego [1], ***Dr. Francois Korbmacher*** [1], ***Dr. Livia Piatti*** [1], ***Mrs. Alina Batzilla*** [1], ***Mr. Dennis Crusius*** [1], ***Dr. Borja Lopez Gutierrez*** [1], ***Dr. Silvia Sanz Sender*** [1], ***Dr. Maria Bernabeu*** [1]

1. *European Molecular Biology Laboratory (EMBL) Barcelona*

Cerebral malaria is a severe and fatal complication of the *Plasmodium falciparum* infection which leads to blood-brain-barrier (BBB) breakdown upon sequestration of infected red blood cells in the brain microvasculature along with the accumulation of immune cells. *P.falciparum* is a human-specific pathogen and consequently, rodent models do not fully recapitulate its interactions with the brain microvasculature and disease mechanisms. This limitation urges and highlights the critical need for the development of advanced human *in vitro* models to enable more physiological relevant studies of infection, pathogenesis and drug discovery.

Our lab has previously generated a 3D-BBB model to understand the molecular disrupted pathways by the malaria parasite. Nevertheless, this model lacks a key component, microglia, the master regulator of the brain immune response. Here we aim to develop a fully human iPSC-derived microvascular BBB model composed by endothelial cells (iEC), pericytes (iPC), astrocytes (iAS) and microglia (iMG). We will then perfuse it with *P.falciparum*-infected red blood cells and the pathogenic pathways will be characterized using single-cell and spatial transcriptomics as well as confocal imaging and functional assays, with a special focus on the microglia inflammatory response. This will allow us to study BBB disruption and pathogenic pathways in a human-specific context and have a better understanding of molecular mechanisms leading to brain inflammation and BBB leakage. These approaches will provide critical insights to guide the development and testing of future therapeutics.

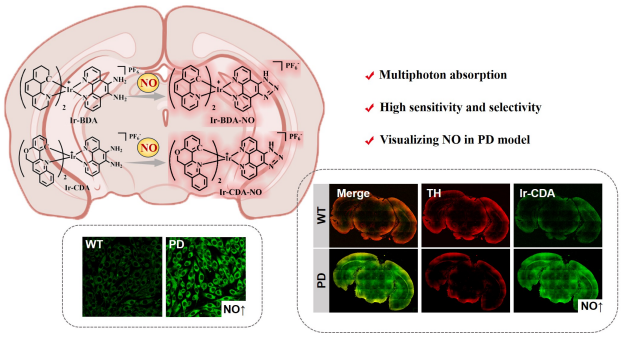
Cyclometalated iridium complexes for monitoring NO fluctuations in Parkinson's disease

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Dandan Chen [1]

1. *Sichuan University West China Hospital*

As an important neurotransmitter molecule, the dysregulation of nitric oxide (NO) homeostasis is closely associated with the pathogenesis of Parkinson's disease (PD) and other neurological disorders. Therefore, imaging NO in the brain is crucial for understanding pathophysiological processes. However, due to the selective permeability of the blood-brain barrier (BBB), most existing NO probes struggle to achieve highly sensitive and selective detection of NO in the brain. To address this challenge, we designed and synthesized two novel cyclometallated iridium(III) phosphorescent complexes, Ir-BDA and Ir-CDA, which are based on the specific reaction of diamine ligands with NO to form a triazole structure, enabling efficient NO detection. These complexes exhibit good biocompatibility, high selectivity, and a low detection limit. Their long-lived phosphorescence effectively minimizes interference from tissue autofluorescence, while their multiphoton absorption properties significantly enhance tissue penetration depth. In this study, we successfully visualized dynamic changes in NO levels during neuroinflammatory processes using a Parkinson's disease cell model. Through quantitative analysis of brain tissue sections from transgenic PD mice, we established a clear correlation between disease progression and elevated cerebral NO concentrations. Most significantly, our research revealed a dose-dependent relationship between the extent of dopaminergic neuron damage and fluctuations in NO concentration within the local microenvironment, providing direct experimental evidence for elucidating the molecular pathological mechanisms underlying Parkinson's disease. This work not only provides novel molecular tools for investigating NO's role in PD pathogenesis, but also offers potential strategies for monitoring neuroinflammation under blood-brain barrier regulation and developing targeted therapeutics. Looking forward, this technology shows promising potential for extension to NO-related research in other neurodegenerative disorders (such as Alzheimer's disease) and brain tumors, which may yield new insights for precision medicine and targeted treatment approaches.



1.jpg

Immunology Innovation Program: Developing Antibodies to Novel Targets via Co-creation

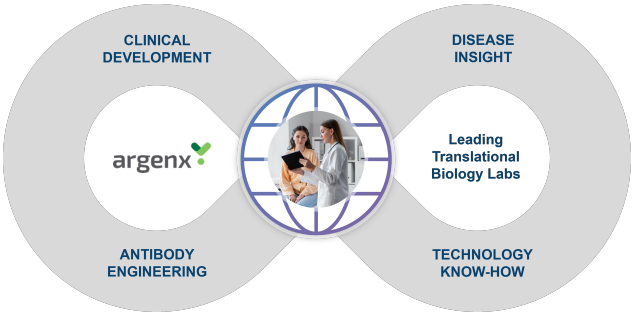
Thursday, 2nd October - 13:45: (Patio Area) - Poster

Mrs. Sofie Meulewaeter [1], ***Mr. Conor Mc Guire*** [1], ***Mr. René Bigirimana*** [1], ***Mrs. Magdalena Sips*** [1], ***Mrs. Ruani Fernando*** [1], ***Mrs. Valentina Lykhopiy*** [1], ***Mr. Bas Van Der Woning*** [1]

1. *argenx BVBA*

argenx is a commercial-stage global immunology company committed to improving the lives of those with severe autoimmune diseases. argenx is developing a broad portfolio of antibody therapies to multiple targets, ranging from early research programs to marketed products. All programs in the argenx pipeline have been initiated in collaboration with leading translational biology laboratories.
argenx leverages its proprietary, cutting-edge SIMPLE Antibody™ Platform and engineering technologies to deliver highly differentiated antibodies. In 2024, the Immunology Innovation Program (IIP) team evaluated over 100 potential opportunities, which resulted in the initiation of five new research programs.
At argenx, we are on a mission to transform the lives of patients with severe autoimmune disease. To deliver on this mission, we are developing a broad pre-clinical and clinical pipeline. Through the Immunology Innovation Program, argenx has developed multiple assets that have reached patients, including one that is approved in various global markets.

To further expand our pipeline, the Immunology Innovation Program team is actively engaging in conversations with leading academic institutes to identify opportunities that can be co-developed into future therapeutic antibodies.



Immunology innovation program.png

In Silico Modeling of Nanoparticles Transport Across the Blood–Brain Barrier: A Systematic Review

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Qianqian Xia [1]

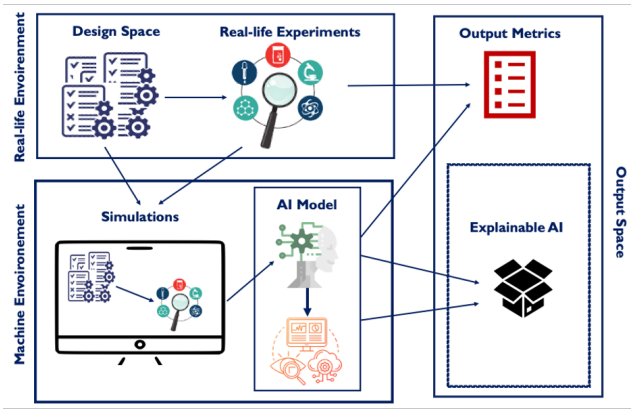
1. *Åbo Akademi University*

Reliable prediction and evaluation of nanoparticle (NP) transport across the blood–brain barrier (BBB) are essential for the rational design of CNS-targeted drug delivery systems. Traditionally, this process has relied heavily on in vivo animal models and in vitro cellular assays. In vivo models offer physiologically relevant insights into NP biodistribution but are costly, time-consuming, ethically constrained, and limited by species differences that reduce translational relevance. In vitro BBB models, such as transwell inserts or microfluidic co-culture systems, offer greater experimental control and higher throughput. Alternatively, in silico modeling and simulation serve as complementary tools that can enhance mechanistic understanding, support high-throughput virtual screening, and inform experimental design. These include simulations of protein corona formation, NP–membrane interactions, nanoparticle translocation via endocytosis, transcytosis, and so on, and data-driven modeling for predicting permeability from experimental or simulated data. Despite their growing importance, few systematic reviews comprehensively assess and categorize existing in silico methodologies for NP–BBB transport study.

In this review, we conduct a systematic literature analysis of 58 peer-reviewed studies that apply in silico approaches to model NP transport across the BBB. Following PRISMA 2020, we extracted data from PubMed, Scopus, and Web of Science using a structured search. Studies were categorized by NP type, transport mechanism, and computational technique. We identify five converging directions of computational modeling: (i) molecular simulations (all-atom molecular dynamics, coarse-grained molecular dynamics, dissipative particle dynamics, hybrid or enhanced sampling, and multiscale approaches), (ii) machine learning and deep learning, (iii) QSAR/QSPR descriptor-based models, (iv) pharmacokinetic/pharmacodynamic (PK/PD) system-level modeling, and (v) nanoinformatics frameworks including databases, ontologies, and workflow platforms. These directions span physics-based and data-driven paradigms, addressing three key stages of NP–BBB transport: protein corona formation in blood, NP–membrane interactions at the BBB interface, and cross-BBB transport through endocytosis, transcytosis, or diffusion.

This review highlights complementary opportunities where simulation-informed models and AI-driven approaches can support BBB permeability prediction, enable NP virtual screening, and accelerate the rational design of CNS-targeted nanomedicines.

Figure 1 shows how in silico simulations complement experiments for scalable design and mechanistic insight, while explainable AI delivers interpretable predictions for NP transport.



In silico ai modeling framework.png

Toxoplasma effector GRA15-driven CCL5 secretion enhances brain parasite load through microvascular sequestration of phagocytes

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Elena Afanaseva [1], ***Dr. Matias Rodriguez*** [1], ***Prof. Antonio Barragan*** [1]

1. *Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm*

The obligate intracellular protozoan *Toxoplasma gondii* infects phagocytes and utilizes them for systemic dissemination and, ultimately, colonization of the central nervous system. However, the mechanisms underlying the interaction between parasitized phagocytes and the endothelium remain poorly understood. Our findings reveal that *T. gondii* infection, but not parasite lysate, induces increased transcription and secretion of C-C motif chemokine ligand 5 (CCL5/RANTES) by endothelial cells and dendritic cells (DCs). This CCL5 expression was triggered by *T. gondii* effector GRA15 and mediated via NF-kB signaling, while effector TEEGR counter-regulated this effect MYR translocon-dependently. Moreover, infected phagocytes exhibited enhanced motility in the presence of recombinant CCL5, demonstrated chemotaxis towards a CCL5 gradient, and maintained transmigration. Intraperitoneally infected mice rapidly elevated *Ccl5* expression in the cerebral microvasculature, thereby increasing the adhesion of parasitized DCs GRA15-dependently. Pre-treatment of mice with recombinant CCL5 dramatically elevated sequestration while treatment with the selective chemokine receptor 5 (CCR5) antagonist Maraviroc reverted sequestration and reduced cerebral parasite loads. These findings offer new insights into the microbial strategies for subverting the host´s CCL5/CCR5 chemotactic signaling axis, revealing a GRA15/CCL5-driven mechanism by which *T. gondii* promotes leukocyte-mediated dissemination and colonization of the CNS.

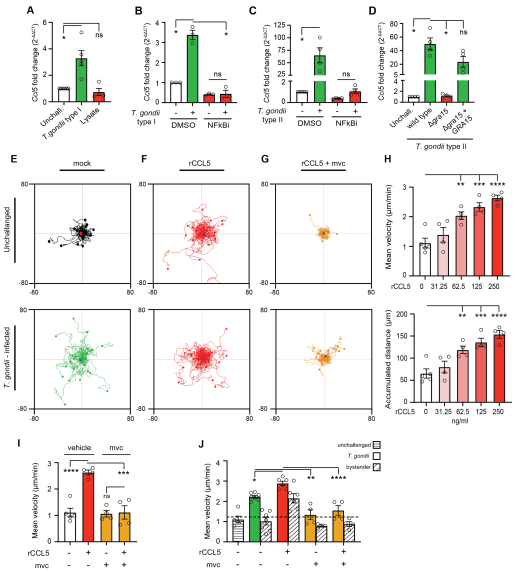


Figure 1. *Ccl5* expression by endothelial cells upon challenge with *T. gondii* and inhibition of cell signaling pathways andimpact of recombinant CCL5 and CCR5 inhibitor on motility of DCs.

(A-D) qPCR analyses of *Ccl5* cDNA from bEnd.3 cells challenged with *T. gondii* type I (RH LDM) tachyzoites or parasite lysate (A), type I (RH LDM) tachyzoites in the presence of NFkB/STAT3 inhibitors (B), *T. gondii* type II (PRU A7) tachyzoites in the presence of NFkB/STAT3 inhibitors (C) or T. gondii type II (PRU A7, parental wild type), GRA15-deficient mutant (Δgra15), and reconstituted mutant (Δgra15+GRA15) (D). Data are presented as mean (±SEM) from 3–4 independent experiments per condition and displayed as fold change in relation to unchallenged condition. (E-G) Representative motility plots of unchallenged or *T. gondii*-infected DCs mock-treated (E), in the presence of recombinant CCL5 (250 ng/ml) (F) or in presence of recombinant CCL5 (250 ng/ml) and CCR5 inhibitor (mvc) (G). (H) Mean velocity and accumulated distance of unchallenged DCs in the presence of recombinant CCL5. n=4-5. (I) Mean velocity of unchallenged or *T. gondii*-infected DCs in the presence of recombinant CCL5 and CCR5 inhibitor maravirоc (mvc). n=4-5. (J) Mean velocity of bystander or *T. gondii*-infected DCs in presence of recombinant CCL5 and CCR5 inhibitor maraaviroc (mvc). n=4-7.

Figure 1.png

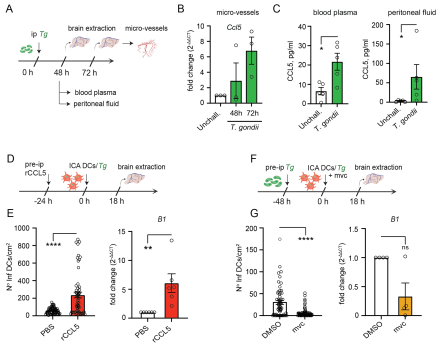


Figure 2. Impact of rCCL5 and CCR5 antagonism on the sequestration of infected DCs and cerebral parasite loads

(A) Experimental set up. Mice were inoculated ip with 3x10⁵ cfu of freshly egressed tachyzoites (type II, ME49-RFP) or control medium. After 48 h and 72h, brains were collected for microvessels purification. (B) Relative mRNA expression (qPCR) of CCL5 in brain microvessels. Samples were collected as shown under (A). Data expressed as mean ± SEM from three interdependent experiments (n= 3 mice). (C) Abundance of CCL5 (pg/ml) in blood plasma and peritoneal lavage, determined by ELISA (n=5 mice). Samples were collected as shown under (A). (D) Experimental setup. Mice were pre-treated ip with rCCL5 (1 µg) or mock treated. CMTMR-labelled DCs were challenged with *T.gondii* (PRU-GFP, MOI 2) to obtain an infection frequency of ~50%. Infected DCs (12x10⁶ DCs / 6x10⁶ cfu Tg) were inoculated in the brain circulation via ICA. Brains were collected at 18 h post-inoculation. (E) The absolute numbers of infected DCs (CMTMR+, GFP+) per cm² cortical tissue (60 cortical sections per condition) and relative expression (qPCR) of *T. gondii* TgB1 gene in brain tissue of mice pretreated and inoculated as in (D). Data are from three independent experiments (n= 6 mice). (F) Experimental setup. Mice were pre-infected ip with 3x10⁵ cfu type II ME49-RFP tachyzoites. Upon inoculation into the brain circulation via ICA, Maraviroc (mvc) or mock treatment was applied. Brains were collected at 18 h post-inoculation. (G) The absolute numbers of infected DCs (CMTMR+, GFP+) per cm² cortical tissue (60 cortical sections per condition from three independent experiments, n=6 mice) and relative expression (qPCR) of *T. gondii* TgB1 gene in brain tissue (three independent experiments, n= 4 mice) of mice pretreated and inoculated as in (F).

Figure 2.png

Development of brain shuttle peptides for the transport of antibodies, proteins, and gene delivery systems

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Cristina Díaz-Perlas [1], Dr. Maria Lucana Meneses [2], Dr. Nathanaël Rakotoarinoro [2], Ms. Ona Bretones Torres [2], Dr. Shambhavi Pandey [1], Dr. Benjamí Oller-Salvia [1]

1. *Institut Quimic de Sarria - University Ramon Llull,* ***2.*** *IQS - Ramon Llull University*

Presenting author: n.rakotoarinoro@iqs.url.edu. Corresponding authors: cristina.diaz@iqs.url.edu, benjami.oller@iqs.url.ed.

ABSTRACT

Receptor-mediated transcytosis represents a promising non-invasive strategy for delivering large therapeutic molecules, such as antibodies and nanocarriers, into the brain. Over the past decade, our research has focused on identifying and engineering peptide-based carriers capable of crossing the blood-brain barrier (BBB).[1] This poster outlines our work on chemically enhanced peptide shuttles, with particular emphasis on a novel class of bicyclic peptides termed BrainBikes.[2] BrainBikes are generated by modifying linear shuttle peptides using a trifunctional linker, which improves their structural stability and binding affinity. Our lead candidate, BrainBike-4, specifically targets transferrin receptor 1 and has been conjugated to poly(β-amino ester) vectors incorporating zwitterionic groups to enhance specificity and BBB transport.[3] Notably, BrainBike-4 achieved a fivefold enhancement in the transport of protein therapeutics, including antibody fragments, across the BBB. Building on insights from previous shuttle designs,[4] we are currently investigating how conjugation site and valency influence the delivery of full-length antibodies.

1. Prades *et al. Mol Pharm.* 2025, 22, 1100

2. Lucana *et al. RSC Chem. Biol*., 2024, 5, 7

3. Lucana *et al. Drug. Deliv. Transl. Res.* 2025. DOI: 10.1007/s13346-025-01902-z

4. Masmudi-Martín *et al. Mol Pharm, 2025, 22, 1384*

Microglia-microvascular endothelial cell heterogeneity and intercellular communication in relation to white matter abnormalities in vascular cognitive impairment and dementia

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Dr. Emina Hayashida [1], ***Dr. Eleni Papachristoforou*** [1], ***Dr. Juraj Koudelka*** [2], ***Dr. Gan Han*** [3], ***Dr. Jack Barrington*** [4], ***Prof. Catherine Hall*** [5], ***Dr. Axel Montagne*** [6], ***Prof. Neil Henderson*** [7], ***Dr. Barry McColl*** [6], ***Prof. Raj Kalaria*** [3], ***Dr. Jill Fowler*** [1], ***Prof. Karen Horsburgh*** [1]

1. *Institute for Neuroscience and Cardiovascular Research, University of Edinburgh,* ***2.*** *Institute for Neuroscience and Cardiovascular Research, UK Dementia Research Institute, University of Edinburgh,* ***3.*** *Translational and Clinical Research Institute, University of Newcastle,* ***4.*** *UK Dementia Research Institute, University of Edinburgh,* ***5.*** *School of Psychology and Sussex Neuroscience, University of Sussex,* ***6.*** *UK Dementia Research Institute, Institute for Neuroscience and Cardiovascular Research, University of Edinburgh,* ***7.*** *Centre for Inflammation Research, Institute for Regeneration and Repair, University of Edinburgh*

White matter abnormalities, linked to vascular dysfunction, are critical determinants of vascular cognitive impairment and dementia (VCID). Substantive preclinical and clinical evidence suggests disruption of the microglia-microvascular endothelial cell axis influences white matter abnormalities leading to cognitive deficits. However, the mechanisms by which microglia-endothelial cell interactions influence white matter abnormalities remain to be defined. To provide further mechanistic insight, we are using single nucleus RNA sequencing (snRNA-seq) approaches to firstly dissect microglia-microvascular endothelial cell heterogeneity and secondly identify alterations in intercellular communication in relation to white matter abnormalities in VCID.

We are studying a unique prospectively and clinically characterised post-mortem cohort (Cognitive Function After STroke, CogFAST study) of aged individuals with and without VCID, alongside age- and sex-matched controls. We have adapted the VINE-seq method to enable the isolation of nuclei from microvessels in parallel with vascular-related cells and microglia from frozen post-mortem white matter tissue. Using snRNA-seq, we aim to investigate the heterogeneity of vascular-related cells and microglia populations and identify differential gene expression profiles between individuals who are vulnerable or resilient to VCID. Further, bioinformatic approaches will be used to investigate ligand-receptor interactions between microglia and endothelial cells within the white matter and how these may be altered in VCID.

Our current approach will enable us to provide unique insights into microglia-microvascular endothelial cell communication and dysfunction in the context of cerebrovascular-mediated white matter disease.

Cognitive Dysfunction Following Burn Injury: The Role of Blood-Brain Barrier Disruption

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Shangke Liu [1]

1. *Sichuan University West China Hospital*

Burn injuries, beyond their immediate physical impact, have been increasingly recognized for their systemic effects, notably on neurological health. Emerging evidence indicates that severe burns can lead to cognitive impairments, including memory deficits and attention disorders, which are not solely attributable to psychological trauma but also to physiological changes within the central nervous system.
A pivotal factor in this process is the disruption of the blood-brain barrier (BBB), a critical structure maintaining cerebral homeostasis. Post-burn systemic inflammatory responses result in elevated levels of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. These cytokines can compromise BBB integrity by affecting tight junction proteins, leading to increased permeability. Consequently, neurotoxic substances and peripheral immune cells may infiltrate the brain parenchyma, triggering neuroinflammation and neuronal damage.

Clinical and experimental studies have corroborated these findings. Neuroimaging in burn patients has revealed structural and functional brain alterations correlating with cognitive deficits. Animal models further demonstrate that burn-induced BBB disruption precedes neuroinflammatory changes and cognitive decline. Understanding these mechanisms underscores the necessity for early interventions targeting systemic inflammation and BBB preservation to mitigate long-term neurological sequelae in burn survivors.

Regulation of brain endothelial fatty acid elongation during development and disease

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Brooke Tran [1], ***Dr. Sean Harvey*** [1], ***Dr. Richard Daneman*** [1]

1. *University of California, San Diego*

Dysregulation of the blood-brain barrier (BBB) is a hallmark of numerous neuroinflammatory disorders, including epilepsy, multiple sclerosis, and stroke. However, the molecular mechanisms underlying BBB breakdown in these conditions remain poorly understood. Using bulk-RNA sequencing to compare central nervous system (CNS) and peripheral endothelial cells as well as CNS endothelial cells across several disease models, we identified the fatty acid elongase enzyme **ELOVL7** as uniquely enriched in brain endothelial cells and dysregulated across neuroinflammatory models. Our findings show ELOVL7 levels decrease in neuroinflammatory conditions and implicate ELOVL7 activity in the resolution of neuroinflammation. Intriguingly, ELOVL7 diverges from other BBB-enriched genes in its developmental timeline—it is not expressed during embryogenesis like most BBB genes, but instead emerges post-natal. This raises fundamental questions regarding the unique upstream factors that regulate ELOVL7's distinct expression pattern. What drives ELOVL7 expression during development? What suppresses it during disease? And what reactivates it during inflammation resolution? To investigate these regulatory mechanisms, we are applying various inhibitors and activators targeting canonical BBB-induction pathways to cultured brain endothelial cells. Preliminary data suggest that **cAMP signaling** may play a key role in initiating ELOVL7 expression. Through understanding the molecular signals that regulate ELOVL7, we aim to uncover potential therapeutic targets to restore BBB function and ameliorate neurological disease.

Lysergic Acid Diethylamide (LSD) Enhances Angiogenesis and Maintains Blood-Brain Barrier Integrity

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. María Jesús Garrido Muñoz [1], ***Ms. Beatriz Guimarães*** [2], ***Dr. Ignacio Casanova-Maldonado*** [1], ***Dr. Bárbara S. Casas*** [1], ***Dr. Stevens K. Rehen*** [2], ***Dr. Verónica Palma*** [1]

1. *Universidad de Chile,* ***2.*** *Instituto D'OR de Pesquisa e Ensino*

Lysergic acid diethylamide (LSD) is a psychedelic compound increasingly recognized for its potent ability to induce neuroplasticity within the central nervous system (CNS). Recent studies have highlighted its efficacy in modulating neuronal connectivity and synaptic plasticity, which underpin potential therapeutic benefits in psychiatric disorders. Neuronal activity and neuroplasticity critically depend on a tightly regulated neurovascular coupling, where metabolic demands of neural tissue are matched with appropriate blood supply. Neurogenesis and angiogenesis occur concurrently during embryonic brain development, coordinated by a complex intercommunication within the neurovascular niche involving neural progenitors and brain endothelial cells (BECs), ultimately leading to the formation and integrity of the blood-brain barrier (BBB). Given this intricate interplay, we investigated whether LSD exerts effects beyond neural plasticity by modulating angiogenesis and BBB function both *in vitro* and *in vivo*. We employed various LSD concentrations and analyzed both direct endothelial responses and indirect influences mediated via human cerebral organoid-derived secretome. Specifically, endothelial tube formation, trans-endothelial electrical resistance (TEER), and permeability were evaluated *in vitro*. Additionally, *in vivo* angiogenic responses were assessed using the chick embryo chorioallantoic membrane (CAM) assay. Our findings reveal that direct LSD treatment of BECs significantly enhances angiogenesis *in vitro*, characterized by increased endothelial tube formation. The CAM assay further corroborated these results, showing that LSD promotes the formation of larger, potentially more mature vessels. LSD did not directly affect BBB integrity, as evidenced by unchanged TEER and permeability measures. Conversely, the secretome derived from LSD-treated organoids did not enhance angiogenesis but notably exerted protective effects against BBB disruption, suggesting indirect neuroprotective signaling mechanisms. Collectively, these findings demonstrate that LSD modulates neurovascular interactions through both direct endothelial stimulation and indirect neural-mediated pathways. Enhanced vessel diameter and preserved BBB integrity could support the establishment of functional vascular networks, potentially beneficial for psychiatric conditions characterized by impaired neurovascular coupling and BBB disruption, such as depression and schizophrenia. This study provides novel insights into the neurovascular dimension of LSD's therapeutic potential, supporting its exploration as a multifaceted intervention for neuropsychiatric disorders.

Development of an In Vitro Model of the Blood-Brain Barrier to Study the Implications of Diabetes Mellitus in Alzheimer's Disease

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Mrs. Amanda Martins Story [1], ***Mrs. Paula Ferreira*** [1], ***Mrs. Geisa Salles*** [1], ***Dr. Márcio da Luz*** [1], ***Dr. Thabata Nakamura*** [1], ***Prof. Marimélia Porcionatto*** [1]

1. *Department of Biochemistry, Escola Paulista de Medicina, Federal University of São Paulo - UNIFESP*

Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) are two chronic diseases with a high prevalence among the aging population and have become major public health issues, with a significant risk to each other. Recently, more attention has been directed to deciphering the role played by advanced glycation end-products (AGEs), whose actions have been highlighted as a strong link between these diseases. AGEs interaction with AGE receptors (RAGE) trigger a series of molecular processes that could lead to the development of AD in T2DM patients, such as blood-brain barrier (BBB) breakdown, beta-amyloid (Aβ) protein aggregation, astrocyte reactivity, and neurodegeneration. To better understand the mechanisms associated with and triggered by AGEs, it is necessary to develop *in vitro* models that capture the complexity of the composition and functioning of BBB. Therefore, we aimed to develop a cellular model of the BBB to mimic a hyperglycemic condition similar to T2DM and investigate the action of AGEs in this structure. The model was organized in a transwell system with endothelial cells and astrocytes derived from hiPSCs, located in the apical and basolateral portion of the insert, respectively, and neurons derived from the SH-SY5Y human neuroblastoma cell line, presented at the bottom of the well. The measurement of transendothelial electrical resistance and permeability to FITC-Dextran revealed no significant damage to the BBB after exposure to 100 µg/mL AGE-BSA in medium with high glucose level (35 mM) for 48 hours. However, an initial qualitative analysis of the neurons using immunofluorescence suggested an increase in the fluorescence intensity of amyloid protein precursor (APP), RAGE, and Aβ oligomers (AβO) markers, in addition to morphological changes (figures 1A and 2). Moreover, the analysis of a single-cell and spatial RNA-seq database for Alzheimer's disease (ssREAD) revealed two differentially expressed genes in human astrocytes involved in the AGE-RAGE signaling pathway; *PLCB1* was upregulated and *F3* was downregulated in prefrontal cortex astrocytes. In conclusion, although our data showed no BBB dysfunction, neurons responded with metabolic changes that may represent the pathogenesis of AD (figure 1B) and astrocytes might contribute to these alterations through their close interaction with neurons.

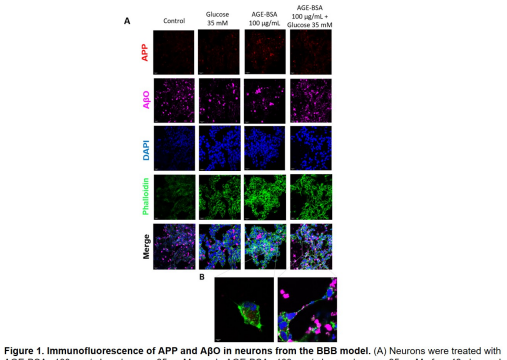


Figure 1. Immunofluorescence of APP and AβO in neurons from the BBB model. (A) Neurons were treated with AGE-BSA 100 µg/mL, glucose 35 mM, and AGE-BSA 100 µg/mL + glucose 35 mM for 48 h, and immunofluorescence was performed after this period. Staining for APP (red), AβO (magenta), DAPI (blue), and phalloidin (green). With phalloidin, we can observe changes in cytoskeletal architecture in each treatment group. (B) In the groups treated with AGE-BSA 100 µg/mL and AGE-BSA 100 µg/mL + glucose 35 mM, we identified neurons in conditions similar to those found in AD. Apparently, we see one degenerating neuron (left) and two neurons with AβO accumulation near the synaptic cleft. (scale bar 20 µm, x63 magnification)

Figure 1 story a.jpg

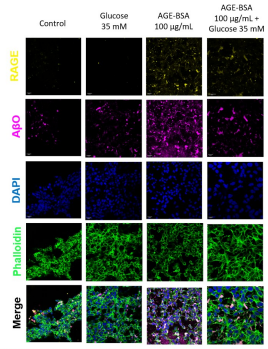


Figure 2. Immunofluorescence of RAGE and AβO in neurons from the BBB model. Neurons were treated with AGE-BSA 100 µg/mL, glucose 35 mM, and AGE-BSA 100 µg/mL + glucose 35 mM for 48 h, and immunofluorescence was performed after this period. Staining for RAGE (yellow), AβO (magenta), DAPI (blue), and phalloidin (green). With phalloidin, we can observe changes in cytoskeletal architecture in each treatment group. (scale bar 20 µm, x63 magnification)

Figure 2 story a.jpg

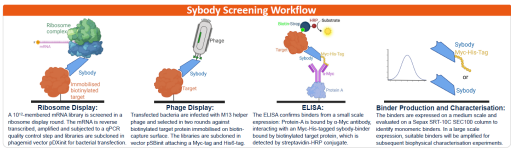
Development of brain shuttles based on synthetic nanobodies targeting CD98hc for receptor-mediated transcytosis

Thursday, 2nd October - 13:45: (Patio Area) - Poster

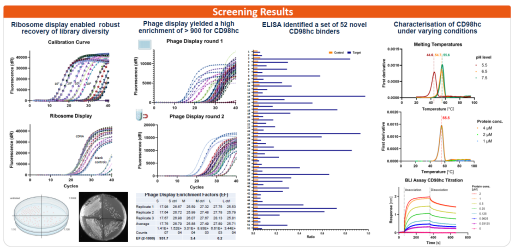
Dr. Elisabeth Rothweiler [1], ***Dr. Matthew Hankins*** [2], ***Dr. Mingda Ye*** [3], ***Mrs. Laura Braig*** [3], ***Dr. Georgia Isom*** [2], ***Dr. David Sauer*** [3], ***Dr. Franziska Guenther*** [1], ***Dr. Emma Mead*** [1], ***Dr. Emma Murphy*** [1]

1. *ARUK Oxford Drug Discovery Institute, Nuffield Department of Medicine Research Building, University of Oxford Old Road Campus , R oosevelt Drive, Oxford, OX3 7FZ,* ***2.*** *Sir William Dunn School of Pathology, University of Oxford, Oxford, OX1 3RE,* ***3.*** *Centre for Medicines Discovery, Nuffield Department of Medicine Research Building, University of Oxford Old Road Campus, Roos eve lt Drive, Oxford, OX3 7FZ*

The permeability of the blood brain barrier (BBB) poses a significant challenge for the development of effective Alzheimer's Disease therapeutics. As a means to facilitate drug delivery to the brain, receptor mediated transcytosis has been explored. CD98hc (SLC3A2) is the heavy chain of LAT1 which functions as a transporter for neutral amino acids across the BBB. CD98hc has been exploited via scFv-vehicles for receptor-mediated transcytosis of anti-amyloid beta antibodies across the BBB(1). In this study, we utilise a sybody screening platform(2) comprising ribosome-display and phage-display to select synthetic nanobody binders against biotinylated CD98hc. Following binder identification, we perform comprehensive biophysical characterisation to assess their binding affinities at varying pH, representing different cellular compartments.



Sb workflow.png



Sb result.png

The uptake of tau across the blood-brain barrier

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Melis Pisiren [1], ***Dr. Anna Masato*** [2], ***Dr. Catia Lopes*** [3], ***Ms. Alessia Santambrogio*** [4], ***Mr. Peifeng Xu*** [4], ***Dr. Amayra Hernandez*** [3], ***Dr. Amel Amara*** [5], ***Prof. Patric Turowski*** [5], ***Prof. Michele Vendruscolo*** [4], ***Prof. Giampietro Schiavo*** [2], ***Prof. Giuseppe Battaglia*** [3]

1. *Department of Chemistry, University College London, United Kingdom,* ***2.*** *UK Dementia Research Institute, University College London, London, United Kingdom,* ***3.*** *Institute for Bioengineering of Catalonia & University of Barcelona,* ***4.*** *Centre for Misfolding Diseases, Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge,* ***5.*** *UCL Institute of Ophthalmology, University College London, United Kingdom.*

The accumulation of neurodegenerative proteins, such as amyloid-beta (Aβ) and tau, plays a key role in the progression of diseases like Alzheimer's. Recent advances in biomarker research have shown that Aβ and tau can be detected in cerebrospinal fluid (CSF) and blood, providing valuable tools for early diagnosis and prognosis. However, the mechanisms by which these proteins bypass the blood-brain barrier (BBB) remain unclear, especially as the BBB becomes disrupted and leaky in disease states.

Our lab has previously shown that dysfunction of the low-density lipoprotein receptor-related protein 1 (LRP1), a crucial receptor for transporting macromolecules across brain endothelial cells, affects the processing of materials based on their avidity. Materials with high avidity are processed into the cell's endo-lysosomal network, while those with mid-avidity are shuttled across using the BAR domain protein, syndapin-2 (Tian et al., 2020). Our research has demonstrated that this dysfunction impairs the clearance of Aβ, leading to its accumulation and disease progression (Leite et al., 2022). This raises an important question about how tau is transported across the blood-brain barrier (BBB), with two main research goals: 1) reducing the burden of tau accumulation in the brain and 2) protecting the BBB from further damage.

To address these aims, we have optimized a human BBB in vitro model to study the uptake of monomeric and fibrillar tau via its binding to LRP1. We hypothesize that tau may be transported through two distinct pathways: 1) the Rab5-mediated endocytic route, which could lead to tau accumulation, and 2) the syndapin-2-mediated tubular pathway, which may promote tau clearance. Understanding these mechanisms may enhance biomarker-based diagnostics and open new therapeutic avenues to mitigate BBB disruption and neurodegenerative disease progression.

Non-parametric analysis of endocytic patterns identifies phenotypic behaviour of endothelial membrane proteins and helps predict aging-related dynamics.

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Valentina Schastlivaia [1], ***Prof. Giuseppe Battaglia*** [2], ***Dr. Daniel Gonzalez Carter*** [2]

1. *Institute for Bioengineering of Catalonia (IBEC),* ***2.*** *Institute for Bioengineering of Catalonia & University of Barcelona*

Endocytic behaviour of cell membrane proteins plays a crucial role in BBB function. Hence, temporal dynamics of protein endocytosis can reveal mechanisms underlying BBB specialization and dysfunction.

Leveraging high-throughput proteomic data quantifying cell membrane abundance of large protein arrays across time, we map the temporal endocytic profile of 3,617 proteins in brain and peripheral endothelial cells in healthy and inflamed conditions. Applying non-parametric methodologies combining Dirichlet Process for automatic clustering and Gaussian Process for trajectory modelling, we group protein behaviour without the need for pre-specified cluster counts or functional forms.

Our analysis identified 110 distinct clusters of endocytic behaviour patterns. Based on pairwise similarity between proteins, we designed a descriptive quantitative and qualitative features analysis, revealing a peripheral-like behaviour shift during inflammation. Statistical analysis showed significant positive correlation between endocytic rate and peripheral-like behavioural expression ($p < 0.05$). Machine learning models trained on the endocytic pattern clusters predicted protein aging dynamics with a weighted accuracy of 0.18 and Cohen's kappa of 0.113, significantly exceeding random chance.

Together, these results indicate short-term endocytic behaviour of cell-membrane proteins identifies phenotypic signatures and contains latent information to predict aging-related behaviour.

Phthalate exposure impacts blood-brain barrier integrity and circadian rhythm regulation

Thursday, 2nd October - 13:45: (Patio Area) - Poster

Ms. Destiny Tiburcio [1], ***Ms. Makenna Parsell*** [1], ***Ms. Michala O'Neill*** [1], ***Ms. Omolade Adeyemi*** [1], ***Dr. Sophia George*** [1], ***Dr. Michal Toborek*** [1]

1. *University of Miami*

The purpose of this study is to determine whether phthalate exposure can impact the integrity and structural function of the blood-brain barrier (BBB), as well as investigate the effects it can have on circadian functioning in the brain. Phthalates are persistent organic pollutants that are ubiquitously found in personal care products, industrial building materials, food packaging, and medical devices, leaving humans vulnerable to exposure via oral ingestion, dermal contact, and even intrauterine exposure. Preliminary data from an *in vitro* BBB model demonstrates aberrant expression of tight junction (occludin, claudin-5) and core clock genes following phthalate exposure. To assess how these chemicals impact the BBB and dysregulate circadian function, adult, wild-type mice were administered a mixture of five phthalates found in the groundwater of a Superfund site in Homestead, FL via oral gavage for 6 weeks. Tissue analysis revealed selective dysregulation of tight junction proteins responsible for the barrier function of the BBB. We also observed changes in the expression of core clock genes and markers of inflammation in the suprachiasmatic nucleus, the master regulator of circadian rhythms within the hypothalamus. These data suggest that chronic phthalate exposure can influence the integrity and permeability of the BBB, as well as impact the highly regulated expression of core clock genes responsible for a wide range of downstream biological processes.

How the brain barriers maintain CNS immune privilege

Thursday, 2nd October - 14:45: (Auditorium 1) - Invited Speaker

Prof. Britta Engelhardt [1]

1. *The University of Bern*

How the brain barriers maintain CNS immune privilege:
The central nervous system (CNS) has a unique relationship with the immune system called CNS immune privilege. CNS immune surveillance is precisely regulated by a compartmentalization of the CNS etablished by distinct anatomical barriers. These include the blood–brain barrier (BBB), blood–cerebrospinal fluid barrier (BCSFB), the artachnoid barrier and the glia limitans, which together form a dynamic interface allowing immune surveillance while protecting the brain parenchyma. The presentation will illustrate how immune cells, particularly activated T cells, access perivascular and meningeal spaces without disrupting the CNS parenchyma and how T cells breach the glia limitans leading to neuroinflammation. Our findings refine the understanding of CNS immune privilege and open new avenues for targeted therapies and biomarker discovery in neurological disorders

Advanced Tools for Studying Blood-Brain Barrier Interactions in Health and Disease

Thursday, 2nd October - 15:25: (Auditorium 1) - Invited Speaker

Prof. Ben Maoz [1]

1. *Tel Aviv University*

The blood-brain barrier (BBB) is crucial for maintaining neural homeostasis, yet its role in disease pathophysiology remains incompletely understood, primarily due to limitations of conventional models. This talk presents cutting-edge organ-on-a-chip technologies developed to precisely investigate metabolic and functional interactions between the human BBB and human neuronal networks. Using these advanced microfluidic platforms, we identified critical metabolic pathways and calcium mediators involved in BBB-neuron communication under physiological conditions. Furthermore, we demonstrate how BBB function is altered during pathological states such as cancer, neurodegenerative disease and traumatic brain injury, highlighting potential targets for therapeutic intervention and novel approaches for personalized medicine.

Blood-brain barrier permeability contributes to cognitive impairment in the aftermath of SARS-CoV-2 infection: a role for cerebrovascular Wnt/beta-Catenin and Caveolin-1

Thursday, 2nd October - 16:05: (Auditorium 1) - Invited Speaker

Prof. Sarah Lutz [1]

1. *University of Illinois at Chicago*

Cognitive impairment persists for years after SARS-CoV-2 infection in many individuals. The mechanisms underlying long-lasting neurological deficits are unclear, and therapeutic interventions are limited. Emergent data correlates blood-brain barrier (BBB) permeability to cognitive impairment in long COVID patients, leading to the possibility that long-lasting brain endothelial cell dysfunction contributes to post-infectious neurological deficits. We studied mechanisms of brain endothelial cell dysfunction in COVID-19 patient samples and in multiple animal models of SARS-CoV-2 infection. Our experiments revealed that mild respiratory SARS-CoV-2 infection caused BBB permeability, leukocyte infiltration, and cognitive impairment that persisted for months post-infection. Unbiased RNA sequencing and immunostaining revealed downregulated cerebrovascular canonical Wnt/beta-catenin pathway activity, thereby increasing Caveolin-1 (Cav-1) expression. In turn, heightened Cav-1 activity led to transcellular BBB permeability through increased vesicular trafficking and to paracellular BBB permeability through destabilization of tight junctions. These structural changes to the BBB facilitated CNS influx of proinflammatory fibrinogen and CD4+ T cells, which closely correlated with cognitive impairment. Importantly, interventions using pharmacologic, genetic, and gene therapy vector strategies targeting Cav-1 or Wnt/beta-catenin pathway components were able to prevent or reverse BBB permeability, intra-CNS leukocyte activation, neuroinflammation, and cognitive impairment after SARS-CoV-2 infection. Together, these studies support that BBB permeability contributes to neuroinflammation and cognitive impairment after COVID-19, and suggest the value of continued exploration of therapeutic strategies targeting the BBB in Long COVID and other chronic neuroinflammatory diseases.

Blood Brain Barrier - Natural Peptide Shuttles for Drug Delivery into the CNS

Thursday, 2nd October - 17:15: (Auditorium 1) - Oral

Dr. Roland Hellinger [1], ***Ms. Paula Schwarz*** [1], ***Prof. Irena Loryen*** [2], ***Prof. Christian Gruber*** [1]

1. *Medical University of Vienna,* ***2.*** *University of Uppsala*

The majority of drug-like molecules are unable to cross the blood-brain barrier (BBB) due to its rigorous transport regulation. (1,2) Hence, the BBB permeability of drug candidates is essential for therapeutic success if the disease-causing factors are within the central nervous system. Despite significant advances in peptide-based drugs in the last decades, the proteolytic instability continues to pose a major challenge in their development. Utilizing natural peptides as a scaffold in the design can enhance stability, affinity to a target, or cell penetration. (3) Our laboratory has established workflows for the rational design of therapeutic peptide probes using ribosomally-synthesized and post-translationally modified peptides as a starting point. (4) We employ peptide scaffold matching and the molecular grafting approach to design and synthesize proteolysis-stabilized peptides. (5) In this study, the prototypic sunflower trypsin inhibitor 1 (SFTI-1) was selected for the design of chimeric molecules incorporating reported BBB shuttles, such as peptide 22 and MiniAp-4. These potential 'Trojan Horse' probes were assessed in a brain microvascular endothelial transport assay. SFTI-1-peptide 22 arose from this transport screening as a bioactive probe, obtaining a permeability (P_{app} 9 x 10^{-6}) that significantly surpassed that of peptide 22 (P_{app} 3.5 x 10^{-6}) and native SFTI-1 (P_{app} 5.3 x 10^{-6}). Further, we evaluated general and CNS-specific pharmacokinetic (PK) parameters with the aim to determine the unbound brain-to-plasma ratio ($K_{p,uu,brain}$) for SFTI-1-peptide 22. Pharmacokinetic characterization with the brain slice method, the plasma- and the brain tissue binding assays predicted a $V_{u,brain}$ of 0.175, $f_{u,plasma}$ of 0.587 and a $f_{u,brain}$ of 0.120, respectively. The *in vivo* BBB permeability evaluation through intravenous administration of the probe in the rat model will inform on the concentration in the brain (K_p) and ultimately allow the derivation of the $K_{p,uu,brain}$ parameter. Our findings indicate that nature-derived peptide scaffolds can be utilized to enhance the stability of an incorporated bioactive peptide and facilitate transcellular transport, providing proof-of-concept for designing stabilized peptide BBB shuttles.

1. doi:10.1158/1078-0432.CCR-19-3258
2. doi:10.1602/neurorx.2.1.
3. https://doi.org/10.1038/s41589-018-0039-y
4. doi: 10.1038/s43586-023-00205-2
5. doi: 10.1021/acs.jmedchem.5c00677

Acknowledgment

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Targeted Delivery of a Highly Specific Anti-pE3–42 Amyloid-β Antibody via Transferrin Receptor Shuttle for Alzheimer's Disease

Thursday, 2nd October - 17:30: (Auditorium 1) - Oral

Dr. Sarah Headland [1], ***Dr. Phillip Liu*** [1], ***Dr. Andi Liu*** [1], ***Dr. Mohsen Karimi*** [1], ***Dr. Jia Hao*** [1], ***Dr. Geoff Norris*** [1], ***Dr. Wei Yan*** [1]

1. *Sound Biologics*

Targeting pyroglutamate-modified amyloid-β (pE3–42 Aβ) species offers a promising strategy for disease-modifying treatment in Alzheimer's disease (AD). We have developed a monoclonal antibody with high specificity for pE3–42 Aβ oligomers and fibrils, exhibiting minimal off-target binding in immunohistochemistry (IHC) compared to benchmark antibodies lecanemab, donanemab, and remternetug. To mitigate Fc-mediated inflammatory responses, the antibody includes a K322A mutation to abrogate C1q binding.

In the FAD4t transgenic mouse model, our antibody significantly reduced Aβ pathology as measured by 6E10-positive plaque area and insoluble Aβ levels, outperforming clinical-stage anti-Aβ antibodies. To enhance brain delivery, we engineered a brain shuttle version by fusing a monovalent anti-human transferrin receptor (TfR) VH domain to the Fc region. This format achieved approximately 20-fold higher brain exposure compared to the unshuttled antibody, confirmed via ELISA and IHC imaging.

We are now advancing this antibody into efficacy testing in mice humanized for both TfR and Aβ expression (FAD4t), followed by GLP toxicology studies in cynomolgus monkeys. These results support further development of this next-generation, brain-penetrant anti-Aβ therapeutic with an improved safety profile.

Brain distribution, Aβ interaction, and immune cell engagement of Lecanemab with a brain shuttle.

Thursday, 2nd October - 17:45: (Auditorium 1) - Oral

Ms. Enrica Cerilli [1], ***Ms. Min Xue*** [1], ***Dr. Sara Lopes van den Broek*** [2], ***Prof. Stina Syvänen*** [1], ***Dr. Dag Sehlin*** [1]

1. *Uppsala University,* ***2.*** *University of Uppsala*

Lecanemab, recently approved as the first disease-modifying therapy for Alzheimer's Disease (AD), effectively targets and clears amyloid-beta (Aβ) aggregates. However, its impact on cognitive decline remains limited, and treatment can be associated with potential adverse effects. To improve its therapeutic efficacy, we engineered a bispecific variant—bi-Lecanemab—with a brain shuttle targeting the transferrin receptor (TfR) to enable active receptor-mediated transcytosis across the blood-brain barrier (BBB). Both Lecanemab and bi-Lecanemab were recombinantly expressed on a human IgG1 scaffold, with bi-Lecanemab carrying a single-chain Fab fragment derived from the TfR antibody 8D3 to facilitate enhanced brain uptake. Antibodies were radiolabelled with iodine-125 (^{125}I) to assess their brain uptake, and fluorophore-conjugated to study their spatial distribution and assess interactions with brain-resident immune cells in the tg-ArcSwe transgenic mouse model of Aβ pathology. Results revealed enhanced (fig.A) and widespread accumulation of bi-Lecanemab throughout various brain regions in tg-ArcSwe mice. Notably, this accumulation strongly co-localized with amyloid-β aggregates (Fig.B), indicating effective targeting of pathological structures. In addition, bi-Lecanemab showed clear and active engagement with brain-resident immune cells — such as microglia (Fig.C) — suggesting potential involvement in immune-mediated clearance mechanisms or modulation of the local neuro-inflammatory response.

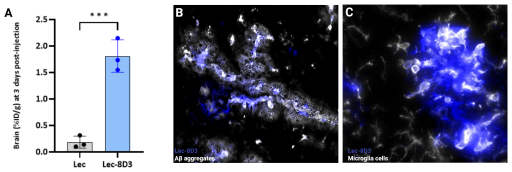

Figure. A. Brain uptake, expressed as percent of injected dose per gram of brain tissue (%ID/g), comparing Lecanemab (Lec) with bi-Lecanemab (Lec-8D3) at 3 days after injection in tg-ArcSwe mice; **B,C.** Immunofluorescence staining of tg-ArcSwe brain sections showing Lec-8D3 and Aβ aggregates (B); Lec-8D3 and microglia cells (C).

Brain uptake and immunofluorescence of bi-lecanemab.png

Novel Insights of Microthrombi-Mediated Blood-Brain-Barrier Dysfunction after Traumatic Brain Injury

Thursday, 2nd October - 17:15: (Auditorium 2) - Oral

Dr. Antonia Wehn [1]***, Dr. Sodai Yoshimura*** [2]***, Dr. Bernhard Groschup*** [3]***, Dr. Joshua Shrouder*** [3]***, Prof. Nicole Terpolilli*** [1]***, Dr. Andrey Klymchenko*** [4]***, Prof. Nikolaus Plesnila*** [5]***, Prof. Igor Khalin*** [6]

1. *Department of Neurosurgery, LMU University hospital, Munich,* ***2.*** *Department of Neurosurgery, Nihon University School of Medicine, Tokyo,* ***3.*** *Institute for Stroke and Dementia Research (ISD), LMU University hospital, Munich,* ***4.*** *Laboratoire de Biophotonique et Pharmacologie, University Strasburg, Strasburg,* ***5.*** *Institute for Stroke and Dementia Research (ISD) University Hospital, LMU Munich, Munich, Germany,* ***6.*** *Normandie University, UNICAEN, INSERM UMR-S U1237, Physiopathology and Imaging of Neurological Disorders (PhIND), GIP Cyceron, Institute Blood and Brain @ Caen-Normandie (BB@C), Caen*

Background: Traumatic brain injury (TBI) is characterized by reduced cerebral blood flow and increased blood-brain barrier (BBB) permeability, yet the mechanisms driving these disturbances and their long-term consequences remain insufficiently understood. Emerging evidence suggests that microthrombi (MTi) may play a critical role in the traumatic penumbra, contributing to BBB dysfunction. This study aims to characterize the formation and cellular composition of post-TBI MTi, their impact on BBB permeability, and their downstream effects.

Methods: MTi and BBB leakage were visualized following controlled cortical impact (CCI) in C57BL/6 mice using the systemic administration of highly fluorescent 30 nm lipid nanodroplets (LnDs). Brain tissue was stained for immune cells, platelets, erythrocytes, and fibrin, and analyzed via confocal fluorescence microscopy. To investigate the mechanisms of MTi-associated BBB disruption, we employed caveolin-1 knockout mice (Cav1tm1Mls/J) alongside wildtype littermate controls. In a rescue experiment, caveolin-1 expression was reintroduced using an adeno-associated virus tropic to endothelial cells (BI30-AAV) carrying a Cav1-eGFP construct, allowing targeted reconstitution of Cav1 in the brain vasculature.

Results: Approximately 50% of MTi within the traumatic penumbra were associated with extravasation of blood-derived molecules, including albumin, fibrinogen, IgG. MTi composition was heterogeneous, consisting of varying proportions of erythrocytes, platelets, fibrin, and leukocytes. The degree of BBB leakage positively correlated with cellular content in MTi (n=426 clots; R=0.31, p=0.02) and inversely with fibrin density (R=–0.64, p<0.001). Microglia located in proximity to MTi showed higher activation compared to distant microglia (p<0.001). Cav1 knockout significantly reduced extravasation at MTi sites compared to wildtype controls (n=5/group, p=0.002), along with reduced microglial activation (p=0.01). Notably, re-expression of Cav1 in endothelial cells of knockout mice restored the pathological phenotype, further supporting the role of Cav1 in MTi-mediated BBB dysfunction.

Conclusions: Our results demonstrate that microthrombi occlude cerebral vessels in the traumatic penumbra, thereby contributing to a focal increase in BBB permeability and subsequent activation of perivascular microglia. Increased BBB permeability is mediated by caveolin-1 in brain endothelial cells. Our results suggest that the increase in BBB permeability in the traumatic penumbra is mediated by increased caveolar transport across brain endothelial cells, offering a new therapeutic approach.

The contribution of endothelial derived lrg1 to blood-spinal cord barrier leakage and immune activation upon peripheral injury

Thursday, 2nd October - 17:30: (Auditorium 2) - Oral

Dr. Deniz Karabag [1], ***Dr. Jóse Ricardo Vieira*** [2], ***Ms. Ann-Kristin Kenkel*** [2], ***Dr. Christian Litke*** [2], ***Dr. Kishore Ravichandran*** [1], ***Dr. Andromachi Karakatsani*** [3], ***Dr. Anke Tappe-Theodor*** [4], ***Prof. Daniela Mauceri*** [2], ***Prof. Carmen Ruiz de Almodovar*** [5]

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Chronic pain is a debilitating condition affecting a significant percentage of the worldwide population. Crucial steps involved in pain chronicity revolve around diverse forms of maladaptive plasticity of both peripheral and central pathways of pain perception and processing. Increasing evidence point out towards a dysfunction of the neurovascular unit (NVU) with leakage of the blood-spinal cord barrier (BSCB), in the development and maintenance of chronic pain. In addition, peripheral as well as CNS immune cell activation has been shown to contribute to the transition from acute to chronic pain. How these different pathological processes are interlinked and influence each other is poorly understood. Here, we utilized the complete freund's adjuvant (CFA)-induced inflammatory pain model and the neuropathic pain induced spared nerve injury (SNI) model to gain more insight into the effect of peripheral pain on the NVU, its molecular mechanisms and its potential contribution to pain sensitivity and chronicity. Our data show that both pain models lead to a dorsal spinal cord specific local opening of the BSCB, without exhibiting significant changes in vessel morphology and density, pericyte coverage or astrocytic end-feet coverage. We identify Lrg1 as a gene highly upregulated in spinal cord endothelial cells upon pain induction. We show that intrathecal delivery of recombinant Lrg1 into wild type mice results in opening of the BSCB and increased mechanical pain sensitivity, while inhibition of Lrg1 expression attenuates mechanical pain sensitivity. Mechanistically, we find that Lrg1 leads to reduced expression of Claudin-5, a tight junction protein regulating BSCB permeability. Linking endothelial cell changes to the CNS innate immune response, we find that upon pain induction there is a significant, and spatially restricted, increase of vessel-associated microglia and that Lrg1 induces release of TNFa in microglia. Altogether, we describe a cellular and molecular mechanism that links peripheral pain stimuli to a local response of the NVU at the spinal cord dorsal horn that impacts on microglia function.

Post-stroke Microthrombi as Size-Selective Gateways for Nanoparticle Delivery Across the Blood-Brain Barrier

Thursday, 2nd October - 17:45: (Auditorium 2) - Oral

Dr. Igor Khalin [1]

1. *INSERM, Blood and Brain @ Caen Normandy Institute*

Clinical translation of nanoscale brain-targeted drug-delivery systems is hindered by limited understanding of how nanocarriers cross the blood-brain barrier (BBB), particularly under pathological conditions. Previous studies demonstrated retention of nanocarriers within injured brain tissue, but the precise entry routes and underlying mechanisms remained unclear, representing a critical translational barrier. In this study, we investigated the dynamics and mechanisms underlying BBB transport of highly fluorescent lipid nano-emulsion droplets (LNDs) of two sizes (30 nm and 80 nm) in mouse models of ischemic stroke. Using in vivo two-photon microscopy and ex vivo confocal imaging at subcellular resolution, we discovered that post-stroke cerebral microthrombi act as specific accumulation as well as extravasation sites for LNDs. Importantly, only 30 nm LNDs extravasated into the brain parenchyma, ultimately reaching neuronal cells, whereas 80 nm LNDs remained confined within the microthrombi in vessel lumen. Real-time Förster resonance energy transfer (FRET)-based imaging revealed significantly faster cargo release from LNDs within microthrombi compared to normal circulation. Correlative light-electron microscopy (CLEM) confirmed vesicle-mediated endothelial transcytosis of 30 nm LNDs at microthrombus, in single nanoparticle resolution. Mechanistically, this was associated with Cav-1 as a crucial mediator of trans-BBB transport. Clinically, such findings suggest a novel therapeutic strategy to target not only large vessel thrombus but also post-stroke microvascular clots potentially reducing detrimental outcome of the no-reflow phenomenon. Collectively, our data establish microthrombi as size-selective gateways facilitating nanoparticle transport across the BBB, offering new mechanistic insights vital for advancing targeted treatments for neurological disorders.

Innovative Solutions for Early Alzheimer's Diagnosis: Pioneering New Tools

Thursday, 2nd October - 17:15: (Sala Blava 2) - Oral

Ms. Patrícia Fraga [1], ***Ms. Beatriz T Simões*** [2], ***Ms. Catarina Chaparro*** [3], ***Dr. Paula Soares*** [3], ***Prof. Pedro Ramos-Cabrer*** [4], ***Prof. Miguel Castanho*** [1], ***Dr. Vera Neves*** [1]

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Alzheimer's disease (AD) poses a significant healthcare challenge due to its fatal nature and lack of effective treatment options, highlighting the urgent need for early detection methods and innovative therapies. The disease progresses through distinct phases, beginning with preclinical accumulation of amyloid beta (Aβ) peptides in the brain. However, detecting biomarkers like Aβ oligomers (AβO) is challenging due to the limited transport across the blood-brain barrier (BBB). To attain a novel diagnostic tool, we propose the use of magnetic nanoparticles (NPs) functionalized with BBB-penetrating peptides and AβO-targeting antibodies that will enable non-invasive detection and monitoring of AD. Here, the optimization of NP preparation, synthesis and functionalization is presented. The obtained NPs were analysed by DLS and TEM, displaying small, stable, homogeneous and reproducible sizes. The final NP formulations are non-toxic up to 50 µg/mL, while being able to internalize in HBEC-5i and bENd.3 cell lines as well as translocate a BBB trans well in vitro model. Ongoing work involves assessing the in vivo biodistribution, brain kinetics and relaxometry of the nanoparticles to evaluate their feasibility as MRI contrast agents. Initial results show that, upon intravenous injection, signal intensity in brain blood vessels drops rapidly and strongly—indicating swift circulation through the brain's vascular network. Importantly, signal in muscle tissue remains unchanged, confirming minimal extravascular leakage in healthy tissue. The tested formulations exhibited a pronounced and sustained signal drop (~10%), suggesting greater accumulation within the brain parenchyma. These findings indicate effective BBB crossing and brain-specific retention, reinforcing the potential of these nanoparticles for early, non-invasive AD diagnosis.

Increasing brain half-life of antibodies by additional binding to myelin oligodendrocyte glycoprotein, a CNS specific protein

Thursday, 2nd October - 17:30: (Sala Blava 2) - Oral

Dr. Marie-Lynn Cuypers [1], ***Mr. Tom Jaspers*** [1], ***Mr. Jarne Clerckx*** [1], ***Mr. Simon Leekens*** [2], ***Dr. Christopher Cawthorne*** [3], ***Prof. Guy Bormans*** [2], ***Prof. Frederik Cleeren*** [2], ***Dr. Nick Geukens*** [4], ***Prof. Bart De Strooper*** [5], ***Prof. Maarten Dewilde*** [4]

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Therapeutic proteins for the treatment of neurological disease are promising although limited by difficulties for passing the blood-brain barrier (BBB). Finding ways to increase brain exposure of antibodies is a critical step to boost the development of innovative antibody drugs for neurological disorders. The most well-studied approach to enhance brain influx of protein therapeutics into the brain, is receptor-mediated transcytosis (RMT) by targeting nutrient receptors to shuttle protein therapeutics over the blood-brain barrier (BBB) along with their endogenous cargos. While higher brain exposure is achieved with this approach, the timeframe is short due to rather fast brain clearance. This project explores the alternative possibility to decrease the brain efflux of antibodies after they have reached the brain by binding to a brain specific protein. We developed mouse/human/cyno cross-reactive anti-myelin oligodendrocyte glycoprotein (MOG) single variable domain antibodies (VHHs). First, we've evaluated the role of the affinity and valency of these anti-MOG VHHs fused to an antibody on the levels and activity of the fused antibody, in both plasma and brain. Next, we studied the systemic clearance, brain pharmacokinetics and pharmacodynamics, CNS and peripheral biodistribution, and brain toxicity for the selected lead molecule. We demonstrate that MOG binding VHHs have the ability to substantially increase the CNS half-life of two different antibodies and to prolong the therapeutic effect of an anti-β-secretase 1/anti-TfR antibody construct. These results mark a significant advancement in the pursuit of brain accumulation of biologicals, addressing a major bottleneck in the search for innovative drugs for neurological disorders.

Extracellular vesicles to fight dementia: a journey across the blood-brain barrier

Thursday, 2nd October - 17:45: (Sala Blava 2) - Oral

Dr. Diogo Fortunato [1], ***Mr. Henrique Nogueira Pinto*** [1], ***Ms. Gabija Danilinaite*** [1], ***Ms. Leontien Bosch*** [2], ***Ms. Susanne van der Pol*** [1], ***Ms. Manon Karsten*** [1], ***Dr. Negisa Seyed Toutounchi*** [1], ***Dr. Remco Klaassen*** [3], ***Prof. Joke den Haan*** [1], ***Prof. August B. Smit*** [3], ***Prof. Ruud Toonen*** [4], ***Prof. Vivi Heine*** [5], ***Prof. Michiel Pegtel*** [2], ***Prof. Helga E. de Vries*** [1]

1. *Department of Molecular Cell Biology and Immunology, Amsterdam UMC location Vrije Universiteit Amsterdam, Amsterdam, The Netherlands.,* ***2.*** *Department of Pathology, Cancer Center Amsterdam, Amsterdam UMC location Vrije Universiteit Amsterdam, Amsterdam, The Netherlands,* ***3.*** *Department of Functional Genomics, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands,* ***4.*** *Department of Molecular and Cellular Neurobiology, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands,* ***5.*** *Department of Complex Trait Genetics, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*

Introduction

Neurodegenerative diseases (NDs) are particularly devastating, with no available cure and only few, largely palliative therapeutic options. ND patients display variable degrees of progressive neuroinflammation, neurodegeneration and blood-brain barrier (BBB) dysfunction. Most promising recently approved therapies are based on monoclonal antibodies. Such therapies manage symptoms but fail to halt disease progression. Extracellular Vesicles (EVs) hold significant potential to revolutionize ND therapeutics. Depending on donor cells and composition, EVs can cross the BBB via transcytosis, dampen inflammation and promote regeneration. Here, we quantified the transport of brain cell-derived EVs across the BBB and characterized their cargo.

Methods

Human induced pluripotent stem cells (hiPSC) were differentiated into brain cells and used for small EV (sEV < 200nm) production. Pre-cleared conditioned medium was concentrated with ultrafiltration and sEVs purified by size-exclusion chromatography. sEVs were characterized using tunable resistive pulse sensing (size, concentration) and cryogenic electron microscopy. EV-associated markers were detected by capillary western blot, single-particle flow analysis (MIFlowCyt-EV compliant) and microscopy.

The BBB was modelled *in vitro* using brain microvascular endothelial cells (BMEC) hCMEC/D3 or hiPSC-derived BMEC barrier monolayers on the apical side of cell culture inserts, with or without brain pericytes on the underside. Uptake, transport and permeability of fluorescently-labelled EVs and liposomes on *in vitro* BBB models was quantified by single-particle flow analysis.

Results

BMEC barrier remained intact upon sEV treatment during the timeframe of transport assays. sEVs quickly bound to the apical BMEC membrane, uptake gradually increased and peaked at 4h. Transcytosis mirrored uptake dynamics, peaking at 6h after 30min CFSE-labelled EV pulses. Blockage of endo/exocytosis and intracellular transport mechanisms using different pharmacological inhibitors evidenced important pathways for sEV transcytosis across BMEC monolayers. EVs derived from BMECs, astrocytes and neuroepithelial stem cells showed higher BBB transcytosis capacity. Proteomics of transcytosed EVs revealed shared pro-transcytotic mediators, but also distinct immunomodulatory cargo across different EV types tested.

Conclusions

We devised a robust screening assay to measure paracellular and transcellular EV transport across *in vitro* BBB with single-vesicle resolution. EV types showing high BBB transcytosis capacity will be tested *in vivo* to assess brain penetrance and anti-inflammatory/regenerative cargo delivery.

Novel engineered BBB receptors for enhanced delivery of biotherapeutics

Thursday, 2nd October - 18:05: (Auditorium 1) - Oral

Dr. Eduardo Ruiz-López [1], ***Ms. Burcu Yaldiz*** [1], ***Mr. Adrià Puigdemont*** [1], ***Mr. Benjamin Max Konecny*** [1], ***Mr. Yael Soria*** [1], ***Dr. Shambhavi Pandey*** [1], ***Dr. Benjamí Oller-Salvia*** [1]

1. *Institut Quimic de Sarria - University Ramon Llull*

Pathologies affecting the Central Nervous System represent a large and growing health problem for society. The efficiency of current diagnostic and therapeutic molecules is limited in large extent due to the presence of the Blood-Brain Barrier (BBB). The BBB is the largest interface for molecule exchange between the brain parenchyma and the rest of the body. It acts as a highly selective physical and molecular sieve by thoroughly regulating the transport of the majority of substances, including drugs. Several strategies have been developed to improve brain delivery. Either by compromising BBB integrity or by utilizing ligands of natural transport mechanisms drug access has been facilitated to a limited extent. The Orthogonal Brain Gate (OBGate) project, funded by the European Research Council, aims to engineer novel BBB synthetic transport systems to efficiently deliver CNS biotherapeutics, while displaying orthogonality, selectivity and site-specific expression on brain endothelium. Here we will introduce our advancements in the development and characterization of one of our novel transport systems based on a natural receptor. This synthetic receptor harbors a synthetic epitope, enabling orthogonal recognition by immunotargeting approaches. We have obtained promising expression and binding results *in vitro*. We are investigating multiple receptor variants to understand and enhance transport efficiency across endothelial cells. For further *in vivo* analysis, we are developing delivery vehicles to allow site-specific expression of this synthetic receptor on brain endothelium. The OBGate concept will shed light into receptor-mediated transcytosis performed by new engineered transport systems which potentially will enable more effective delivery of biotherapeutics to the brain.

Expanding sources and formats for enhanced brain drug delivery

Thursday, 2nd October - 18:20: (Auditorium 1) - Oral

Dr. Maria C Lucana [1], Dr. Roberta Lucchi [1], Dr. Shambhavi Pandey [1], Dr. Cristina Díaz-Perlas [1], Dr. Benjamí Oller-Salvia [1]

1. *Institut Quimic de Sarria - University Ramon Llull*

The blood-brain barrier (BBB) represents a significant physiological impediment, severely restricting the passage of most small-molecule drugs and virtually all large therapeutic agents into the brain. Nonetheless, intrinsic transport mechanisms at the BBB can be strategically used through the application of brain shuttles to enhance drug transport. Among non-invasive methodologies, receptor-mediated transcytosis has been consistently demonstrated as the most effective pathway for the cerebral delivery of large therapeutics. Over the past decade, our research has focused on identifying novel sources and formats of peptides capable of overcoming the BBB. We pioneered the use of venoms as a source of brain shuttles, successfully developing cyclic peptidomimetics. These were inspired by neurotoxic miniproteins found in bee and scorpion venoms.[1] Our research further assessed the influence of multivalency on brain shuttling efficacy.[2]

In this contribution, we will report on BrainBikes, a novel family of bicyclic peptide shuttles.[3] These are derived from the modification of a linear shuttle peptide using a trifunctional linker. Our lead candidate, BrainBike-4, has demonstrated improved stability and affinity for transferrin receptor 1 (TfR1). It also displays a remarkable 5-fold increase in the transport of proteins, including antibody derivatives, across a human cell-based model of the BBB. BrainBike-4 was conjugated to gene delivery vectors to improve brain selectivity.[4] A simplified poly(β-aminoester)s (pBAE) formulation using a single polymer was developed, incorporating zwitterionic moieties for reduced transfection and enhanced brain targeting. Functionalization with BrainBike-4 increased transfection efficiency in cells with high TfR1 levels. The shuttle also boosted the vectors' ability to transmigrate across a human BBB model. Overall, BrainBike-4 displays promising features of a versatile brain shuttle to transport proteins and gene delivery systems across the BBB.

1. Oller-Salvia *et al. Angew Chem Int Ed.* 2016, 55, 572
2. Díaz-Perlas *et al. Chem. Sci.* 2018. 9, 8409
3. Lucana *et al. RSC Chem. Biol*., 2024, 5, 7
4. Lucana *et al. ChemRxiv.* 2025

Next-Generation Nanocarriers for Brain-Targeted Therapies

Thursday, 2nd October - 18:35: (Auditorium 1) - Oral

Prof. Francesca Re [1], ***Dr. Michela Patrucco*** [1], ***Dr. Francesco Saverio Sica*** [1], ***Dr. Davide Ferrario*** [1], ***Dr. Susanna Comi*** [1], ***Dr. Veronica Fontanini*** [1], ***Dr. M. Silvia Sesana*** [1], ***Dr. Giulia Sierri*** [1]

1. *University of Milano-Bicocca*

Delivering therapeutic agents to the brain remains one of the most challenges in modern medicine. The blood-brain barrier (BBB), a highly selective endothelial interface, effectively protects the central nervous system (CNS) but also restricts the passage of most systemically administered drugs. Despite the enormous potential of nanotechnology in this field, to date no nanoparticle-based formulations have been approved specifically for brain-targeted therapies, underlining the urgent need for novel and more effective strategies.

The most explored approach in recent years has been the functionalization of nanoparticles with targeting ligands aimed at facilitating transcytosis across the BBB. However, this method has shown limited success due to the complex and dynamic nature of the barrier. To address these limitations, we have investigated alternative strategies focused on optimizing the physicochemical properties of nanoparticles—such as size, shape, and surface charge—to enhance their interaction with the BBB and improve brain penetration.

Furthermore, we have developed brain metastasis-derived hybrid nanoparticles using membranes derived from brain-metastatic cancer cells. These membranes carry surface proteins that naturally interact with the brain endothelium, thereby facilitating the transport of the nanoparticle payload across the BBB via endogenous pathways.

We also emphasize the importance of disease-induced BBB alterations, which may be leveraged to enhance drug delivery in pathological conditions. In parallel, we explored alternative routes of administration such as intranasal delivery, which bypasses the BBB but suffers from low bioavailability. Additionally, we evaluated implantable biomaterials as localized drug delivery platforms capable of releasing nanoparticles directly into the brain parenchyma.

Our preliminary results demonstrate the superior efficacy of hybrid nanoparticles and the key role of the nanoparticles shape in delivering therapeutic agents to the brain, compared to traditional functionalized systems. These findings pave the way for a new generation of nanocarriers with the potential to revolutionize the treatment of CNS disorders.

The stroke risk gene Foxf2 maintains brain endothelial cell function via Tie2 signaling and protects against neuronal ferroptosis

Thursday, 2nd October - 18:05: (Auditorium 2) - Oral

Dr. Katalin Völgyi [1], Dr. Judit Gonzalez-Gallego [1], Mrs. Luise Schröger [1], Dr. Mihail Todorov [2], Dr. Stephan Müller [3], Dr. Burcu Seker [1], Mr. Simon Frerich [1], Dr. Filippo Cernilogar [4], Prof. Ali Ertürk [5], Prof. Arthur Liesz [6], Prof. Gunnar Schotta [4], Prof. Nikolaus Plesnila [6], Prof. Stefan Lichtenthaler [3], Prof. Dominik Paquet [7], Prof. Martin Dichgans [6]

1. *Institute for Stroke and Dementia Research (ISD) University Hospital LMU Munich, Munich, Germany,* ***2.*** *Institute for Stroke and Dementia Research (ISD) University Hospital LMU Munich, Munich, Germany, Institute for Tissue Engineering and Regenerative Medicine (iTERM), Helmholtz Zentrum München, Neuherberg, Germany,* ***3.*** *German Center for Neurodegenerative Diseases (DZNE) Munich, Germany,* ***4.*** *Biomedical Center Munich Molecular Biology Planegg-Martinsried, Germany,* ***5.*** *Institute for Stroke and Dementia Research (ISD) University Hospital LMU Munich, Germany, Institute for Tissue Engineering and Regenerative Medicine (iTERM), Helmholtz Zentrum München, Neuherberg, Germany,* ***6.*** *Institute for Stroke and Dementia Research (ISD) University Hospital, LMU Munich, Munich, Germany,* ***7.*** *Institute for Stroke and Dementia Research (ISD), University Hospital LMU Munich, Munich, Germany*

Forkhead transcription factor f2 (Foxf2) has emerged as a key transcription factor in brain endothelial cells (BECs). FOXF2 further represents a major risk locus for stroke and ischemic white matter lesions in humans but the mechanisms linking FOXF2 to vascular dysfunction and neuronal injury are unknown. We show that Foxf2 maintains BEC function via Tie2 signaling and identify excessive trans-endothelial iron influx and neuronal ferroptosis as a key mechanism underlying parenchymal injury. RNA sequencing in combination with ChIP-Seq reveal that FOXF2 acts as a transcriptional activator of Tie2 and other endothelial lineage-specific genes. EC-specific inactivation of Foxf2 in adult mice results in BBB leakage, which is exacerbated in the context of experimental stroke. Proteomics on BECs from mice with endothelial Foxf2-deficiency and on human induced pluripotent stem cell (iPSC)-derived ECs lacking FOXF2 reveal a downregulation of proteins involved in Tie2 signaling. We further show that endothelial Foxf2 deficiency reduces endothelial NO production, compromises functional hyperemia, and increases infarct size in experimental ischemia via Tie2 signaling. Treatment with the Tie2 activator AKB-9778 rescued the effects of Foxf2 deficiency on key outcomes. These results highlight a critical role of Tie2 signaling for BEC dysfunction in SVD and stroke, thus offering new perspectives for therapeutic interventions. Data related to the mechanisms underlying iron overload and neuronal ferroptosis will be presented at the meeting.

Chronic exposure to prescription opioids and ischemic stroke outcomes: focus on microvascular dysfunction and inflammasome activation

Thursday, 2nd October - 18:20: (Auditorium 2) - Oral

Dr. Silvia Torices [1], ***Dr. Enze Sun*** [1], ***Dr. Olivia M. Osborne*** [1], ***Dr. Michal Toborek*** [1]

1. *University of Miami*

The opioid epidemic endangers not only public health but also social and economic welfare. Growing clinical evidence indicates that chronic use of prescription opioids may contribute to an elevated risk of ischemic stroke and negatively impact post-stroke recovery. In addition, NLRP3 inflammasome activation has been related to several cerebrovascular diseases, including ischemic stroke. Interestingly, an increase in NLRP3 inflammasome activation has also been reported in chronic opioid exposure. Given the pivotal roles of the blood-brain barrier (BBB) and oxidative stress in ischemic stroke pathophysiology, this study focuses on the impact of chronic exposure to prescription opioids on the integrity of cerebrovascular microvasculature, endothelial mitochondrial homeostasis, and the outcomes of ischemic stroke in male wild type and NLRP3-deficient mice. Our results demonstrate that chronic opioid exposure can compromise the integrity of the BBB and elevate the generation of reactive oxygen species (ROS), resulting in endothelial mitochondrial dysfunction and apoptosis activation. We also provide evidence that opioid exposure enhances inflammasome activation, inflammatory responses, and increases the severity of an ischemic stroke. The antioxidant N-acetylcysteine (NAC) ameliorated these opioid-induced alterations and accelerated the post-stroke tissue restoration and functional recovery processes in opioid-exposed mice. Importantly, there was also a significant decrease in ischemic stroke damage in the NLRP3-deficient mice with chronic opioid exposure as compared to wild-type controls. These findings indicate that chronic exposure to prescription opioids impacts the outcome of ischemic stroke by damaging microvascular cerebral integrity through inflammasome activation and mitochondrial dysfunction.

Therapeutic Potential of Salicornia ramosissima Polyphenols to Preserve BBB Integrity in a 3D Human Stroke Spheroid Model

Thursday, 2nd October - 18:35: (Auditorium 2) - Oral

Ms. Marina Romero Bernal [1], ***Dr. Carmen del Rio*** [2], ***Ms. Ángela González-Díaz*** [1], ***Ms. Carmen Ortiz Salguero*** [1], ***Dr. Joan Montaner*** [3]

1. *Instituto de Biomedicina de Sevilla,* ***2.*** *Universidad de Sevilla,* ***3.*** *Hospital Universitario Virgen Macarena*

The disruption of the blood-brain barrier (BBB) plays a central role in the pathophysiology of stroke. When the BBB is compromised, its selective permeability is lost, allowing potentially neurotoxic substances, immune cells, and plasma proteins to infiltrate the brain parenchyma. This breach contributes to the formation of cerebral edema, triggers robust neuroinflammatory responses, and can lead to elevated intracranial pressure, all of which exacerbate neuronal injury. Preserving the integrity of the BBB is therefore essential for maintaining central nervous system homeostasis and limiting secondary brain damage following a stroke.

To better understand these mechanisms and support therapeutic development, reliable and physiologically relevant in vitro models of the BBB are urgently needed. In this study, we present a standardized, reproducible 3D spheroid model of the human BBB composed of endothelial cells, astrocytes, and pericytes. Stroke-like conditions were simulated by subjecting the spheroids to 24 hours of hypoxia, which led to increased levels of pro-inflammatory cytokines (IL-6, VEGF, IL-1α, and TGF-β), disrupted expression of tight junction proteins (Claudin-5 and ZO-1), and enhanced albumin permeability—hallmarks of BBB breakdown. This model provides a robust platform for studying stroke-induced BBB dysfunction and for testing potential neuroprotective and BBB-permeable therapeutics.

In the search for effective therapeutic strategies to counteract stroke-induced BBB damage, polyphenols have emerged as promising candidates thanks to their potent antioxidant and anti-inflammatory properties. We thus evaluated both individual and combined treatments of *Salicornia ramosissima*-derived polyphenols in our hypoxia-induced 3D BBB spheroid model. While single compounds showed modest reductions in pro-inflammatory markers, the polyphenolic combination—whether as crude plant extract or defined cocktail—achieved statistically significant decreases (e.g., IL-6 and VEGF, $p < 0.05$) alongside marked improvements in Claudin-5 and ZO-1 expression ($p < 0.01$). This synergistic effect underscores the potential of *S. ramosissima* polyphenols as a platform for novel neurovascular therapies in stroke and other hypoxia-related disorders

Key words: Blood-Brain Barrier, stroke, polyphenols, Salicornia, prevention

Research on Multivalent Targeting of LRP1 for the Treatment of Parkinson's Disease

Thursday, 2nd October - 18:05: (Sala Blava 2) - Oral

Ms. pan xiang [1], ***Prof. Xiaohe Tian*** [2]

1. *Sichuan University,* ***2.*** *Sichuan University West China Hospital*

Impaired trans-blood-brain-barrier transport and diminished clearance of α-synuclein in the brain have been identified as one of the core pathological mechanisms underlying Parkinson's disease (PD) and Lewy body dementia (LBD). The abnormal aggregation of α-synuclein forms Lewy bodies, which can spread between neurons in a prion-like manner, triggering oxidative stress, mitochondrial dysfunction, and synaptic toxicity, ultimately leading to the apoptosis of dopaminergic neurons in the substantia nigra pars compacta. Studies have shown that the efflux transport and inter-tissue diffusion of α-synuclein are closely dependent on the mediation of low-density lipoprotein receptor-related protein 1 (LRP1), suggesting that this pathway may provide a potential therapeutic target for disease intervention.

This study successfully constructed a bifunctional polymeric nanovesicle system capable of crossing the blood-brain barrier and targeting the regulation of LRP1. The nanosystem specifically recognizes the LRP1 receptor on the surface of brain microvascular endothelial cells and utilizes PACSIN2-mediated transcytosis to promote the transmembrane transport of LRP1 from the vascular side to the brain parenchyma, significantly enhancing the clearance efficiency of α-synuclein in brain tissue. Within the brain parenchymal microenvironment, the nanovesicles employ clathrin-dependent endocytosis and Rab5-regulated endosomal sorting mechanisms to direct neuronal surface LRP1 into the lysosomal degradation pathway, thereby effectively blocking the trans-neuronal spread of α-synuclein.

Furthermore, this system exhibits multi-drug co-delivery capabilities, enabling the simultaneous loading of neurotrophic factors (e.g., GDNF) and antioxidants (e.g., curcumin) to modulate the levels of neuroinflammatory cytokines (TNF-α, IL-6) in Parkinson's disease models, thereby reducing the apoptosis rate of dopaminergic neurons. This study is the first to reveal the mechanism by which nanocarriers achieve bidirectional intervention of pathological proteins through spatiotemporal differential regulation of LRP1 metabolic pathways, providing an innovative nanomedical strategy for the precise treatment of neurodegenerative diseases.

Ligand topology matters: optimised arrangement of T7 ligand on polymersomes for enhanced blood-brain barrier transcytosis

Thursday, 2nd October - 18:20: (Sala Blava 2) - Oral

Dr. Catia Lopes [1], ***Mr. Marco Basile*** [2], ***Dr. Valentino Barbieri*** [2], ***Dr. Vanina Cosenza*** [2], ***Mr. Peter Pfeifer*** [2], ***Prof. Giuseppe Battaglia*** [1]

1. *Institute for Bioengineering of Catalonia & University of Barcelona,* ***2.*** *Institute for Bioengineering of Catalonia (IBEC)*

Ligand-receptor interactions play a pivotal role in modulating cellular responses, with spatial characteristics such as ligand/receptor density, ligand orientation, and accessibility critically influencing receptor activation and downstream signalling. In the context of blood-brain barrier (BBB) targeting, optimising these parameters is essential to enhance nanomedicine-mediated brain drug delivery. This work focuses on the systematic screening of T7 peptide-functionalised polymersomes, where the density and accessibility of ligands were precisely tuned to maximise brain endothelial transferrin receptor (TfR) engagement and transcytosis efficiency. We highlight our recent advances in controlling ligand spatial arrangement to promote receptor engagement while mitigating steric hindrance and hydrodynamic instability. By leveraging engineered polymersomes with well-defined ligand architectures, we demonstrate how rational nanocarrier design can improve BBB penetration. Key topics to be presented include i) the impact of ligand density on TfR binding and cellular uptake, ii) strategies to maintain ligand accessibility and orientation, and iii) translational insights for brain-targeted nanomedicines. This work underscores the importance of spatially optimised ligand display in overcoming biological barriers and advancing precision brain drug delivery.

Brain drug delivery innovation through blood-brain barrier-on-a-chip technology

Friday, 3rd October - 09:00: (Auditorium 1) - Keynote speakers

Dr. Tae-Eun Park [1]

1. *Ulsan National Institute*

Organ-on-a-chip technology, which faithfully mimics the structural and dynamic characteristics of in vivo vasculature, has emerged as a promising platform for identifying vascular-targeted drug delivery systems (DDS). In this study, a blood–brain barrier (BBB) chip model demonstrated superior performance over conventional transwell systems in screening nanocarriers, due to its accurate reproduction of the endothelial glycocalyx and shear stress conditions. This physiological relevance enabled the discovery of BBB shuttles with enhanced in vivo functionality. Building on this approach, we implemented a microphysiological system (MPS)-based SELEX strategy (MPS-SELEX) to identify BBB-penetrating aptamers. Using a dual-channel BBB chip composed of human brain microvascular endothelial cells, astrocytes, and pericytes, we successfully screened and validated a novel aptamer, hBS01, which promotes protein transport across the BBB via clathrin-mediated endocytosis. hBS01 showed high targeting specificity and efficient brain accumulation in both in vitro and in vivo settings. These findings underscore the potential of organ-on-a-chip systems not only for DDS screening but also for the functional discovery of BBB-penetrating aptamers under physiologically relevant conditions. Additionally, we developed an iPSC-derived BBB organoid model incorporating vasculature, pericytes, and astrocytes by recapitulating the developmental process of the human BBB. In this seminar, I will also introduce this BBB organoid model to highlight its utility in studying the structural characteristics of pathological BBB conditions.

Receptor-Mediated Trafficking at the Blood-Brain Barrier

Friday, 3rd October - 10:00: (Auditorium 1) - Invited Speaker

Prof. Morten Nielsen [1]

1. *Aarhus University*

The Blood-Brain Barrier (BBB) is a tightly regulated and polarized interface that plays a critical role in maintaining the microenvironmental homeostasis of the central nervous system (CNS) and protecting it from circulating xenobiotics. However, this structural integrity of the BBB significantly restricts the entry of biologics into the brain. As a result, extensive research efforts within academia and the biotech industry are focused on developing effective therapeutic strategies for the growing population of patients with CNS disorders.

Among these strategies, receptor-mediated transcytosis (RMT) has emerged as one of the most promising approaches for targeted delivery of biotherapeutics across the BBB. Recent advances in antibody engineering—particularly those targeting transferrin receptor (TfR) and CD98, either individually or as dual-targeting constructs—have shown encouraging results. Deepit the progress, Brain Endothelial Cells (BEC) harbor undisclosed regulatory trafficking mechanisms of TfR and other sorting receptors, including apical vs basal membrane localization, endocytic mechanism and sorting in the polarized endosomal system.

Studying these mechanisms has been challenging due to the flat morphology of BECs and the lack of robust in vitro models, which limits conventional trafficking stuides. To address this, we employed a primary porcine BBB model combined with advanced imaging techniques to investigate the bidirectional trafficking of the recycling TfR and the retrograde transport of the mannose-6-phosphate receptor.

Investigating Busulfan's effect on the Blood-Brain Barrier to enhance CNS engraftment in hematopoietic stem cell gene therapy for neurometabolic disorders

Friday, 3rd October - 11:10: (Auditorium 1) - Oral

Dr. Sara Benedetti [1], ***Dr. Bethan J Critchley*** [2], ***Dr. Giorgia Santilli*** [1], ***Prof. Adrian Thrasher*** [2], ***Prof. Bobby H Gaspar*** [3]

1. *UCL Institute of Child Health & NIHR Great Ormond Street Hospital Biomedical Research Centre, London, UK,* ***2.*** *UCL Institute of Child Health, London,* ***3.*** *UCL Institute of Child Health & Orchard Therapeutics, London*

Neurological lysosomal storage disorders (LSDs) are metabolic diseases caused by dysfunctional enzymes leading to toxic accumulation of macromolecules, especially within the central nervous system (CNS). Haematopoietic stem cell (HSC) gene therapy has shown potential in alleviating neurological symptoms and preventing further neurodegeneration, primarily through the CNS engraftment of HSC-derived microglial-like cells. However, the treatment's success depends on the HSC's ability to cross the blood-brain barrier (BBB). Experimental and clinical evidence indicates that the conditioning agent busulfan, used prior to transplantation to clear the bone marrow niche, can enhance HSC engraftment in the CNS, though the underlying mechanisms are not fully understood. Beyond its critical role in depleting resident microglia, prior research suggests that busulfan may also cause vascular injury and potentially involving BBB disruption. We hypothesise that a disrupted BBB may partly contribute to improved HSC engraftment. To this end, we conducted in vivo transplantation studies of lin- murine HSCs in mice conditioned with either busulfan or irradiation, finding that busulfan increases HSC engraftment exclusively to the CNS, and not to other tissues. This suggests that busulfan's mechanism(s) of action is CNS-specific and may involve BBB remodelling. Additionally, we performed comprehensive immunofluorescent imaging and quantification of CNS microvasculature in vivo at various time points post-conditioning. We specifically assessed both brain microvasculature structure and brain endothelial tight junction integrity and organization. Although we did not observe acute macroscopic brain vascular injury or gross disruption of the BBB structure in busulfan-treated mice compared to irradiated ones, we found a significant downregulation of Claudin-5, a key brain endothelial tight junction protein highly expressed in the BBB, in busulfan-treated mice. Claudin-5 downregulation has been previously shown to be associated with increased BBB permeability, suggesting that a subtle and rapid, but reversible, BBB remodelling may occur following busulfan treatment. These findings offer new insights into the effects of pre-conditioning regimes on CNS vasculature, paving the way for new investigations into BBB manipulation to increase permeability and thereby enhance the therapeutic efficacy of HSC based gene therapy for neurometabolic disorders.

Mechanisms underlying obesity-induced damage to the neural vasculature

Friday, 3rd October - 11:25: (Auditorium 1) - Oral

Dr. Jesús Rodríguez-Aguilera [1], ***Dr. Olga Bondareva*** [2], ***Mr. Hugo Martin*** [2], ***Dr. Bilal Sheikh*** [2]

1. *Helmholtz Munich,* ***2.*** *Helmholtz Institute for Metabolic, Obesity and Vascular Research (HI-MAG) of the Helmholtz Center Munich*

Metabolic diseases such as obesity and diabetes increase the risk of developing dementia by up to 6-fold. However, the underlying reasons remain unknown. Here, we report neurovascular damage and breakdown in an animal model of metabolic disease. We develop and utilise a FACS-based strategy to isolate neurovascular cells including endothelial cells, pericytes, astrocytes, microglia, perivascular macrophages and fibroblasts, and undertake scRNA-seq analyses to analyse the impact of metabolic disease on the neural vasculature. We uncover unique deregulation of transcriptional and molecular networks across all cell types. Through tracer-dye analyses, confocal imaging, and a customised spatial transcriptomics panel on the Visium platform, we find that the blood vessels in the hippocampus to be particularly vulnerable to metabolic disease. Mechanistically, we uncover a retinoid acid based signalling network that invokes endothelial dysfunction, reduction of cell-junction proteins, and breakdown of barrier function. Together, our study provides important molecular insights governing the integrity of the blood brain barrier, and how metabolic disease promotes neural dysfunction.

Blood-brain barrier permeabilization and reduced hippocampal blood flow impairs cognitive function in AAV-PCSK9DY atherosclerotic mice

Friday, 3rd October - 11:40: (Auditorium 1) - Oral

Mr. Luis Daniel Hernandez Torres [1], ***Dr. Walter Raasch*** [1], ***Dr. Zouhair Aherrahrou*** [1], ***Dr. Ümit Özorhan*** [1], ***Ms. Eva Peschke*** [2], ***Dr. Olga Will*** [2], ***Dr. Jan-Bernd Hoevener*** [2], ***Dr. Oliver Müller*** [3]

1. *Lübeck University,* ***2.*** *Kiel University,* ***3.*** *UKSH*

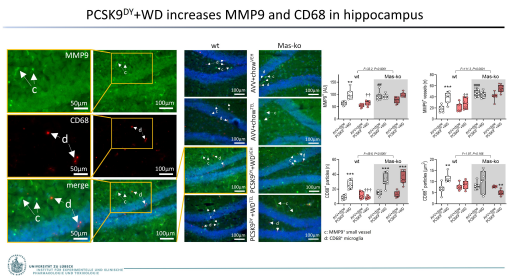
Introduction: We recently showed that mice exhibit increased anxiety behavior as a result of diet-induced obesity while cognitive function remained normal. We speculated that cognitive dysfunction may require additional vascular damage.

Objectives: Thus, we aimed to investigate whether atherosclerotic mice have impaired learning and memory and whether this is associated with small vessel alterations. Furthermore, we also investigate whether Telmisartan (TEL) improves cognitive function and whether TEL effect persist on MasKO mice.

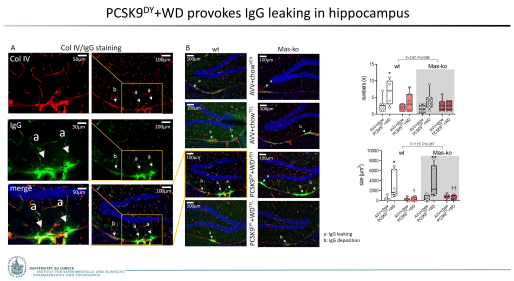
Materials & methods: We induced atherosclerosis by an AAV-PCSK9^{DY} (2x10^{11} VG) injection plus cholesterol-rich Western diet (verum mice). For this experiment we had used WT and Mas-receptor KO mice. Mice received daily treatment with TEL (8 mg/kg$_{bw}$). After 4 months, we examined these mice and chow-fed controls for cognition using Barnes maze procedure for spatial learning (5 days training) and memory at d6 (long term memory) and at d17 (remote memory). Cerebral blood flow was determined by using ASL-MRI. Using ColIV/IgG, ColIV/CD31 and MMP9 immunofluorescence into brain slices, we determined vascular morphology in the hippocampus.

Results: Compared with controls, verum mice developed obesity, aortic plaque burden, and dyslipidaemia. TEL protected against the metabolic insult. By immunofluorescence, we detected BBB impairment in verum mice and TEL protective effect but only in WT and not MasKO mice; those mice had increased MMP9, IgG permeability and hippocampus inflammation. Verum mice also have small vessel morphological changes, there is reduced ColIV/CD31 ratio, increased development of string vessels and higher number of pericytes normalized by TEL. Due to this, hippocampus blood flow was reduced and mice shown memory function decline. TEL protection was partial in MasKO mice.

Conclusions: We conclude that as consequences of atherosclerotic lesions and obesity, the spatial learning and long-term memory of the animals were decreased due to the formation of string vessels which further causes decreased blood flow in certain brain areas. The atherosclerotic model also provokes BBB permeabilization by the action of MMP9 and hippocampus inflammation. In addition, TEL vascular protective effects are partially mediated by Mas-receptor. These findings make the AAV-PCSK9^{DY} mouse model particularly valuable for research in obesity-associated dementia.



Pcsk9 dy wd increases mmp9 and cd68 in hippocampus.jpg



Pcsk9 dy wd provokes igg leaking in hippocampus.jpg

A novel approach to understand blood-brain barrier leakage and neurovascular dysfunction in schizophrenia: molecular insights and functional validation

Friday, 3rd October - 11:10: (Auditorium 2) - Oral

Mr. Benjamin Reuse-Benavente [1], ***Mr. Jesús Juárez-Balarezo*** [1], ***Ms. María Jesús Garrido Muñoz*** [1], ***Dr. Bárbara S. Casas*** [1], ***Dr. Catalina Prieto*** [1], ***Dr. Ignacio Casanova-Maldonado*** [1], ***Mr. Yerko Suazo*** [2], ***Dr. Katherina Llanos*** [2], ***Dr. Verónica Palma*** [1]

1. *Universidad de Chile,* ***2.*** *Instituto Psiquiatrico Dr. Jose Horwitz Barak*

Schizophrenia is a chronic psychiatric disorder with a heterogeneous clinical presentation that affects approximately 1% of the global population. It is associated with high disability, reduced life expectancy, and significant socioeconomic burden, causing a profound impact on patients' quality of life. While the origin of the disease lies in neurodevelopment, primarily affecting the central nervous system (CNS), recent findings indicate a neurovascular dysfunction, including alterations and damage to the blood-brain barrier (BBB) in its pathophysiology. Structural and functional disruptions of the BBB suggest schizophrenia may involve multisystemic and vascular components beyond canonical CNS mechanisms. While antipsychotic medications offer partial symptom relief, a substantial subset of patients remains treatment-resistant, highlighting the need for novel biomarkers and therapeutic targets. In this study, we investigated circulating neurovascular-associated proteins in a Chilean cohort of schizophrenia patients, with a focus on BBB-relevant biomarkers. Plasma levels of Brain-Derived Neurotrophic Factor (BDNF), Vascular Endothelial Growth Factor (VEGF) and the axonal guidance cue Netrin-1 were quantified by ELISA and correlated with patients' clinical data. Among these, Netrin-1 levels were significantly dysregulated and showed treatment-dependent variability, suggesting potential as a pharmacodynamic biomarker. In parallel, a metabolic profiling study is currently ongoing to detect drug signatures and identify dysregulated metabolic pathways associated with disease or treatment response. In addition, functional validation experiments were performed using BBB models, including chick embryo brains and the chorioallantoic membrane (CAM). Exposures to patient serum and antipsychotic drugs were used to assess BBB integrity through angiogenic response and vascular permeability assays. Together, these findings underscore the involvement of the neurovascular unit in schizophrenia and support the utility of combined biomarker discovery and model validation strategies to uncover novel targets for therapeutic intervention.

In vivo imaging of gadolinium-based contrast agent leakage in patients with cerebral amyloid angiopathy

Friday, 3rd October - 11:25: (Auditorium 2) - Oral

Dr. Hilde van den Brink [1], Dr. Mariel Kozberg [1], Dr. Nazanin Makkinejad [1], Dr. John Kirsch [2], Dr. Michael Thrippleton [3], Dr. Thijs van Harten [1], Ms. Sabine Voigt [4], Dr. Whitney Freeze [4], Dr. Matthias van Osch [5], Dr. Anand Viswanathan [1], Dr. Susanne van Veluw [1]

1. *Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston MA, USA,* ***2.*** *Athinoula A. Martinos Center, Massachusetts General Hospital, Charlestown MA, USA,* ***3.*** *Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom,* ***4.*** *Department of Neurology, Leiden University Medical Center, Leiden, the Netherlands,* ***5.*** *Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands*

Background

Cerebral amyloid angiopathy (CAA) is a cerebrovascular disease characterized by deposition of amyloid β in the walls of leptomeningeal and small cortical vessels. CAA is a leading cause of lobar intracerebral hemorrhages and cognitive decline. Leakage of the blood-brain barrier (BBB) is thought to be an early pathophysiological phenomenon in the disease, possibly preceding the debilitating lobar intracerebral hemorrhages. In this study we used gadolinium-based contrast enhanced MRI to study leakage of the BBB from leptomeningeal and parenchymal small vessels, and related leakage with hemorrhagic manifestations of CAA (i.e. cortical superficial siderosis and cortical cerebral microbleeds).

Methods

Fourteen patients with probable CAA without prior intracerebral hemorrhage (age 68±9 years, 57% female) and 7 non-CAA controls with mild cognitive impairment (age 70±7 years, 29% female) were included. 3T-MRI included SWI, pre-and postcontrast T2-FLAIR and a Dynamic Contrast Enhanced (DCE) scan. Participants received a gadolinium-based contrast agent (Dotarem, 0.2mL/kg) during the DCE scan. Leakage from leptomeningeal vessels was assessed on post- versus precontrast T2-FLAIR as either focal or sulcal cerebrospinal fluid (CSF) enhancements (see Figure 1 for examples). DCE scans were analyzed to quantify permeability-surface area product (*PS*): a measure of leakage from parenchymal small vessels.

Results

Focal CSF enhancements were observed similarly often in patients with CAA (7 [50%]) and non-CAA controls (4 [57%], p=0.98), while sulcal CSF enhancements were only seen in patients with CAA (5 [36%] vs. 0 [0%], p=0.03). In patients with CAA, focal and sulcal CSF enhancement count were associated with higher cortical superficial siderosis volume (B=2.61, p=0.003; B=1.02, p=0.02). *PS* was numerically higher in the cortex in patients with CAA (5.08±4.02 x$10^{-4}$$min^{-1}$) than non-CAA controls (1.29±4.08 x$10^{-4}$$min^{-1}$, p=0.07), but was not associated with any hemorrhagic manifestations of CAA (p>0.67).

Discussion

Gadolinium-based contrast agent leakage through the BBB can be measured *in vivo* in patients with CAA. Leakage is observed from the leptomeningeal vessels and likely from cortical small vessels as well. Leakage from leptomeningeal vessels was associated with cortical superficial siderosis. Studies with follow-up data need to determine if these measures could serve as a predictors of disease progression in CAA.

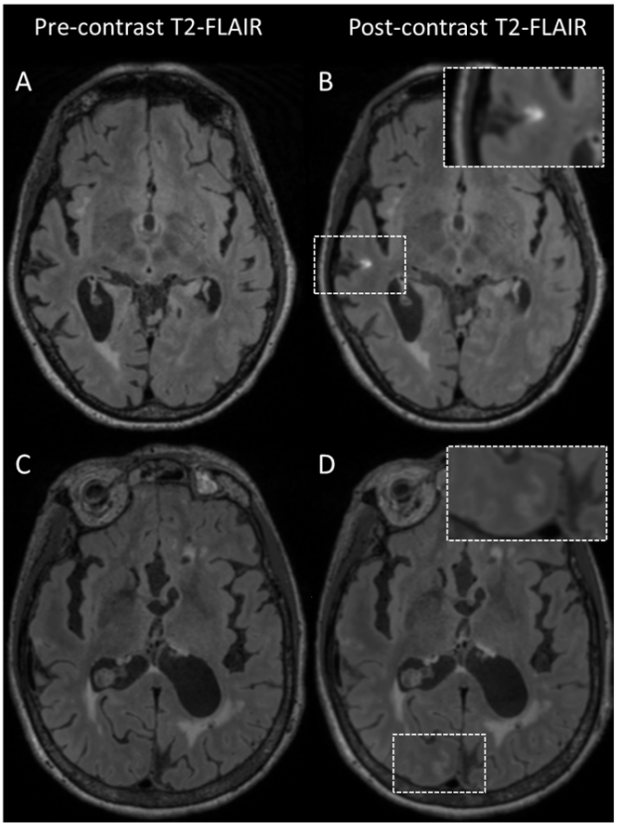


Figure 1 An example of focal (A and B) and sulcal (C and D) CSF enhancement in two participants with probable CAA.

Bbb meeting figure1.png

Brain vascular regulation of normal, diseased, and resilient human brain aging

Friday, 3rd October - 11:40: (Auditorium 2) - Oral

Dr. Madigan Reid [1], ***Ms. Tammie Tam*** [2], ***Mr. Alex Pennacchio*** [1], ***Ms. Bella Ding*** [1], ***Ms. Brittany Davidson*** [2], ***Ms. Jessica Tsui*** [2], ***Ms. Amanda Apolonio*** [1], ***Mr. Hao Liu*** [1], ***Dr. Shih-Hsiu Wang*** [3], ***Dr. Alexis Combes*** [2], ***Mr. Andrew Yang*** [1]

1. *Gladstone Institutes, Gladstone Institute of Neurological Disease, San Francisco, CA, USA,* ***2.*** *University of California, San Francisco, Department of Pathology, San Francisco, CA, USA,* ***3.*** *Department of Pathology, Duke University Medical Center, Durham, NC, USA*

The blood-brain barrier (BBB), crucial for brain health, becomes dysfunctional with aging and Alzheimer's disease (AD). Understanding the cellular underpinnings of this dysfunction is important, but existing brain atlases have lacked the necessary age-span coverage and underrepresent crucial neurovascular cell populations, hindering insights into normal aging processes and the earliest neurovascular triggers of AD. To address this critical gap, we investigated cerebrovascular changes across the full spectrum of human aging and cognitive health. We performed VINE-seq (Vessel Isolation and Nuclei Extraction for Sequencing) on postmortem cortical gray matter (GM) and white matter (WM) tissue from a unique cohort of 105 individuals (ages 20-100). This cohort uniquely includes normal aging, Mild Cognitive Impairment (MCI), AD, and cognitively resilient individuals. Our workflow successfully yielded >1 million nuclei, including an extensive collection of >150,000 vascular cells, enabling robust analysis. Comprehensive analysis revealed distinct, compartment-specific aging trajectories in GM and WM. Notably, critical windows of accelerated molecular aging were identified, occurring earlier in GM (40-50s) compared to WM (60-70s). Trajectory analyses focusing on normal aging further uncovered diverse, cell-specific temporal patterns. Common molecular programs with ascending expression across diverse cell types involved heightened immune signaling (including antigen presentation and interferon pathways), protein folding/stress responses, and extracellular matrix remodeling, particularly prominent within vascular and immune cell populations. Comparative analyses of MCI, AD, and resilient individuals relative to normal aging identified unique molecular hallmarks of cognitive resilience. Specifically, gray matter brain endothelial cells (BECs) in resilient individuals displayed distinct transcriptional signatures vs. normal aging. For instance, resilient BECs exhibited evidence of adaptive mechanisms, such as enhanced proteostasis pathways involving genes for protein degradation as well as enhanced transporter activity. These findings indicate that cognitive resilience may arise from specific neurovascular adaptations rather than passive resistance to age-related changes. We are further employing spatial transcriptomics (10x Xenium) to map these cellular changes in situ relative to aging and AD pathologies. This study provides a vital resource characterizing neurovascular aging across the human lifespan and disease states. Our approach enhances comprehension of BBB cellular dynamics and lays the groundwork for exploring neurovascular contributions to aging and AD pathogenesis.

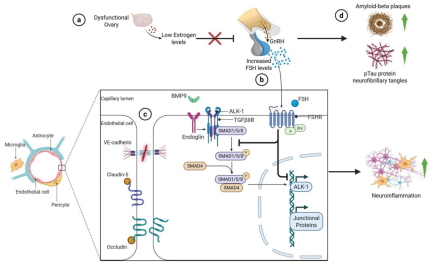
HPG axis dysregulation in aging impacts blood-brain barrier integrity and Alzheimer-like disease pathology

Friday, 3rd October - 11:10: (Sala Blava 2) - Oral

Dr. Hezi Elyahu [1], Dr. Ekaterina Eremenko [1], Mr. Omri Kogman [1], Mr. Amit Shicht [1], Mrs. Anna Nemirovsky [1], Mrs. Talia Indig [1], Mrs. Danit Eidelson Parker [1], Prof. Alon Monsonego [1]

1. *The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences; The School of Brain Sciences and Cognition; Ben-Gurion University of the Negev, Beer Sheva 8410501, Israel.*

Age-related dysfunction of the blood-brain barrier (BBB) is increasingly linked to Alzheimer's disease (AD) pathogenesis. In addition, women exhibit higher AD susceptibility at the post-menopause stage, a phenomenon which is often associated with dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis and elevated levels of the gonadotrophins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Here we investigated the direct role of FSH in modulating the BBB integrity as a potential mechanism linking age-related dysregulation of the HPG axis to AD vulnerability. BBB structural integrity was evaluated with Western blot and immunohistochemistry of key endothelial regulatory and junctional proteins, while functionality was analyzed using trans-endothelial permeability assays. We show that aging in mice was associated with increased levels of circulating FSH and that FSH levels negatively correlated with endothelial expression of the transforming growth factor β (TGFβ) family receptors activin-like kinase 1 (ALK1) and Endoglin, the adherent junction protein VE-Cadherin, and the tight junction protein Occludin. Notably, these age-related vascular changes occurred in female but not in male mice. In vitro, FSH treatment downregulated the expression of ALK1, Endoglin, VE-Cadherin, and Occludin in primary brain microvascular endothelial cells (PBMECs) as well as compromised their permeability to low- and high-molecular weight dextran molecules. Mechanistically, FSH impaired the canonical ALK1 signaling, evidenced by reduced SMAD1/5 phosphorylation in response to its ligand BMP9. Our findings identify FSH, a key indicator of HPG axis dysregulation in female aging, as a potent negative regulator of BBB integrity. Our study may thus highlight a novel hormonal mechanism potentially underlying the heightened female vulnerability to AD via cerebrovascular pathology following menopause.



HPG axis dysfunctionality during ageing promotes FSH-mediated BBB permeabilization via ALK1/Endoglin pathway. (a) In postmenopausal women, ovarian dysfunction results in reduced synthesis and secretion of estrogen, leading to the loss of the negative feedback loop of the HPG axis. (b) Increased levels of FSH are released into the circulation. FSH interacts with its receptor (FSHR) on various tissues, including brain microvascular endothelial cells. (c) Endothelial FSH signaling downregulates key regulatory receptors and junctional proteins, promoting BBB permeabilization. (d) BBB dysfunction is associated with AD pathology, including amyloid-beta plaques and neurofibrillary tangles composed of phosphorylated Tau (pTau), highlighting a novel hormonal mechanism potentially underlying the heightened female vulnerability to AD.

Hpg axis dysfunctionality during ageing promotes fsh-mediated bbb permeabilization via alk1endoglin pathway..jpg

Multi-omics integration of proteomics and single nucleus RNA-Sequencing reveals shared mechanism of blood-brain barrier dysfunction in dementia

Friday, 3rd October - 11:25: (Sala Blava 2) - Oral

Mr. Jannis Heuer [1], ***Mr. Thomas M. Rust*** [2], ***Dr. Peter van Veelen*** [3], ***Mr. Rayman Tjokrodirijo*** [3], ***Mr. Yassene Mohammed*** [3], ***Prof. Annemieke J. Rozemuller*** [4], ***Prof. Bart J. L. Eggen*** [2], ***Prof. Helga E. de Vries*** [1], ***Dr. Nienke M. de Wit*** [1]

1. *Dept. of Molecular Cell Biology and Immunology, Amsterdam UMC,* ***2.*** *Dept. of Biomedical Sciences, UMC Groningen,* ***3.*** *Dept. of Center for proteomics and metabolomics, Leiden UMC,* ***4.*** *Dept. of Pathology, Amsterdam UMC*

Frontotemporal dementia associated with progranulin mutations (FTD-GRN) and capillary cerebral amyloid angiopathy (capCAA) are two distinct neuropathological entities that manifest with cellular inclusions and neuronal loss. Despite their differing causes and protein accumulations, both conditions exhibit significant levels of neuroinflammation, which influences disease onset and progression. Neuroinflammation, together with blood-brain barrier (BBB) dysfunction, are recognized as key players in the early pathological processes. Understanding the common and unique pathways leading to BBB dysfunction in these diseases can reveal critical aspects of vascular contributions to cognitive decline and identify potential therapeutic targets to mitigate neuroinflammation and improve brain health.

To study the BBB, we isolated and characterized capillaries from post-mortem human brain tissue of capCAA, FTD-GRN, and non-demented controls. The isolated vessels were subjected to proteomic analysis (e.g., mass spectrometry) to obtain a comprehensive profile of the pathological changes. In addition, we performed single-nucleus RNA sequencing on the same post-mortem human tissue, where we enriched for vascular and glial cells by depleting the nuclei of neurons and oligodendrocytes. By integrating these two modalities, we identified a number of shared pathways on the proteomic and transcriptomic level. Currently, we are validating these findings via immunohistochemistry and in situ hybridization in post mortem tissue and iPSC-derived vascular cells. The key shared processes indicated mitochondrial dysfunction, accompanied by the differential expression of mitophagy genes in the capillaries. Some of the shared processes showed a stronger phenotype in one of the two dementias. In capCAA we find evidence of vascular remodeling, characterized through changes in the extracellular matrix whereas the capillaries of FTD-GRN brains show RNA/DNA damage. Taken together, we observe an aging phenotype in the capillaries of both dementias, independent of age at death and partially characterized through different processes. Here we provide a comprehensive analysis of the BBB in two forms of dementia, discovering a number of specific pathways altered that can be further investigated for treatment interventions and biomarker research.

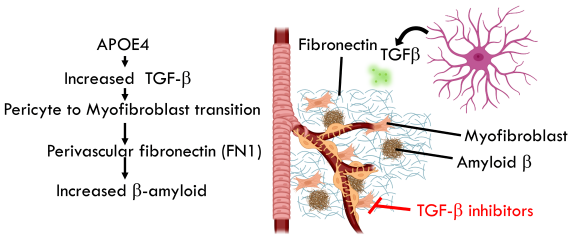
A novel myofibroblast-like cell population drives cerebrovascular degeneration in the APOE4 brain

Friday, 3rd October - 11:40: (Sala Blava 2) - Oral

Mr. Braxton Schuldt [1], ***Dr. Diede Broekaart*** [1], ***Mr. Leon Wang*** [1], ***Mr. Dominic Haworth-Staines*** [1], ***Dr. Alison Goate*** [1], ***Dr. Ana Pereira*** [1], ***Dr. Joel Blanchard*** [1]

1. *Nash Family Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai*

Cerebrovascular pathology is central to Alzheimer's disease (AD) and promotes cognitive decline. *APOE4*, the largest genetic risk factor for AD, is strongly associated with cerebrovascular degeneration. Recent studies showed that genetic variants in vascular basement membrane proteins (*FN1*) can promote cognitive resilience in *APOE4* populations, yet the mechanisms remain unknown. To determine how *APOE4* impacts vascular degeneration in the brain, we generated a high-resolution single-cell transcriptomics atlas of human cerebrovasculature from *APOE4* carriers and non-carriers. In *APOE4* individuals, the number of microvascular pericytes were significantly reduced. Strikingly, this pericyte loss coincided with the emergence of a unique population of *APOE4* smooth muscle cells co-expressing high levels of contraction and extracellular matrix (ECM)-related genes—hallmarks of myofibroblasts. Immunostaining of the post-mortem brain revealed that non-vascular myofibroblast-like cells are present in the *APOE4* human hippocampus, correlating with increased vascular fibrosis and amyloid accumulation. To determine the mechanisms and functional consequences of myofibroblast-like cells in the *APOE4* brain, we developed a vascularized human brain tissue (miBrain) from induced pluripotent stem cells. Similar to the post-mortem human brain, *APOE4* miBrain pericytes detach from microvasculature and transition into myofibroblast-like cells co-expressing ECM and contractile genes. We further found that *APOE4* myofibroblasts secrete fibronectin (*FN1*), promoting vascular and parenchymal amyloid accumulation. To determine how myofibroblast-like cells arise in the *APOE4* brain, computational analysis (NicheNet) predicted TGF-β as the causal driver of the pericyte-to-myofibroblast-like transition. Consistently, TGF-β ligands in astrocytes and receptors in mural cells are significantly upregulated in the *APOE4* brain. Chemical and genetic inhibition of TGF-β signaling in *APOE4* human brain tissue reduced myofibroblast-like cells, increased pericyte microvascular coverage, and reduced vascular fibrosis and amyloid accumulation. We are confirming these findings in mouse models. Our study identifies a novel *APOE4* disease-associated cell type influencing cerebrovascular pathology. We show that upregulated TGF-β signaling causes *APOE4* pericytes to detach from vasculature and transform into myofibroblast-like cells, promoting amyloid deposition via fibronectin secretion. Importantly, TGF-β inhibition reverses *APOE4*-associated cerebrovascular pathologies. Our study thus uncovers mechanisms underlying *APOE4* cerebrovascular dysfunction that may drive age-related cognitive decline and resilience, highlighting new therapeutic strategies for a major AD risk population.



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Neural stem cells sculpt vascular properties in the adult subventricular zone neurogenic niche

Friday, 3rd October - 12:00: (Auditorium 1) - Oral

Prof. Carmen Ruiz de Almodovar [1], ***Dr. María Isabel Alvarez*** [1], ***Dr. Andromachi Karakatsani*** [2], ***Dr. Jóse Ricardo Vieira*** [3]

1. *University of Bonn,* ***2.*** *European Center for Angioscience, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany.,* ***3.*** *Department of Neurobiology, Interdisciplinary Centre for Neurosciences (IZN), Heidelberg University, Germany*

The neurovascular unit (NVU) and the blood-brain barrier (BBB) are unique features of the CNS vasculature. NVU and BBB properties appear to vary across different brain regions; however, the mechanisms underlying these differences remain poorly understood. In this study, we characterize vascular heterogeneity and NVU properties in the neurogenic niche of the subventricular zone (SVZ). We provide evidence highlighting that the NVU in the SVZ exhibits distinct anatomical and morphological characteristics compared to the cortex. We further show that endothelial cells of the SVZ vasculature have different expression of BBB transporters, and metabolic functions. Finally, we provide data demonstrating that NVU properties change when neural stem cells are altered, indicating
that neural stem cells instruct NVU/BBB dynamics in their own niches.

From Resilience to Recovery: Microvascular Adaptations to Pericyte Loss in two Murine Models of Brain-Specific Conditional Depletion

Friday, 3rd October - 12:15: (Auditorium 1) - Oral

Dr. Audrey Chagnot [1], ***Ms. Dorota Stefancova*** [1], ***Ms. Nela Fialova*** [1], ***Dr. Justyna Cholewa-Waclaw*** [2], ***Mr. Ross Lennen*** [3], ***Dr. Owen Dando*** [4], ***Dr. Andrea Corsinotti*** [5], ***Dr. Meryam Beniazza*** [5], ***Dr. Bethany Geary*** [6], ***Dr. Michael Sewell*** [7], ***Dr. Axel Montagne*** [1]

1. *1. UK Dementia Research Institute, The University of Edinburgh, UK; 2. Institute for Neuroscience and Cardiovascular Research (INCR), The University of Edinburgh, UK; 3. BHF-UK DRI Centre for Vascular Dementia Research,* ***2.*** *High Content Screening Facility, Institute for Regeneration and Repair, The University of Edinburgh, Edinburgh, EH16 4UU, UK,* ***3.*** *Centre for Cardiovascular Science, University of Edinburgh, Queen's Medical Research Institute, Edinburgh EH16 4TJ, UK,* ***4.*** *Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, UK.,* ***5.*** *Institute of Regeneration and Repair, Centre for Regenerative Medicine, University of Edinburgh, 5 Little France Drive, Edinburgh EH16 4UU, UK,* ***6.*** *University of Dundee,* ***7.*** *1. UK Dementia Research Institute, The University of Edinburgh, UK*

Pericytes, mural cells lining the endothelium of the microvasculature, play key roles in maintaining the blood-brain barrier (BBB) phenotype and facilitating neurovascular coupling. Loss of pericytes in ageing has been associated with reduced cerebral blood flow, BBB disruption, and impaired neurovascular coupling.

Pericytes and endothelial cells engage in a complex molecular crosstalk that ensures their mutual stabilisation. The loss of pericyte coverage disrupts this balance, leading to the breakdown of the brain-specific endothelial phenotype and compromising the integrity of the BBB. Without the contractile function of pericytes, local vascular tone diminishes, causing reduced blood flow, red blood cell stalling, and ischaemic conditions. In addition, the absence of pericyte cell bodies creates unregulated "dark pockets", where neuronal metabolic demands are no longer matched to blood supply. However, evidence suggests that vessels can adapt to pericyte loss by extending the processes of surviving pericytes or recruiting new ones.

To investigate these mechanisms, we present here two mouse models with brain-specific, conditional pericyte depletion. In the *Atp13a5;ROSA-DTA* model, tamoxifen administration induces a gradual, chronic loss of pericytes. In contrast, the *Atp13a5;iDTR* model exhibits a rapid, acute depletion of pericyte following diphtheria toxin injection, which is then followed by a swift recovery.

In this study, we examine the morphological and functional alterations in brain vasculature across the acute, chronic, and recovery phases of pericyte depletion, using magnetic resonance imaging, transcriptomics, proteomics, and immunohistochemistry. Leveraging in-house analytical tools, we assessed changes in pericyte subtype morphology and soma distribution.

Clinically, pericyte loss and compensatory mechanisms often occur in parallel. Our findings reveal that sustained imbalance in this dynamic can drive maladaptive vascular changes, contributing to hallmarks of cerebrovascular ageing.

Pericyte immaturity leads to fibrovascular anomalies and long-term retinal dysfunction

Friday, 3rd October - 12:30: (Auditorium 1) - Oral

Dr. Pilar Villacampa [1], ***Ms. Nekane Maritorena-Hualde*** [1], ***Ms. Catalina Prats-Lluís*** [1], ***Mr. Hielke van Splunder*** [2], ***Mr. Xabier Perosanz*** [2], ***Dr. Anabel Martinez-Romero*** [2], ***Mr. Xavier Vallvé*** [1], ***Ms. Emma Cerrato*** [1], ***Prof. Ana Mendez*** [1], ***Dr. Luis Arias-Barquet*** [3], ***Prof. Eloi Montanez*** [4], ***Dr. Mariona Graupera*** [2]

1. *Universitat de Barcelona,* ***2.*** *Josep Carreras Leukaemia Research Institute (IJC),* ***3.*** *Hospital Universitari de Bellvitge,* ***4.*** *University of Barcelona*

Pericytes are an essential component of BBB, controlling the passage of fluid and substances into the parenchymal space. Vessel formation and maintenance require an optimal interaction between pericytes and endothelial cells. Sustained immature state in pericytes results in vascular hyperplasia and blocks vascular remodelling during the first angiogenic wave in the postnatal retina, but its consequences during inner retinal vascularization remain unknown. Pericyte immaturity was induced by PTEN deletion in mural cells upon Pdgfrb-Cre^{ERT2} postnatal activation (P1, P2; PTEN^{iDMC}). After a short period of vascular normalization, two weeks-old mutant mice showed abnormal proliferation of retinal vascular cells, associated with ectopic αSMA expression and increased ECM protein deposition. Microvascular abnormalities spanned through the superficial and intermediate vascular plexus of PTEN^{iDMC} retinas, both in central and peripheral areas. These alterations led to intraretinal haemorrhages, disturbing overall retinal homeostasis in terms of gliosis and inflammation. Single-cell transcriptomics in PTEN^{iDMC} retinas at P15 revealed a cluster of cells showing reduced expression of pericyte markers and a strong enrichment in profibrotic genes and myofibroblast markers as potential mediators of the phenotype. Unlike other models, all these pathological features persisted in adult PTEN^{iDMC} mice leading to overt retinal dysfunction assessed by electroretinography. We aim to unravel the molecular programmes of those immature and pathological mural cells that trigger retinal vascular abnormalities and to identify key players involved. We postulate that the study of the molecular cues that govern that process will provide an innovative perspective to tackle neovascular aspects of retinal disease.

Higher blood-brain barrier leakage in young and middle-aged adults with early stages of cerebral small vessel disease.

Friday, 3rd October - 12:00: (Auditorium 2) - Oral

Prof. Frank-Erik Leeuw [1], ***Ms. Esther Janssen*** [1], ***Dr. Joost de Jong*** [2], ***Dr. Esmee Verburgt*** [1], ***Dr. Annemieke ter Telgte*** [3], ***Dr. Danielle van den Berg*** [4], ***Dr. Jose Marques*** [5], ***Dr. Marnix Maas*** [6], ***Dr. Anton Meijer*** [7], ***Dr. Anil M Tuladhar*** [1], ***Prof. Niels Riksen*** [8], ***Dr. Jaap Deinum*** [8], ***Prof. Walter Backes*** [9]

1. *department of Neurology, Radboudumc,* ***2.*** *Research institute for Mental Health & Neuroscience (MHeNs), Maastricht University, Maastricht, the Netherlands,* ***3.*** *VASCage – Centre on Clinical Stroke Research, Innsbruck, Austria,* ***4.*** *Department of Internal Medicine, Rijnstate Hospital, Arnhem,* ***5.*** *Donders Institute for Brain, Cognition and Behaviour, Centre for Cognitive Neuroimaging, Radboud University Nijmegen, PO Box 9101, 6500, HB, Nijmegen,* ***6.*** *Department of Medical Imaging, Radboud University Medical Center, Nijmegen,* ***7.*** *7. Department of Medical Imaging, Radboud University Medical Center, Nijmegen,* ***8.*** *8. Department of Internal Medicine, Radboud University Medical Center, Nijmegen,* ***9.*** *Research institute for Mental Health & Neuroscience (MHeNs), Maastricht University, Maastricht, the Netherlands and Maastricht University Medical Center (MUMC+), Department of Radiology & Nuclear Medicine, Maastricht,*

Background: Hypertension is the major risk factor for cerebral small vessel disease (SVD), but the underlying mechanisms remain incompletely understood. BBB leakage is hypothesized to be involved early in the pathogenesis of SVD, but as this was mainly demonstrated in older patients with already SVD for decades on MRI, proof thereof is lacking. We therefore investigated BBB leakage in young patients with hypertension, at risk for SVD, but yet without visible SVD, compared to controls.

Methods: BBB leakage of gadolinium contrast agent was measured using dynamic contrast-enhanced (DCE)-MRI at 3 Tesla in 59 patients with hypertension and 21 healthy controls. Voxel-wise pharmacokinetic modelling using the Patlak approach was applied to obtain leakage rate *Ki* [min-1] and fractional plasma volume *Vp* [-]. Linear regression adjusted for age and sex was used to compare BBB leakage in the white matter (WM), grey matter (GM), deep GM, and cortex between patients and controls. MRI markers of SVD were rated according to established STRIVE criteria.

Results: Mean age of patients (54% female) was 35.8 years (SD 9.7) and 29.9 years (SD 4.8) in controls (female 52%) ($p<0.01$). Patients had a significantly higher systolic blood pressure than controls (146.4 mm Hg (SD 17.3) versus 119.9 mm Hg (SD 11.0)). Patients with hypertension had significantly higher WM BBB leakage (*Ki*) than controls ($2.8\ 10^{-4}$ min $^{-1}$ vs 2.410^{-4} min $^{-1}$, $p<0.05$), adjusted for age, sex and MRI markers of SVD (example figure 1). There was a trend towards a positive relation between BBB permeability (*Ki)* and higher systolic blood pressure in the white matter (fig 2; standardized $\beta = 0.207$, $p=0.075$). There were no differences in *Vp* between groups.

Conclusion: Young and middle-aged patients with the earliest manifestations of SVD caused by hypertension already have significantly higher BBB leakage independent of pre-existing SVD MRI markers. This suggests a possible role of BBB dysfunction in SVD pathogenesis. Restoration of BBB integrity may be a relevant target for interventions in SVD, but longitudinal studies are needed to confirm causality.

Figure 1: Color-coded gadolinium leakage in healthy control (A) and age-matched participant with hypertension (B). T1, FLAIR and leakage map overlayed on T1 images of 26-year-old control with blood pressure 125/79 mmHg (A) and 26-year-old participant with hypertension with blood pressure 145/94 mmHg (B). Lighter colours reflect increased leakage rate.

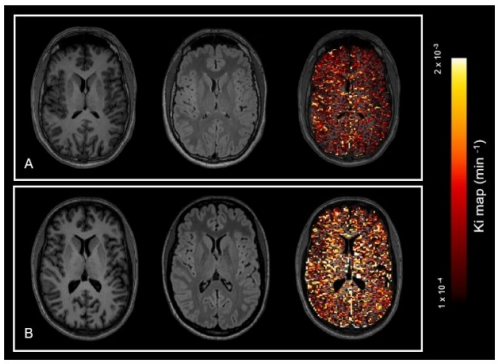


Figure 1.jpeg

Figure 2: scatterplot of the BBB leakage rate in the white matter versus the systolic blood pressure. Pink dots indicate control participants and blue dots indicate patients with hypertension. The regression line is for visualization purposes only and is not corrected for age and sex.

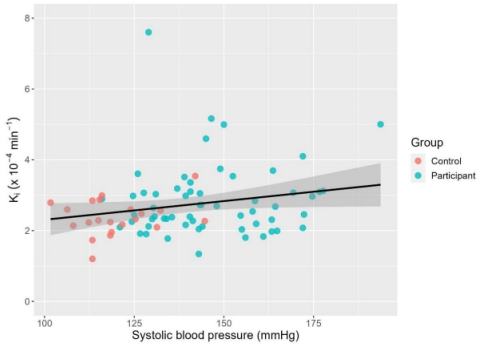


Figure 2.jpeg

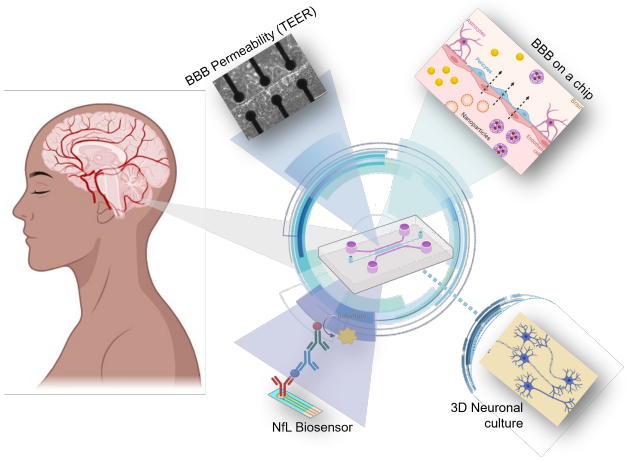
Monitoring systems in biological models to recapitulate a neurovascular unit-on-a-chip

Friday, 3rd October - 12:15: (Auditorium 2) - Oral

Dr. Monica Mir [1], ***Ms. Anna Panteleeva*** [1], ***Dr. Sujey Palma-Florez*** [1], ***Ms. Carla Arroyo*** [1], ***Dr. Anna Lagunas Targarona*** [1], ***Prof. Josep Samitier Martí*** [1]

1. *IBEC*

Neurodegenerative diseases (NDDs) represent a serious health threat. Unfortunately, drugs targeting the central nervous system (CNS) have much higher failure rates than non-CNS-targeted drugs, both preclinically and clinically. One of the reasons implicated is the high restriction of the blood-brain barrier (BBB).
In the search for innovative tools for studying NDDs and drug delivery, brain-on-chip (BoC) technology has recently emerged. It is a powerful tool for studying the behavior of neural networks in a controlled environment. Compared to in vivo analysis, BOCs are more cost effective, easier to use, and animal-free alternatives, allowing for the use of human cells and personalized models. Incorporating a BBB component into a BoC model, further enhances its potential as a physiologically relevant model and adds the unique properties of the BBB [2]. BoCs and organ-on-chip technology in general have traditionally been characterized by optical detection methods, but they require expensive equipment, a large investment of time, and skilled personnel. Electronic monitoring systems (i.e., electrochemical biosensors) offer numerous advantages, such as the ability to monitor in real time and automate the detection of a wide range of analytes [3].
In this context, we present the development of a neurovasculature unit-on-a-chip (NVUoC) model with integrated monitoring systems. The chip contains a coculture of human endothelial cells in close interaction with human astrocytes and pericytes, in combination with cortical neurons. The sensing platform combines electrodes to analyze blood-brain barrier (BBB) permeability using transendothelial electrical resistance (TEER) and an electrochemical immunosensor to detect neurofilament light (NfL), providing a noninvasive method for continuous monitoring of neuronal activity, neurotoxicity, and disease progression. The monitoring system supports optical characterization, and the chip was analyzed in parallel using live/dead cell assays, immunofluorescence, and dextran-based permeability assays.



Brainoc sensors.png

Impact of hypertension on cerebral small vessel disease: A post-mortem study of microvascular pathology from normal-appearing white matter into white matter hyperintensities

Friday, 3rd October - 12:30: (Auditorium 2) - Oral

Dr. Gemma Solé-Guardia [1], ***Ms. Anne Janssen*** [1], ***Mr. Rowan Wolters*** [1], ***Mr. Tren Dohmen*** [1], ***Dr. Benno Küsters*** [2], ***Dr. Jurgen AHR Claassen*** [3], ***Dr. Anil M Tuladhar*** [4], ***Prof. Frank-Erik Leeuw*** [4], ***Dr. Maximilian Wiesmann*** [1], ***Dr. Jose Gutierrez*** [5], ***Prof. Amanda Kiliaan*** [1]

1. *Department of Medical Imaging, Anatomy, Radboudumc,* ***2.*** *Department of Pathology, Research Institute for Medical Innovation, Radboud University Medical Center,* ***3.*** *Department of Geriatrics, Radboudumc,* ***4.*** *department of Neurology, Radboudumc,* ***5.*** *Department of Neurology, Vagelos College of Physicians and Surgeons, Columbia University Medical Center*

Cerebral small vessel disease (SVD) is the major vascular risk factor for stroke and dementia. SVD is diagnosed through imaging hallmarks like white matter hyperintensities (WMH). Novel hypotheses imply that endothelial dysfunction, blood-brain barrier (BBB) disruption and neurovascular inflammation are amongst the earliest contributors to the conversion of normal-appearing white matter (NAWM) into WMH in hypertensive individuals. Aiming to unravel the association between chronic hypertension and the earliest WMH pathogenesis, we characterized microvascular pathology in periventricular NAWM into WMH in post-mortem brains of individuals with (n=17) and without hypertension (n=5). Specifically, we evaluated microvascular pathology using GLUT1 and Masson's trichrome staining; blood-brain barrier (BBB) damage by assessing immunoglobulin G (IgG) extravasation; and neurovascular inflammation by analyzing matrix metalloproteinase 9 (MMP9) staining in NAWM and WMH. Our second aim was to delineate the NAWM-WMH transition from NAWM towards the center of WMH using deep learning to refine WMH segmentation thereby capturing increases in FLAIR signal. Such approach may facilitate the use of imaging, supported by AI, to capture gradients of microvascular pathology. Finally, we aimed to demonstrate, by performing voxel-wise correlations between MRI and microvascular pathology, whether these processes, particularly BBB damage and neuroinflammation, may synergistically contribute to WMH pathogenesis. Larger endothelium disruption ($p<0.001$), BBB damage ($p=0.018$) and neurovascular inflammation ($p=0.004$) were observed in individuals with hypertension. We did not observe gradual BBB damage nor neurovascular inflammation along the NAWM-WMH transition. We found a strong correlation between BBB damage and neurovascular inflammation in all individuals in both periventricular NAWM and WMH ($\rho = 0.370$, $p<0.001$). These novel findings suggest that endothelium disruption, BBB damage and neurovascular inflammation are major contributors to SVD progression, being already present in NAWM in hypertension.

Involvement of the synaptic protein Synapsin III in Parkinson's disease-associated neurovascular damage

Friday, 3rd October - 12:00: (Sala Blava 2) - Oral

Mr. Francesco Amodeo [1], Dr. Viviana Brembati [1], Dr. Francesca Longhena [2], Dr. Arianna Bellucci [1], Dr. Gaia Faustini [1]

1. *Department of Molecular and Translational Medicine, University of Brescia,* ***2.*** *Department of Clinical Neurosciences-Clifford Allbutt Building, University of Cambridge*

Parkinson's disease (PD) is the most common movement disorder. The main hallmarks are the loss of nigrostriatal dopaminergic neurons and the deposition of Lewy bodies (LB), insoluble inclusions mainly composed of fibrillary alpha-synuclein (aSyn) (Spillantini et al., 1997, Nature, 10.1038/42166). Compelling evidence indicates that the deposition of aSyn aggregates is the main driver of neurodegeneration in PD. Of note, aSyn pathology-linked neuroinflammation, the associated vascular changes and lymphocyte infiltration play a central role in PD onset and progression (Wu et al., 2024, ACTA Neuropathol 10.1007/s00401-024-02696-z; Brochard et al., 2009, J Clin Invest, 10.1172/JCI36470). Recently, the synaptic protein synapsin III (Syn III) has emerged as a new key player in PD, as it participates in the formation and stabilization of aSyn fibrillary aggregates and is tightly bound to aSyn fibrils purified from post-mortem brains of PD patients (Longhena et al., 2018, Brain Pathol 10.1111/bpa.12587; Faustini et al., 2018; ACTA Neuropathol 10.1007/s00401-018-1892-1; 2022 Mol Ther 10.1016/j.ymthe.2022.01.021).

In this study, we addressed whether reproducing Syn III increase by adeno associated viral vector-mediated overexpression in the nigrostriatal system of C57BL/6J mice may result in the onset of PD-like phenotype including neurovascular alterations.

Remarkably, we found that accumulation of Syn III in the nigrostriatal system resulted in aSyn aggregation and pathological deposition and associated with dopamine transporter loss in the striatum and motility deficits. In addition, mice overexpressing Syn III developed significant astrocyte activation, alterations of vascular integrity with fibrinogen extravasation and peripheral immune cell infiltration.

In parallel, we observed a marked accumulation of Syn III within the vessel walls of post-mortem PD brain samples. Complementary in vitro experiments demonstrated that human brain microvascular endothelial cells (hBMEC) can endocytose recombinant Syn III, and that their exposure to Syn III could reduce the levels of the tight junction proteins ZO-1 and occludin.

Collectively, our findings support that the increase of Syn III observed in the vessel walls of post-mortem PD brains may participate in the induction of blood brain barrier leakage suggesting that Syn III may be crucially involved in PD neurovascular damage.

Loss of endothelial CD2AP causes sex-dependent cerebrovascular dysfunction

Friday, 3rd October - 12:15: (Sala Blava 2) - Oral

Mrs. Milène Vandal [1], ***Mr. Adam Institoris*** [1], ***Mrs. Louise Reveret*** [2], ***Mr. Sotaro Hirai*** [1], ***Mrs. Yulan Jiang*** [1], ***Mrs. Suzie Lee*** [1], ***Mr. Philippe Bourassa*** [2], ***Mr. Govind Peringod*** [1], ***Mrs. Faye Arellano*** [1], ***Mrs. Cyntia Tremblay*** [2], ***Mrs. Kelsea Gorzo*** [1], ***Prof. Grant Gordon*** [1], ***Prof. Frederic Calon*** [2], ***Prof. Minh Dang Nguyen*** [1]

1. *University of Calgary,* ***2.*** *Université Laval*

Polymorphisms in CD2-associated protein (CD2AP) predispose to Alzheimer's disease (AD), but the underlying mechanisms remain unknown. Here, we show that loss of CD2AP in cerebral blood vessels is associated with cognitive decline in AD subjects and that genetic downregulation of CD2AP in brain vascular endothelial cells impairs memory function in male mice. Animals with reduced brain endothelial CD2AP display altered blood flow regulation at rest and during neurovascular coupling, defects in mural cell activity, and an abnormal vascular sex-dependent response to Abeta. Antagonizing endothelin-1 receptor A signaling partly rescues the vascular impairments, but only in male mice. Treatment of CD2AP mutant mice with reelin glycoprotein that mitigates the effects of CD2AP loss function via ApoER2 increases resting cerebral blood flow and even protects male mice against the noxious effect of Abeta. Thus, endothelial CD2AP plays critical roles in cerebrovascular functions and represents a novel target for sex-specific treatment in AD.

Flow-induced shear stress protects against vascular dysfunction in a stem cell-derived microfluidic model of the blood-brain barrier in Alzheimer's disease

Friday, 3rd October - 12:30: (Sala Blava 2) - Oral

Ms. Lily Takeuchi [1], *Ms. Jennifer Lam* [1], *Mr. Kent Qi* [1], *Dr. Uros Kuzmanov* [1], *Dr. Anthony Gramolini* [1], *Dr. Craig Simmons* [1]

1. University of Toronto

Background: Growing evidence has identified vascular dysfunction as an early event in Alzheimer's Disease (AD), preceding even the formation of pathological hallmarks such as amyloid deposition. However, the mechanisms underlying vascular pathology in AD have yet to be fully understood, in part, due to the lack of physiologically relevant models of the cerebral vasculature. To address this unmet need, we applied a novel microfluidic blood-brain barrier model to discover mechanisms of vascular dysfunction in AD.

Methods: Stem cells derived from a patient with familial AD and a control line (fControl) were differentiated into brain endothelial-like cells (BECs) and neurons **(Fig. 1A)**. BECs were cultured statically or exposed to 12 dynes/cm^2 of shear stress for 72 hours prior to functional assessment by efflux transporter inhibition and monocyte adhesion assays. Proteomics was conducted in BEC-neuron cocultures to identify paracrine mediators of vascular dysfunction.

Results: BECs from the patient with AD (AD-BECs) demonstrated impaired efflux transport by p-glycoprotein, breast cancer resistant protein, and multidrug resistant protein compared to controls (fControl-BECs, p = 0.0015, p = 0.0004, p = 0.0002, respectively) **(Fig. 1B)**. Under shear stress, no differences were observed suggesting shear maintains efflux transport function. AD-BECs exhibited increased monocyte adhesion (1.9-fold; p<0.01) which was reduced under shear stress in both lines (AD: p<0.001; fControl: p<0.01) **(Fig. 1C)**. Proteomic assessment revealed differential protein expression in BECs and neurons **(Fig. 2A)** related to mitochondrial dysfunction and oxidative phosphorylation in AD models **(Fig. 2C-E)**. Secretome analysis identified insulin degrading enzyme (IDE) as a potential shear-sensitive protective paracrine factor, with IDE reduced in conditioned media of AD cocultures in static conditions compared to fControl cocultures in both static and shear (p<0.0001) **(Fig. 2B)**.

Conclusion: We report the lack of shear stress (reflective of hypoperfusion) dysregulates BEC efflux transport function, immune interactions, proteome, and secretome in a model of the cerebral vasculature in AD. The identification of IDE as a protective factor upregulated by shear stress suggests a novel mechanism by which hypoperfusion could lead to loss of protection, opening new avenues to combat vascular dysfunction in neurodegeneration.

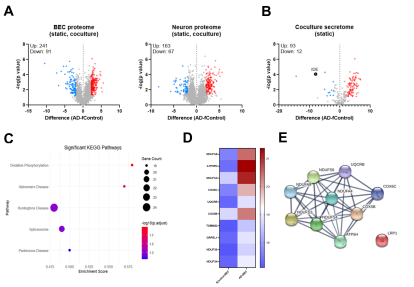


Figure 2: Proteome and secretome analysis of static BEC-neuron cocultures. A) Volcano plots demonstrating differential protein expression between AD and fControl cell lysates from BECs (left) and neurons (right). B) Volcano plot demonstrating differential protein expression in conditioned media between AD and fControl cocultures, highlighting insulin degrading enzyme as a protein significantly downregulated in AD. C) Significant KEGG pathways identified in lysates of AD-BECs. Top 10 core enriched proteins of the AD KEGG pathway plotted D) a heat map of label-free quantification values and E) a protein interactome.

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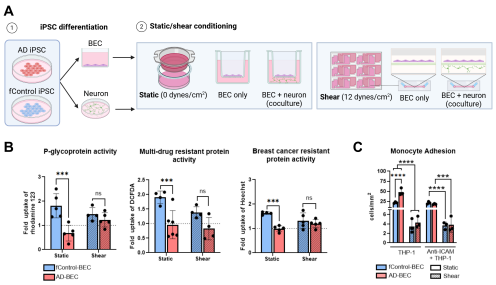

Figure 1: Characterization of iPSC-derived BECs in AD. A) Workflow of iPSC differentiation and static and shear conditioning methods. C) Static conditions reveal alterations to efflux transport activity of p-glycoprotein, multi-drug resistant protein, and breast cancer resistant protein in AD-BECs. C) AD-BEC monolayers show increased adhesion of THP-1 monocytes compared to fControl-BEC monolayers under static conditions. Adhesion is decreased for both groups upon application of shear stress.

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The Blood-Brain Barrier: Insights into Modeling, Aging, and Transport Opportunities

Friday, 3rd October - 13:45: (Auditorium 1) - Invited Speaker

Prof. Lino Ferreira [1]

1. *university of coimbra*

TBC

The effects of pericyte-mediated capillary constriction in Alzheimer's disease and how to reverse them

Friday, 3rd October - 14:25: (Auditorium 1) - Invited Speaker

Prof. David Attwell [1]

1. *University College London*

It is common when studying Alzheimer's disease (AD) to focus mainly on generation of amyloid beta (Abeta) and hyperphosphorylated tau, and the changes of synaptic function and loss of cognitive power that they eventually produce. In this talk I will emphasise an Abeta-driven factor that probably contributes early in the disease to later cognitive decline: a decrease of cerebral blood flow.

We previously used imaging experiments to show that, in vivo in human AD and in a mouse model ($APP^{NL\text{-}G\text{-}F}$) of AD, capillaries (but not arterioles or venules) become constricted as a result of pericytes contracting (Nortley et al., 2019, Science). This was predicted to roughly halve cerebral blood flow. Human and mouse brain slice experiments suggested that the capillary constriction reflects oligomeric Abeta evoking, in microglia and pericytes, generation of reactive oxygen species that then trigger the release of endothelin-1 (ET, possibly from endothelial cells or astrocytes) which evokes pericyte contraction. We have also shown that contraction of pericytes is amplified by a mechanism in which ET-evoked Ca^{2+} release from intracellular stores activates the TMEM16A chloride channel, generating a depolarization that opens voltage-gated calcium channels (Korte et al., 2022, JCI).

We now report that giving to AD mice the voltage-gated calcium channel (CaV) blocker nimodipine in their drinking water from early in AD increased capillary diameter at pericytes, reduced leukocyte stalling at pericyte somata, improved CBF and attenuated brain hypoxia. Aβ-evoked pericyte contraction in human cortical tissue was also greatly reduced by CaV block.

Thus, awareness of the possibility of glia- and pericyte-mediated capillary constriction reveals new therapeutic targets to increase blood flow in AD, and possibly other neurological pathologies.

Circadian and sleep regulation of the BBB

Friday, 3rd October - 15:05: (Auditorium 1) - Invited Speaker

Prof. Amita Sehgal [1]

1. *The University of Pennsylvania*

Amita Sehgal, HHMI, University of Pennsylvania Perelman School of Medicine
Almost all physiological processes display circadian regulation, manifest as a 24 hour rhythm, on some level. Often these processes are also affected by behavioral state, e.g. sleep. We showed that the activity of efflux transporters in the BBB is controlled by a circadian clock within the BBB that drives high efflux from the brain during the animal's waking hours. As a result, small lipophilic molecules, targets of these transporters, are more likely to be retained by or transported into the brain during sleep. This rhythm of efflux occurs in both Drosophila and mammals and while the mechanisms driving the rhythm are a bit different in the two species, both involve the cycling of magnesium ions. We showed that this rhythm can regulate the permeability of a specific drug into the brain.
In other work, we found that endocytosis through the BBB is dependent on sleep. Again, this is true in Drosophila and mice and likely serves to clear the brain of metabolic products.

Vessel Types Determine Extent of Blood-Brain Barrier Paracellular Leakage, Adsorptive Transcytosis Increase, Responses to Treatments, and Glial Activation in Huntington's Disease

Friday, 3rd October - 16:15: (Auditorium 1) - Invited Speaker

Dr. Krzysztof Kucharz [1]

1. *University of Copenhagen*

TBC

Authors Index