1ST YOUNG GLIAL CELL DAY

GRAND AMPHITHEATRE – 18 Quai Claude Bernard

LYON, FRANCE

MAY 23, 2023

Young Researchers Institut NeuroMyoGene

NEUR ATRIS
Innovation for Translational Neuroscience

Table des matières

WELCOME !

Dear colleagues,

It's a great pleasure to welcome you to our 1st Young Glial Cell Day, co-organized with the French Glial Cell Club, YRIN and NEURATRIS.

Through this day, we would like to bring together researchers and students working on the multifaceted role of glial cells (astrocytes, microglia, oligodendrocytes, Schwann cells, tanycytes...), both in the central and peripheral nervous systems.
This day aims to promote and encourage exchanges/collaborations within this scientific community.
Finally, contrary to other usual congresses, this day is dedicated to students (PhD students, postdoctoral fellows) and engineers in order to give them the opportunity to present their work.

During this day of meeting, no less than 52 speakers will present their most recent findings, through one plenary lecture, four symposia and two poster sessions.
The meeting will be punctuated by the Glial Cell Club's general assembly and the social event, taking place in the Maker of Stories bar.

The day will end with our social event and the general assembly with the aim of providing information of the Club's past and future activities, news, and collecting ideas guiding future orientations of the Club.

Finally, we would like to thank our sponsors: Vizgen, NSCo Neurosciences et Cognition, UFR Biosciences Université Claude Bernard Lyon 1, and Labex Cortex Université de Lyon, thanks to whom the organization of this meeting was possible.
We wish you an excellent meeting with many and fruitful interactions, hoping that you will have as much pleasure in participating in this meeting as we had in organizing it.

ACKNOWLEGEMENTS

The French Glial Cell Club gratefully acknowledges the collaboration and financial support of the following partners:

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MEETING PROGRAM

Program

10:00-10:20 **Reception**

10:20-10:30 **Opening Ceremony**

10:30-11:30 **Symposium 1: Astrocytes signaling in physiopathology**

Chairs: Martine COHEN-SALMON & Tom LAKOMY

Katia-Raquel AVILA-GUTIERREZ (Paris, FRANCE)
Alteration of astrocyte local translation: A pathogenic mechanism in Alzheimer's disease

Angel BAUDON (Strasbourg, FRANCE)
Central amygdala astrocytes oxytocin receptor Gai pathway mediates behavioral adaptation in mice

Yiannis POULOT (Paris, FRANCE)
Astrocytes heterogeneity in Alzheimer's disease

11:30-11:40 **Vizgen Presentation**

Sébastien BELLOW
Mapping the Future with Spatial Genomics

11:40-12:25 **Coffee break + Poster session 1**

12:25-13:25 **Symposium 2: Oligodendrocyte differentiation and maturation in the developing brain and pathologies**

Chairs: Brahim NAIT OUMESMAR & Jeflie TOURNEZY

Mary-Amélie MASSON (Paris, FRANCE)
A role for the post-synaptic density protein PSD-95 in CNS myelination

Cristobal IBACETA (Paris, FRANCE)
Developmental cell death of oligodendroglia is required for cognitive flexibility in mice

Nina POTTIER (Paris, FRANCE)
Functional consequences of IDH1 and CIC mutations on oligodendroglioma cells of origin

13:25-15:00 **Lunch break + "Free" Poster session**

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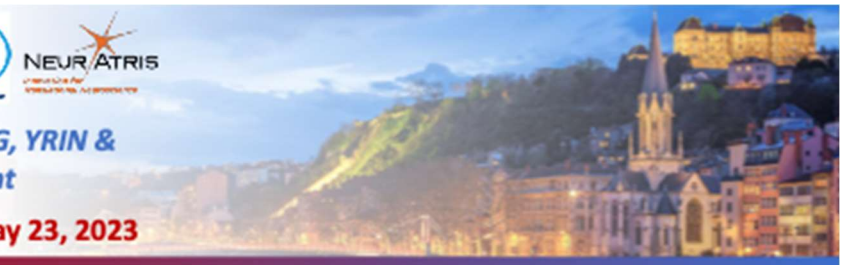

Young Researchers of CCG, YRIN & NeurATRIS present

I[st] Young Glial Cell Day - May 23, 2023

Program

15:00-16:00 **Plenary Lecture**

Chairs: Kassandre COMBET, Chloé DIAS, Sarah MOUNTADEM & Nina POTTIER

Pr. Mikael SIMONS (Munich, GERMANY)
Mechanisms of (re)myelination in the CNS

16:00-17:00 **Symposium 3: Revisiting cellular communication through microglia from extracellular vesicles to activity dependent interactions**

Chairs: François RASSENDREN & Nissrine BALLOUT

Kassandre COMBET (Lyon, FRANCE)
Microglia morphodynamics regulation by neuronal activity: A daytime matter?

Chloé DIAS (Toulouse, FRANCE)
Microglia-derived extracellular vesicles promote neuropathology in Sanfilippo syndrome

Clément PERROT (Paris, FRANCE)
Neuronal activity modulates microglia phenotype in repair through microglia-node of Ranvier interaction

17:00-17:45 **Coffee break + Poster session 2**

17:45-18:45 **Symposium 4: Role of glial cells in brain functions and homeostasis**

Chairs: Etienne AUDINAT & Mary-Amélie MASSON

David GUENOUN (Paris, FRANCE)
Premature brain ageing, the aftermath of an early-life inflammatory event

Mahalakshmi DHANASEKAR (Paris, FRANCE)
Investigation of mechanisms underlying glia-dependent motor arrest

Barbara DELAUNAY-PIEDNOIR (Paris, FRANCE)
Impact of gestational exposure to pesticides in gliovascular interactions establishment during brain development

18:45-19:15 **Closing and Awards ceremony**
19:15-19:30 **General assembly**
20:00 **Social Event**

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PLENARY PRESENTATION

Mechanism of myelination and remyelination in the CNS

Pr. Mikael Simons

We study the mechanisms of myelination and remyelination in the CNS. Our goal is to understand why myelin repair fails in multiple sclerosis and to develop regenerative medicines for the nervous system. A central obstacle for progress in this area has been the complex biology underlying the response to CNS injury. Acute CNS damage is followed by a multicellular response that encompasses different cell types and spans different scales. Currently, we do not understand which factors determines lesion recovery. Failure of inflammation to resolve is a key underlying reason of poor regeneration, and one focus is therefore on the biology of microglia during de- and remyelination, and their cross talk to other cells, in particular oligodendrocytes and the progenitor cells. In addition, we are exploring the link between lipid metabolism and inflammation, and its role in the regulation of regeneration. I will report about our recent progress in our understanding of myelination and remyelination in the CNS.

SYMPOSIA PRESENTATION

Alteration of astrocyte local translation: a pathogenic mechanism in Alzheimer's disease

Katia-Raquel AVILA-GUTIERREZ
Avila K.[1], Oudart M.[1], Carrillo-de Sauvage M.[2], Poulot-Becq-Giraudon Y.[2], Garcia V.[3], Hirbec H.[3], Escartin C.[2], Cohen-Salmon M.[1]
[1] Center for Interdisciplinary Research in Biology (CIRB), College de France, CNRS, INSERM, Université PSL, Paris, France.
[2]Université Paris-Saclay, CEA, CNRS, MIRCen, Laboratoire des Maladies Neurodégénératives, Fontenay-aux-Roses, France.
[3]Institut de Génomique Fonctionnelle, Département de Neurosciences, Montpellier, France.

Astrocytes are complex, highly ramified, and voluminous glial cells in the brain. They possess a unique polarization with processes simultaneously contacting the synapses and the blood vessels. Via their perisynaptic astrocytic processes (PAPs), astrocytes are able to regulate and contribute to the formation, maturation and activity of synapses. Whereas the perivascular astrocytic processes (PvAPs) fully sheathe the brain vasculature and influence the perivascular homeostasis, blood brain barrier integrity, neurovascular coupling among other functions. In Alzheimer's disease (AD), the most common form of dementia worldwide, astrocytes become reactive and undergo important morphological and molecular changes, provoking perturbations in their perisynaptic and perivascular properties, but the molecular mechanisms behind these effects are poorly described. Our lab recently suggested that astrocyte perisynaptic and perivascular functions are sustained by local translation, a well-known and conserved evolutionary mechanism important for cell polarity. We hypothesized that mRNA transport and local translation changes in astrocytes may contribute to AD-linked astrocytic vascular and synaptic dysfunctions. We extracted ribosome-bound mRNAs by translating ribosome affinity purification (TRAP) in hippocampal astrocytes, isolated PAPs in wild type mice and in the APP/PS1 mouse model of AD, and analyzed them by RNA sequencing. Our preliminary results indicate that major translation changes related to astrocyte reactivity occur specifically in PAPs at an early stage of the disease. We are currently validating these results and analyzing earlier stages of the disease to uncover when these mRNA expression modifications start in PAPs during AD. These observations are the first steps to provide important insights into the molecular alterations of astrocytes in Alzheimer's disease and to identify interesting new targets for future therapeutic treatments.

Central amygdala astrocytes oxytocin receptor Gαi pathway mediates behavioral adaptation in mice

Angel BAUDON
Baudon A.[1], Althammer F.[2], Grelot V.[1], Wang K-Y[1]. Krabichler Q.[3], Clauss--Creusot E.[1], Rouach N.[4], Breton R.[4], Darbon P.[1], Charlet A.[1]
[1]*Institute of cellular and Integrative Neurosciences-CNRS, Strasbourg France*
[2]*Institute of Human Genetics, Heidelberg University Hospital, Heidelberg, Germany*
[3]*Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany*
[4]*Center for Interdisciplinary Research in Biology, Collège de France, Paris, France*

In order to survive, living beings have to detect, analyze and react to their environment. To weight the importance of sensorial stimuli, life developed emotions, deep and raw feeling that trigger an attraction or a repulsion toward something. These emotional values are then computed to create elaborate feelings such as fear, anger, joy or disgust. The attribution of the emotional valence of things take place in a brain regions buried deep in the temporal cortex named amygdala. This structure is under the regulation of many neuromodulators among which we can find the neuropeptide oxytocin. To evaluate whether oxytocin play a role in the encoding of the fear memory in mice, we evaluate the activity of hypothalamic oxytocin neurons during a fear conditioning protocol and observed an increased activation of these neurons after the conditioning phase. Knowing that oxytocin can modify the astro-neuronal functional network in the amygdala, we evaluate the oxytocin-induced astrocyte and neuronal activity in this structure. Importantly, the oxytocin receptor can couple two distinct intracellular pathways that depend on the Gi and the Gq protein. In light of this information, we used biased agonists to recruit one pathway or the other and we found that the OTR-Gq pathway stimulation triggers the activation of astrocytes and of neurons whereas the activation of the Gi pathway downstream the oxytocin receptor only trigger calcium transient in astrocytes but no change in amygdala neurons' activity. Disconcerted, we decided to evaluate the long-term effect of these Gi-mediated calcium transients in amygdala astrocytes. To do so, we fear conditioned control animals and animals that do not express the oxytocin receptor in amygdala astrocytes. Interestingly, we observed that fear conditioning triggers important morphological changes in amygdala astrocytes and that these changes were correlated with a strong modification of nearby neurons' excitability. Crucially, these data were reproduced with brain slices incubated in the biased agonists and indicates that the activation of the Gi but not the Gq pathway downstream the oxytocin receptor is responsible for these morpho-functionnal changes. In addition to show for the first time that the oxytocin receptor can couple the Gi pathway in the brain at a cellular level, we also provide an original engram mechanism to explain how fear is stored in the brain and how emotional perception are modified after an episode of intense fear.

Astrocyte heterogeneity in Alzheimer's disease

Yiannis POULOT
Poulot Y.[1], Guillemaud O.[1], Møller Clausen M.[1], Brohard S.[2], Muret K.[2], Juricek L.[1], Lakomy T.[1], Derbois C.[2], AureganG.[1], Guillermier M.[1], Ben Haim L.[1], Dufour N.[1], M.-C. Gaillard [1], Bemelmans A.-P.[1], Carrillo de-Sauvage M.A.[1], Milior G.[3], Bonnet E. [2], Rouach N.[3], Escartin C.[1]
[1]Université Paris-Saclay, CEA, CNRS, MIRCen, Laboratoire des maladies neurodégénératives, Fontenay-aux-roses, France
[2]Université Paris-Saclay, CEA, CNRGH, Evry, France
[3]Center for Interdisciplinary Research in Biology, Collège de France, CNRS, INSERM, Labex Memolife, Université PSL, Paris, France

Astrocytes are involved in several neurodegenerative diseases such as Alzheimer's disease (AD). We and others have demonstrated that in AD, astrocytes develop a reactive phenotype and display changes in their molecular, morphological and functional characteristics, which can have deleterious but also beneficial effects, depending on the disease [1]. Histological studies focusing on few markers and more recent single cell/nucleus RNAseq analysis show that different astrocyte populations with specific molecular markers coexist in AD brains [2–4]. However, the signalling cascades controlling these subpopulations as well as their functional characteristics and impact on neurons are still unknown.

The JAK2-STAT3 and NF-κB signalling pathways have been described as important astrocyte regulators in neurodegenerative diseases, including AD [5–7]. To monitor the activity of these two signalling cascades in astrocytes during AD, we have developed astrocyte-specific reporter systems, whereby a promoter with specific responsive elements for the STAT3 or NF-κB pathways controls the expression of the fluorescent proteins GFP or CFP, respectively.

We show that there are at least three distinct astrocyte subpopulations, depending on their active signalling cascades, in the prefrontal cortex (PFC) of 1-year-old APP/PS1dE9 mice (APP mice, a standard AD mouse model [8]). We show that these subpopulations have different morphologies, as assessed by Sholl analysis on brain sections, and specific molecular features, as shown by RNAseq and RT-qPCR on FACS-sorted astrocyte subpopulations. Functional experiments on sorted astrocyte subpopulations or acute slices (e.g. proteostasis, gap junction coupling) reveal that these three subpopulations also have distinct functional profiles.

Using novel viral reporters, we show the existence of three astrocyte subpopulations in the PFC of APP mice, with unique molecular, morphological and functional characteristics. Specific inhibition of the signalling cascade controlling a defective or deleterious subpopulation would represent a more efficient and targeted therapeutic strategy for AD.

Acknowledgements
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A role for the post-synaptic density protein PSD-95 in CNS myelination

Mary-Amélie MASSON
Masson M-A.[1], Graciarena M.[1], Deboux C.[1], Nait Oumesmar B.[1]
[1]Institut du Cerveau - ICM, Sorbonne Université, CNRS, INSERM, Paris, France

Oligodendrocytes (OLs) are the myelinating cells of the central nervous system (CNS) and are derived from oligodendrocyte precursor cells (OPCs). The main function of myelin is to insulate axons, thus allowing saltatory conduction of action potentials and providing trophic and metabolic supports to axons. Interestingly, oligodendroglial cells have the capacity to sense neuronal activity, which regulates myelin sheath formation via the vesicular release of neurotransmitters. Neuronal activity-dependent regulation of myelination is mediated by specialized interactions between axons and oligodendroglia, involving both synaptic and extra synaptic modes of communications. These interactions require the formation of specialized domains at axon oligodendroglial contact sites, with clusters of synaptic vesicles, trans-synaptic and post-synaptic density (PSD) proteins. We previously showed that PSD-95, encoded by the *Dlg4* gene and required for the clustering of glutamatergic receptors at neuronal synapses, is also expressed by oligodendroglial cells. However, the function of PSD-95 in OL development and myelination remains largely unknown. In this study, we investigated the expression pattern of PSD-95 in oligodendroglial lineage cells *in vitro* and *in vivo* during mouse CNS development. The expression profile of PSD-95 was analyzed by immunostaining of mouse brain sections at different developmental stages, using confocal microscopy and 3D imaging reconstruction with the IMARIS software. Our data showed that PSD-95 puncta on oligodendroglial cells increased with their differentiation into mature OLs during active myelination of the mouse brain. PSD 95 puncta were mainly located in myelin sheaths rather than in cell soma or processes of OLs, suggesting that this protein could regulate myelin sheath growth and/or stabilization. We also examined the functional role of PSD-95 in OPC differentiation by miRNA- and CRISPR-mediated loss-of-function in rat primary oligodendroglial cells. Strikingly, our data showed that PSD-95 deletion in oligodendroglia leads to an increase of *Mbp* and *Plp1* gene expression as early as 2 days *in vitro* (DIV) in differentiation condition, and to an increase in MBP+ cells number and surface at 5 DIV. Moreover, we are currently investigating the impact of CRISPR-Cas9 mediated PSD-95 deletion, specifically in oligodendroglia, using *in vivo* live imaging of myelination in the zebrafish larva. Overall, our study should provide better insights of axo-glial communications underlying activity dependent regulation of myelination under physiological and pathological conditions.

This study was supported by ANR, IHU-A-ICM and Sorbonne Université.

Cognitive flexibility impairment in mouse due suppression of developmental cell death in lineage-related interneuron and oligodendroglia

Cristobal IBACETA
Ibaceta-González C.[1], Khelfaoui H.[1], Angulo MC.[1].
[1]Université Paris Cité, Institute of Psychiatry and Neuroscience of Paris (IPNP), Paris, France.

The programmed cell death (PCD) is critical in cortical development, specifically in the elimination process of interneurons and oligodendroglia precursor cells (OPCs). However, the PCD role in the establishment of cortical network and adulthood behavior has been poor understood. In this work we used two transgenic models: the first one suppresses PCD on a subpopulations of cortical interneurons and OPCs, by a triple transgenic: $Nkx2.1^{Cre+/-}$, $Bak^{-/-}$, $Bax^{lox/lox}$, $TdTomato^{lox/lox}$ (NKX2.1:BAX). The second one suppresses only cortical OPCs, also by a triple trangenic: $NG2^{CreERT2+/-}$, $Bak^{-/-}$, $Bax^{lox/lox}$, $TdTomato^{lox/lox}$ (NG2.1:BAX). Therefore, we investigated the impact of these phenotype on adult animal behavior (P90-120). First, we evaluated the locomotor activity and anxiety level using Open Field. We did not find any difference between mice control and NKX2.1:BAX in terms on distance traveled and center/periphery exploration nor controls and NG2:BAX mice. Second, we assessed the object memory state (using the Nobel object recognition test) related with the hippocampus, where we did not find difference between their controls for both transgenic models. Finally, we used the Barnes maze test, associated with spatial memory and hippocampus, as well as the reversal Barnes. Briefly, Barnes maze consists in two main parts: Learning phase (LP) and memory test (MT). Reversal Barnes test has been related with medial prefrontal cortex (mPFC) and cognitive flexibility. For the NKX2.1:BAX mice and their controls, both have learned in LP and we did not find difference in MT. In the reversal Barnes, both groups did not shown difference in LP. Nevertheless, NKX2.1:BAX mice shown a significant lesser performance in MT than controls (t-Test $p<0.05$). Moreover, they expended more time in the previous quadrant where was located the escape-box in the normal Barnes test (t-Test $p<0.05$). These results show us a cognitive flexibility impairment for NKX2.1:BAX mice. In other hand, NG2:BAX mice shown similar results than their controls in normal Barnes (both LP and MT). However, we found difference in LP. Control mice learned, according to a comparison through training days (ANOVA test $p<0.0001$) but NKX2.1:BAX mice could not learn ($p=0.406$). Also in MT, control mice expended significantly more time in the quadrant where was located the escape-box (ANOVA test $p<0.0001$). It did not occur with NG2:BAX mice ($p=0.283$), showing cognitive flexibility impairment, similar to NKX2.1:BAX mice. Thus, NG2:BAX mice seem to be a stronger phenotype than NG2:BAX, where the main difference between them are the interneurons. The next step will be recording in vivo electrophysiology to understand the effect of interneurons and OPCs in mPFC neuronal network.

Functional consequences of Idh1 and CIC mutations on oligodendroglioma cells of origin

Nina POTTIER
Pottier N.*, Joppé S.*, Magne N., Paris S., Delannoy L., Bardella C., Huillard E.
Sorbonne Université, Institut du Cerveau - Paris Brain Institute - ICM, Inserm, CNRS, APHP, Hôpital de la Pitié Salpêtrière, Paris, France
**These authors contributed equally to this work*

Oligodendrogliomas are primitive tumors of the central nervous system characterized by mutations in isocitrate dehydrogenase 1 ($IDH1^{R132H}$) gene, codeletion of 1p and 19q chromosomic arms and mutations in Capicua (CIC) gene. Oligodendrocyte precursor cells (OPCs) are potential cells of origin for oligodendrogliomas and OPC-like tumor cells are responsible for tumor amplification at early stages of gliomagenesis. Genetic alterations are well characterized for these tumors, but little is known about the roles of IDH1 and CIC in oligodendroglioma initiation and development. Individually, IDH1 and CIC mutations can induce neoplastic characteristics but are not sufficient for tumoral development.

We aim to understand how IDH1 and CIC mutations affect OPC development and whether these alterations are sufficient for tumoral initiation. To this end, we have generated an inducible mouse model allowing endogenous expression of $IDH1^{R132H}$ and inactivation of CIC in postnatal OPCs. We analyzed by immunofluorescence the consequences of IDH1 and CIC mutations on the proliferation and differentiation of OPCs and other brain cells. We describe distinct effects of these mutations on oligodendroglial cell number and proliferation, at early and late time points. In addition, we found that $IDH1^{R132H}$ increases the proliferation of other brain cell types, suggesting paracrine effects of this mutation in the brain.

Our study describes for the first time the phenotypic impact of the combined expression of IDH1 and CIC mutations on oligodendroglial lineage cells and other brain cells, leading to a better understanding of oligodendroglioma development.

Microglia morphodynamics regulation by neuronal activity: a daytime matter?

Kassandre COMBET
Combet K. [1,2], Honnorat J. [1,2,3], Comte J.C.[1,4], Pascual O.[1,2]
[1]*Université Claude Bernard Lyon 1, Lyon, France*
[2]*Institut NeuroMyoGène (INMG), MeLiS, Equipe SynatAc, INSERM U1314, CNRS UMR 5284, Lyon, France*
[3]*Centre maladies rares sur les syndromes neurologiques paranéoplasiques, Hospices Civils de Lyon, Lyon, France*
[4]*Centre de Recherche en Neurosciences de Lyon (CRNL), Equipe Forgetting, INSERM U1028, CNRS UMR 5292, Lyon, France*

Microglial cells, the resident immune cells of the brain, have particularly dynamic processes. Various studies suggested that beyond a possible role in surveillance, microglial dynamics could be linked to synaptic mechanisms or, at least, to neuronal activity. However, signaling pathways modulating neuronal control of microglial motility remain largely unknown.
We recently showed that sleep episodes decrease microglial motility and complexity, depending on fractalkine receptor expression. To better assess the possible involvement of microglia in neuronal homeostasis occurring during sleep, we decided to study their morphodynamics along the inactive period in mice. We also looked at how delta and sigma oscillations, which are involved in memory consolidation during sleep, could impact microglial cells.
Microglial morphodynamic changes were monitored through in vivo transcranial imaging using two photon microscopy on Cx3cr1-eGFP mice, while electroencephalogram and electromyogram were simultaneously recorded. We then evaluated the impact of sleep-wake episodes along the inactive period, and fractalkine receptor Cx3cr1 deletion, to figure out their role in microglial dynamics. Consequently, we performed morphodynamics analysis to evaluate process motility and cell complexity.
Our results indicate a decrease in microglial morphodynamics during slow wave sleep that correlated independently with delta and sigma oscillations, depending on daytime during the light phase. We also found that depletion of the fractalkine receptor abolished these sleep-induced morphodynamics changes, indicating that fractalkine could be involved in microglia's detection and/or response to neuronal activity changes.
In conclusion, this work highlights a fine regulation of microglial motility involving the Cx3cr1 receptor during a precise phase of the inactive period. This study will lead to a better understanding of microglial functions in the context of synaptic transmission and plasticity.

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Microglia-derived extracellular vesicles promote neuropathology in Sanfilippo syndrome

Chloé DIAS
Dias C.[1], Morla G.[1], Ballout N.[1], Santiago C.[1], Ausseil J., Trudel S.[1,2]
1 Infinity Center, INSERM UMR1291, Université Toulouse III Paul Sabatier, Toulouse, France
2 CHU Toulouse, Institut Fédératif de Biologie, Service de Biochimie et Biologie moléculaire, Toulouse, France

Sanfilippo syndrome (mucopolysaccharidosis type III, MPSIII) is a severe neurological disease that shares common features with adult neurodegenerative disorders. In this syndrome, the progressive accumulation of partially degraded heparan sulphate oligosaccharides (HSO) activates the production of pro-inflammatory cytokines by astrocytes and microglia leading to severe neuroinflammation and oxidative stress, in turn leading to neuronal death. Consistent with our finding that abnormal extracellular HSO accumulation do not induce a direct activation of primary neurons, we hypothesize that the triggering and the propagation of neuroinflammation could be enhanced by the secretion of extracellular vesicles (EVs) by the activated microglia, as previously demonstrated in adult neurodegenerative disorders.
The objective of the present study was to characterize the cargo of microglia-derived EVs in an *in vitro* model of MPS III. We first isolate EVs sub-populations from culture supernatants of the murine BV-2 microglial cell line treated with urinary GAGs from MPS III patients. By label-free quantitative proteomic using LC-MS/MS and RNA sequencing analysis, we showed that MPS-EVs are enriched with proteins and miRNAs involved in the inflammatory response and more specifically in neuroinflammation. On the other hand, MPS-EVs drive less proteins and miRNAs involved in neurodevelopment pathways, like axonal guidance, myelination or synaptogenesis. Preliminary results obtained by treating primary cortical neurons with EVs extracted from brain tissues of MPS III B mouse model showed a fragmentation of neurites and a higher level of inflammation and oxidative stress mRNA markers compared to brain EVs extracted from wildtype mouse.
Our results strongly suggest that MPS-EVs can deliver specific molecular messages to the surrounding naive cells and can actively participate to the propagation of neuroinflammation in Sanfilippo syndrome. MPS-EVs may also play a role in the regression and loss of cognitive and motor skills that occurs in childhood, by directly deprive neurons of neurodevelopmental molecules.

Neuronal activity modulates microglia phenotype in repair through microglia-node of Ranvier interaction

Clément PERROT
Perrot C.[1], Ronzano R.[1], Aigrot M-S.[1], Stheneur P.[1], Ballbé J.[3], Lubetzki C.[1,2], Desmazières A.[1]
[1] Paris Brain Institute, ICM, Sorbonne University, Inserm U1127, CNRS UMR 7225, Pitié-Salpêtrière hospital, Paris, France.
[2] Assistance Publique des Hôpitaux de Paris (APHP), Neurology Department, Pitié-Salpêtrière hospital, Paris, France.
[3] Centre Borelli, UMR 9010, ENS Paris-Saclay, France

Myelination of axons ensures the rapid propagation of action potentials by saltatory conduction. This process relies on axonal segments insulated by myelin alternating with the nodes of Ranvier, short unmyelinated domains highly enriched in sodium and potassium channels. In Multiple Sclerosis (MS), nodal structures are altered even prior demyelination. Similarly, microglia, the immune resident cells of the central nervous system, are activated early in the disease. Following demyelination, an endogenous repair process exists, which ensures the restoration of myelinated fiber organization and fast axonal conduction. This process is however partial and, with time, neurodegeneration occurs. Understanding the mechanisms promoting remyelination and neuroprotection in MS is thus essential.
It has been shown that nodal reclustering is an early event during remyelination and it could thus participate in repair. Microglia are also key players in MS repair processes, as they either participate in tissue damage or promote neuroprotection and remyelination. We recently identified nodal structures as preferential sites of microglial interaction along axons, in both mouse and human. We showed that microglia-node interaction is modulated by neuronal activity and stabilized by axonal potassium release. Disrupting the axonal potassium efflux or its read-out by microglia following demyelination impaired the microglial switch towards its pro-regenerative state and alters remyelination, suggesting a functional role of this interaction in repair.
Using two complementary approaches, optogenetics and DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) in *ex vivo* and *in vivo* models, we show that increasing neuronal activity promotes microglia-node contact and the microglial switch towards a pro-regenerative phenotype. Our preliminary data further suggest that neuronal activity patterns could influence this crosstalk and its functional output. Taken together, these findings identify neuronal activity as a key player in remyelination, by directly acting not only on the oligodendrocyte lineage, but also on microglia and by allowing them to adapt their behavior to the status of the surrounding neurons during repair.

Premature brain ageing, the aftermath of an early-life inflammatory event

DAVID GUENOUN

Guenoun D.[1,2], Sautet I.[1], Ruinet S.[1,3], Faivre V.[1], Hua J.[1], Young Ten P.[1], Jacquens A.[1], Tanter M.[3], Van Steenwinckel J.[1], Gressens P.[1], Adle-Biassette H.[4], Bokobza C.[1]

[1]Inserm, UMR1141 Neurodiderot, Paris, France,
[2]Department of Pharmacy, AP-HP, Robert Debré Hospital, Paris, France,
[3]Physics for Medicine Paris – Inserm, ESPCI Paris-PSL, CNRS – Paris, France
[4]Université de Paris, service d'Anatomie Pathologique, AP-HP, Hôpital Lariboisière, DMU DREAM, UMR 1141, INSERM, Paris, France

Background: World's population is ageing, with an expected count of 426 million, the amount of people over 80 will triple by 2050. Ageing is the result of the accumulation of various damages over time, leading to a gradual decline in physical and mental performance. A part of this ageing population consists of former preterm infants (born before 37 gestational weeks). As survival rate is increasing, new deficits, directly linked to their birth inflammatory status, are appearing. A growing body of evidence demonstrates that the effects of inflammation are long-lasting, increasing the risk of further neurological damages and disorders. Preterm birth-associated brain injuries (PBI) have been linked toglial cells' (microglia and astrocytes) reactivity. Recent literature highlights the pivotal role of this glial reactivity on the severity of pathological ageing. Since glial cells could respond faster and stronger to a second inflammatory stimulus, we hypothesize that PBI induced a glial "priming" leading to their over-reactivity during ageing. Such phenomenon could worsen or accelerate the long-term outcome of brain damage.
Aims: We sought to determine whether a perinatal inflammatory challenge could accelerate the process of pathological brain ageing.
Methods: We used a mouse model of perinatal inflammation (interleukin-1-beta injections on postnatal days 1-5) responsible for PBI-like lesions. 7 months after the onset of PBI, we performed transcriptomic, functional, and behavioural analyses to evaluate the impact of perinatal inflammation on age-related inflammation and neuronal impairments.
Results: Bulk RNA sequencing (RNAseq) as well as flow cytometry analysis demonstrated a central (microglial reactivity) and peripheral inflammatory activation (monocyte and PMN) in middle-aged PBI mice. Furthermore, RNAseq's most significant pathways were linked to pathological brain ageing. Finally, exposure to perinatal inflammation is correlated with brain connectivity defects, higher susceptibility to epilepsy-like strokes and behavioural impairments.
Conclusion: Beyond its early consequences, perinatal inflammation causes a long-lasting increase in the inflammatory response related to cellular and functional defects suggesting early ageing. This project illustrates the benefits of normalizing persisting glial dysfunction to improve brain structure and health. Moreover, by promoting a more precise follow-up of former preterm, this study paves the way for new therapeutic and preventive strategies for this specific paediatric population.

Investigation of mechanisms underlying glia-dependent motor arrest

Mahalakshmi DHANASEKAR
Mahalakshmi Dhanasekar[1], Elias Lunsford[1], Camille Lejeune[1], Martin Carbo-Tano[1], Claire Wyart[1]
[1] Sorbonne Universite, Paris Brain Institute, France

Animals need to avoid threats in their environment to survive. They do so by responding to aversive cues by deploying complex avoidance responses to flee and/or by freezing. While the escape circuitry has been well characterized, the circuit mechanisms contributing to motor arrests have remained elusive. A recent study showed that noradrenergic (NA) signaling can trigger motor arrest by eliciting a massive glial wave that blocks motor output. It still remains unknown whether and how debilitating threats can induce motor arrest via NA-glial wave. Our working hypothesis is that avers

ive stimuli trigger a NA-glial wave which inhibits motor neurons in the spinal cord to induce motor arrest. To tackle this question, we exploit the optical and genetic accessibility of larval zebrafish exposed to mechanical or chemical aversive stimuli. Upon both aversive stimulations, we observed a recruitment of NA neurons that was followed by a glial wave initiating in the rostral spinal cord and propagating anterior in the brain and posterior in the spinal cord. Accordingly,some noradrenergic neurons in the medulla oblongata project precisely in the rostral spinal cord through the lateral fasciculus. In order to distinguish the sensory response from the effect of neuromodulation, we established paradigms to elicit motor arrest upon optogenetic activation of NA neurons. Three second-long optogenetic activation of NA in freely-swimming larvae elicited effective motor arrest lasting at least 10 seconds. We confirmed in paralyzed animals that such activation was effective in eliciting a glial wave initiating in the rostral spinal cord as well. 2D optogenetic stimulation combined with single-cell electrophysiological recordings will be performed to further dissect the modulation of motor circuitry by the NA-glial wave. Altogether our study illustrates that upon encountering aversive threats, animals employ a NA-glial wave to elicit effective motor arrest to avoid threats in the environment. This opens on the involvement of complex neuro-glial interactions in generating adaptive and maladaptive stress responses.

Impact of gestational exposure to pesticides interactions establishment during brain development

Barbara DELAUNAY-PIEDNOIR
Delaunay-Piednoir B.[1], Lemale L.[1], Cisternino S.[2,3], Salvador-Prince L.[4], Perrier V.[5], Loulier K., Cohen-Salmon M.[1].
[1] Center for Interdisciplinary Research in Biology (CIRB), Collège de France, CNRS, INSERM, Université PSL, Paris, France.
[2] Optimisation Thérapeutique en Neuropsychopharmacologie, INSERM Université de Paris, Paris, France.
[3] Service Pharmacie, Assistance Publique – Hôpitaux de Paris, Hôpital Universitaire – Necker – Enfants Malades, Paris, France.
[4] Institute for Neurosciences of Montpellier (INM), Univ Montpellier, INSERM, Montpellier, France.
[5] U1198 MMDN (Molecular Mechanisms of Neurodegenerative Diseases), Inserm (National Institute for Health and Medical Research), MGX (Montpellier GenomiX), BioCampus, University of Montpellier, Montpellier, France.

The contamination of our drinking water resources and food with environmental pollutants, such as pesticides residues is a major public health issue. Studies in the dosage of pesticides in biological fluids on humans have revealed the extent of exposure and contamination of organisms to these products. The French Food Report conducted by ANSES (TDS2, June 2011) has shown that the anilinopyrimidine (AnPy) fungicides are among the five pesticides most commonly detected in our diet (37% in fruits, 18% vegetables, 25% in alcohols (Nougadère et al., 2012). To protect the European population from potential side effects, EU has defined an accepted maximum residue limit dose in tap water (EU directive 80-778-EEC). However, a recent article reports an altered neurogenesis and an exacerbated neuroinflammation upon AnPy gestational exposure (Wang et al., 2021).
AnPy residues may access to the brain perinatally through the blood circulation and could first alter the gliovascular unit (GVU), a specific interface formed by astrocytes and the vascular compartment, where important brain functions such as the blood brain barrier (BBB), the immune homeostasis, the brain drainage and the neurovascular coupling are set (Cohen-Salmon et al., 2021).
The objective of our study is therefore to further investigate GVU pathophysiological modifications induced by AnPy gestational exposure to wild-type (WT) as well as genetically predisposed to autism spectrum disorder (ASD) mice. ASD is a neurodevelopmental pathology which may result from genetic predisposition associated to environmental assaults (Bölte et al., 2019). To do so, WT and Cntnap2$^{-/-}$
(mice model of ASD) are exposed to a cocktail of AnPy or to an equivalent volume of DMSO (control) during mating, gestation and lactation (Wang et al., 2021). And then we investigated the GVU development on the cortex of male and female offspring. We first analyzed the vascular architecture on cleared brain by using the iDISCO methods and imaging the whole brain vessels by light sheet microscopy. Moreover, we characterized the mural cells (i.e. pericytes, perivascular macrophages, fibroblasts) and the perivascular astrocytic processes covering the blood vessels by immunohistochemistry. And then we assessed the BBB integrity by in situ brain perfusion (collaboration with Salvatore Cisternino, Faculty of Pharmacy, Paris, France).
Our preliminary results indicate that cortical astrocyte reactivity, as well as the vascular organization are changed in neonates upon AnPy gestational exposure but without BBB integrity defects at adulthood. Our next investigations will be focused on the GVU molecular properties and GVU functions by assessing neurovascular coupling and brain drainage by cerebrospinal fluid.

POSTER PRESENTATIONS

ALL POSTERS WILL BE DISPLAYED THROUGHOUT THE MEETING
POSTERS ATTRIBUTED TO **SESSION 1** WILL BE PRESENTED FROM **11:40 – 12:25**
POSTERS ATTRIBUTED TO **SESSION 2** WILL BE PRESENTED FROM **17:00 – 17:45**

Session 1	n°	Session 2	n°
Ahmadi	1	Bancel Vega	2
Aubert	3	Benkeder	4
Barbay	5	Carracedo	6
Barbot	7	Clua Provost	8
Bokobza	9	Den Garcia	10
Burgard	11	Gosset	12
De Gea	13	Grgurina	14
Debacq	15	Heydari Olya	16
Falque	17	Hippauf	18
Frère	19	Hovhannisyan	20
Garcia	21	Khelfaoui	22
Goumon*	23	Laisné	24
Guille	25	Magneron	26
Hekking	27	Mercier	28
Lakomy	29	Moreno-Montana	30
Lavaud	31	Oudart	32
Léger	33	Pereira	34
Mora	35	Plaisier	36
Prieur	37	Riquelme-Pérez	38
Winterberg	39		

***: not concerned by the evaluation**

1. Tools for deciphering the molecular mechanisms involved in atp release by astrocytes using biosensors

Ahmadi N.[1,2], Linck N.[1], Compan V.[1,2], Rassendren F.[1,2]

[1]Institut de Génomique Fonctionnelle, Université de Montpellier, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Montpellier, France. LabEx Ion Channel Science and Therapeutics, Montpellier, France.

Besides providing energy in living cells, adenosine triphosphate (ATP) is also an extracellular signaling molecule that acts on two main classes of membrane receptors, metabotropic P2Y receptors and ionotropic P2X receptors. In the central nervous system, purinergic receptors are involved in diverse functions such as modulation of synaptic transmission or neuron-glia communication. In epileptic conditions, astrocytes release ATP, which through autocrine and paracrine loops, triggers glutamate release from astrocytes thus contributing to network excitability. Yet, the mechanisms underlying astrocytic ATP release are still poorly characterized and are likely diverse.

Our aims are to identify the molecular mechanisms involved in ATP release by astrocytes in pathological conditions. Our hypothesis is that Volume-Regulated Anion Channels (VRAC) encoded by the LRRC8 family of protein could be involved in astrocyte evoked ATP release. Indeed, i) several studies demonstrated the presence of VRAC in astrocytes; ii) sustained neuronal activity can result in local change of extracellular osmolarity that can activate VRAC; iii) our preliminary results show that LRRC8 channels can release extracellular ATP.

To explore the release of ATP by LRRC8 in astrocytes, we combined RNAi approach targeting LRRC8A, the mandatory subunit of LRRC8 channel, with two newly developed fluorescent biosensors based on either P2X or P2Y receptors that allow tracking ATP release in space and time.

We first demonstrated the expression of LRRC8A in primary cultures of cortical astrocyte by western blotting. Using viral-mediated RNAi approaches with either lentiviral or AAV vectors, we were able to achieve knock-down of LRRC8A expression by approximatively 80% in primary astrocyte culture. In parallel, we express both ATP biosensors in astrocytes and confirm that these tools can detect extracellular ATP at different concentration ranges.

To conclude, validation of functional ATP biosensors and iRNA tools should allow to decipher molecular mechanisms involved in ATP release by astrocytes. First, using biosensors we will explore if inhibition of LRR8A expression leads to any potential impairment in ATP release from astrocytes in hypo-osmolar conditions. Second LRRC8 involvement in ATP release by astrocytes will be studied ex vivo and in vivo in different pathological conditions such as epilepsy.

2. Dynamic calcium signals of oligodendroglia in demyelinated lesions.

Bancel Vega R.[1], *Maas D. A.*[1], *Angulo M. C*[1,2].

[1] Université Paris Cité, Institute of Psychiatry and Neuroscience of Paris (IPNP), INSERM U1266, "Team: Interactions between neurons and oligodendroglia in myelination and myelin repair", F-75014 Paris, France. [2] GHU PARIS psychiatrie & neurosciences, F-75014 Paris, France.

Calcium is a key intracellular messenger that regulates many vital cellular processes. Calcium signals in oligodendrocytes, the myelinating glia of the CNS, are known to transduct environmental information to cellular processes such as proliferation, differentiation and myelination. In parallel, our team has shown that neuronal activity in vivo enhances remyelination in demyelinated lesions (Ortiz et al., 2019, JCI Insight). How neuronal activity is linked to calcium signals from oligodendroglia and what role these signals play in activity-dependent remyelination remain unknown. Here we are using PDGF CreERT;Gcamp6f/f mice to perform ex vivo and in vivo calcium imaging of oligodendroglial during myelin repair using demyelination/remyelination models. On the one hand, ex vivo brain slices are used to determine the calcium signaling mechanisms and, on the other hand, in vivo microendoscopy is used to analyze calcium signals during different behavioral tasks. We have recently set up all the experimental and analytical framework for the analysis of ex vivo and in vivo calcium signals in oligodendroglia (Maas et al., 2022, BiorXiv). We expect that behavioral intervention will increase these signals and improve remyelination. This project should shed light on the role of calcium activity in oligodendroglia during myelin repair and, as such, contribute to our understanding of the repair mechanisms in the context of demyelinating diseases such as multiple sclerosis.

3. A Systems biology approach for addressing the role of Microglia in Alzheimer's disease

Aubert A., Bramoulle A., Colombo B., Mendoza-Parra M.A.
UMR 8030 Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, University of Evry-val-d'Essonne, University Paris-Saclay, 91057 Évry, France.

Microglial cells are responsible for both the control of homeostasis and the immune response of the brain to injury and infection. The role of these cells is predominant in the onset and development of neurodegenerative diseases, such as Alzheimer's disease (AD).
Microglia are likely to govern the onset and progression of AD. While they seem to act as a protective layer against the formation and propagation of amyloid beta (Ab) plaques by phagocyting Ab42 aggregates, their role as an immune response conductor in the brain tend to have an ambivalent role in the onset of neurodegeneration. In homeostatic conditions, neuro-inflammation promotes the clearance of dead cells and debris and protects the brain. Nevertheless, in the context of AD, a self-maintained imbalance in neuroinflammation could lead to neurodegeneration (S.Merighini et al 2022 Int J mol Sci.)
Recent genome-wide association studies (GWAS) have deepened the link between AD and microglial cells, as some AD risk loci have been found near or within genes known to be expressed - even sometimes specifically - in microglia (Hansen et al 2018 J cell Biol.). To question this newfound connection, we have implemented microglia differentiation from inducible pluripotent stem cells (iPSC) presenting a healthy genomics background, or harboring AD-related mutations.
With the help of global transcriptome profiling, as well as inflammation response assays, we are scrutinizing potential defects in this derived microglia cells, as a way to reveal their role in Alzheimer's disease.

4. Development of a new automated pipeline for the characterization and description of microglial morphology.

Benkeder S., Dinh S.M., Comte J-C., Pascual O.
MeLiS laboratory, Institut NeuroMyoGène, Claude Bernard University Lyon, France.

Microglial cells are the immune resident cells of the brain. They are found in a wide variety of morphologies, mainly defined by a complex arborization of highly ramified processes, around a small cell body. When microglia detect a damage associated molecular pattern, they rapidly react and adapt their function to respond to this signal. These changes of functions are translated into major morphological modifications. Many studies have shown a high diversity of microglial morphotypes in physiopathological conditions, ranging from amoeboid to hyper-ramified. For years, morphology has been used as a proxy indicator for microglial reactivity, and is employed to describe a broad range of pathological contexts. To date, a plethora of criteria has been used to quantify microglial morphology in the literature, from simple descriptive measures to complex indexes computing different parameters. However, the parameters' selection can be arbitrary and lack objectivity. Moreover, it is difficult to combine several of them to have a complete description of the cells and some of them require time consuming manual inputs. The need to automatize has risen to minimize the time spent in analysis, to limit experimenter biases, and to be able to perform high throughput studies on large populations. Here, we propose a new pipeline applicable to all kind of binarized microglia. We put together around 60 parameters characterizing microglial morphology, among which the most used in the literature and new ones developed by our team. The use of principal component analysis (PCA) allowed us to select the most appropriate parameters to describe the morphology of a chosen dataset. An approach combining Andrews plots and k-means allowed us to rank the microglia according to their morphological complexity level, and to discriminate cell populations based on morphological criteria. We successfully tested this pipeline on different types of samples: live or fixed tissue, and on various datasets obtained from diverse types of microscope (epifluorescence, two-photon and confocal), and in different pathological conditions (Alzheimer and ischemia), showing that our method can be used in a wide variety of contexts. Once finalized, this new automated, quick and easy-to-use tool could homogenize analysis practices among laboratories.

5. Astrocytic contribution in spasticity after spinal cord injury

Barbay T.

Spasticity is a common motor disorder following spinal cord injury (SCI) characterized by motoneurons (MNs) hyper-excitability resulting in muscle hypertonia and for which there is no satisfactory cure and an urgent need. Most of the proposed mechanisms of spasticity are focused on intrinsic neuronal elements. Astrocytes are the most abundant glial cell types and in a unique position to regulate central nervous system (CNS) activity. In response to neuronal activity, astrocyte prevent network hyper-excitability by taking up extrasynaptic potassium mainly via the inwardly-rectifying K+ channel, Kir4.1. We demonstrated that after thoracic spinal cord injury, mice display (1) lumbar astrogliosis which is characterized by astrocytic morphological and inflammatory changes. By using calcium imaging couple with electrophysiology experiments we found that those changes lead (2) to altered calcium and electrical properties in astrocytes following spinal cord injury. In this context it appears that spinal astrocytes (3) display difficulties to re-uptake K+ which leads to (4) abnormal K+ accumulation following neuronal activity and (5) promotes MNs hyper-excitability. Finally, capillary western blot, electrophysiology, and astrocytic intracellular pH imaging experiments (6) highlight Kir4.1 channel dysfunction as the main responsible for this lake of K+ re-uptake. These data pave the way for novel therapeutics strategies to better understand astrocytic functions in motoneuron hyper-excitability in spasticity and broaden the spectrum of therapeutic targets for restoring tri-partite synapse homeostasis (epilepsy, Hungtington, Rett syndrome, depression ...).

6. Implication of neuronal and microglial P2X4 receptor in ALS pathogenesis.

Carracedo S.[1], Bertin E.[1], Quilgars C.[2], Fayoux A.[2], Riffault C.[1], Le Masson G.[3], Bertrand S. S.[2], Boué-Grabot E[1].

[1]Univ. Bordeaux, CNRS, IMN, UMR 5293, F-33000 Bordeaux, France. [2]Univ. Bordeaux, CNRS, EPHE, INCIA, UMR 5287, F-33000 Bordeaux, France, [3]CHU Bordeaux, Reference center of ALS and motorneuron diseases.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a selective loss of motor neurons (MN), leading to muscular weakness, progressive paralysis and death within 3-5 years after diagnosis. The aggregation of misfolded proteins such as SOD1 or TDP-43 is the pathological hallmark of ALS and has been associated with neuroinflammation and cellular degeneration. Growing evidence point ALS to be the result of a complex interplay between the nervous and the immune system. ATP released by neurons and glial cells modulate the neuroglial communication via activation of P2X receptors. Among these, P2X4 receptor, which is a non selective cationic channel has been recently involved in ALS pathogenesis using SOD1G93A ALS mouse model (SOD1).

In this study, we showed that P2X4 expressed in MN at presymptomatic stages is upregulated over the disease progression in spinal cord microglia of SOD1 mice. In addition, we demonstrated that misfolded SOD1 proteins impair P2X4 receptor endocytosis machinery leading to a significant increase in its surface density. Interestingly, we found a significant surface increase of P2X4 in peripheral macrophages of SOD1 mice at presymptomatic stages, which may position P2X4 as a putative early biomarker of ALS.

To evaluate the impact of P2X4 modulation in ALS, we generated double transgenic SOD1 mice expressing either P2X4 internalization-defective knockin gene (SOD1:P2X4KI) or lacking the P2X4 gene (SOD1:P2X4KO). Surprisingly, both genotypes resulted in improved motor performance and survival of SOD1 mice pointing out a complex cell-specific function of P2X4.

To address the neuroglial role of P2X4 in ALS, we have developed new SOD1 mice, expressing either P2X4KI or P2X4KO, selectively in macrophage/microglia or neurons. Currently, we are assessing by motor performance and survival the cell-specific role of P2X4 in the progression of ALS symptoms of the different mice lines. Using histological and biochemical approaches, we are analyzing the cell specific contribution of P2X4 in the interplay between MN death, spinal microglia reactivity and peripheral macrophage over the disease progression. Further, we are investigating by flow cytometry possible changes in the surface trafficking of P2X4 in human monocytes from peripheral blood of ALS patients. This work may not only provide valuable insights into the cellular role of P2X4 to fight this fatal disease, but also define P2X4 as a promising ALS biomarker.

7. Glial communication through connexin 43 in the dorsal vagal complex and energy intake.

Barbot M., Lebrun B., Troadec J-D.
Aix Marseille University, Cognitive Neurosciences Laboratory, UMR CNRS 7291, Marseille, France.

Understanding energy homeostasis control continues to be a crucial global topic since the metabolic syndrome, which includes obesity and diabetes, remains a serious public health issue. The energy balance is finely regulated by the central nervous system, notably through neuronal networks located in the dorsal vagal complex (DVC). These structures integrate nutritional, humoral, and nervous information from the periphery and, in turn, adjust energy expenditure and food intake. Glial cells in the brainstem interact with neuronal networks to finely tune energy homeostasis. However, the underlying mechanisms and the neuron-glia interactions in the context of food intake control remain to be deciphered.

Astrocytes in the DVC strongly express Cx43, a membrane protein belonging to the connexin family. Cx43 hexamers form hydrophilic channels called connexons. Connexons from two adjacent cells can be paired to form intercellular channels, which are gathered in gap junctions and allow for direct cytoplasmic exchanges. . Unpaired Cx43 connexons, called hemichannels (Cx43 HCs), also allow the release of neuroactive substances in the extracellular space. We assumed this glial communication based on connexins within the DVC could be involved in the control of energy balance. Previously, we focused on the contribution of glial HCs to the energy balance of rodents using GAP19, a mimetic peptide blocking the opening of HCs Cx43. After GAP19 infusion into the lateral ventricles, we observed a reduction in food intake and cellular activation of neural networks dedicated to food intake. These results provide the first evidence that HC activity interferes with the control of food intake and attests to a possible tonic glial delivery of orexigenic molecules via Cx43 HCs.

Hence, we sought to better understand how interactions between DVC astrocytes via connexins impact energy balance. We built adeno-associated viral vectors (AAV) expressed under the control of the GFAP gene promoter and carrying shRNAs directed against Cx43 or inactive shRNAs. Adult mice receive bilateral AAV delivery into the DVC parenchyma. Our approach was verified as successful in specifically

lowering Cx43 expression in DVC GFAP+ cells for at least 9 weeks after injection. To determine the impact of Cx43 suppression on energy balance, we recorded the animals' weight increase and food consumption over time, as well as a variety of tests that allowed us to examine the mice's energy metabolism.

These insights may improve our comprehension of the central mechanisms governing our energy balance. It may open new therapeutic avenues for treating overweight, obesity, and their co-morbidities.

8. iPSC-derived human microglia-like cells in neural coculture to study microglia heterogeneity in Alzheimer's disease.

Clua Provost C.[1,3], Auboyer L.[2], Monzo C.[1], Thanawat P.[3], Hirbec H.[3,], Crozet C.[1,*].*

[1]Institut des Neurosciences de Montpellier, Université de Montpellier, INSERM, Montpellier, France. [2]Institut de Médecine Régénératrice et de Biotherapies, Université de Montpellier, CHU de Montpellier, INSERM, Montpellier, France. [3]Institut de Génomique Fonctionnelle, Université de Montpellier, INSERM, CNRS, Montpellier, France

*The authors contribute equally to this project

Microglia are the tissue-resident macrophages of the brain and are involved in many functions in both physiological and pathological contexts. Recent genome-wide association studies have established their critical role in the pathogenesis of Alzheimer's disease (AD) but their precise contribution is not yet clarified. It is still unclear whether microglia are protective but insufficient at early stages or if they became inefficient or even harmful as the disease progresses.

Most studies are performed in animal models, especially mouse models, however murine and human microglia have important discrepancies in their transcriptomic profiles with aging. To improve the transition from preclinical studies to clinical studies, it is crucial to develop new cellular models mimicking as closely as possible the reality and the complexity of the human brain. Recent technological advances in induced pluripotent stem cells (iPS) have paved the way for the development of such models.

In this study, we present a human iPSC-derived model composed of neural networks (NN) supplemented with iPS-derived microglia-like cells (iMGL). To characterize microglia heterogeneity in the context of AD, iPS were generated from healthy individual, patients with autosomal dominant and their isogenic controls or sporadic forms of AD. First, we assessed the relevance of our model by characterizing at the functional level (phagocytosis, cytokines profile, motility and migration assays) healthy control iMGL, whether alone or in coculture in NN. Then, the functionality and the gene expression patterns of iMGL has been studied in control- and patient-derived cocultures to identify potential microglial alterations.

Deciphering microglia heterogeneity is a required step to design innovative therapeutic strategies. With our model, we are expecting to improve our understanding of the role of microglia in the early stages of AD.

9. Additive deleterious effects of delivery mode on perinatal brain injuries: microbiota's fault.

*Morin C.[*1], Bokobza C.[*1], Guenoun D.[1,2], Faure F.[3], Sautet I.[1], Diao S.[1], Faivre V.[1], Hua J.[1], Demene C.[3], Tanter M.[3], Schwendimann L.[1], Young-Ten P.[1], Bourgeois T.[1], Ramanantsoa N.[1], Matrot B.[1], Gallego J.[1], Auger S.[5], Van Steenwinckel J.[$1], Gressens P.[$1]*

*/$co-authors

[1]Inserm, UMR1141 Neurodiderot, Paris, France. [2]Department of Pharmacy, AP-HP, Robert Debré Hospital, Paris, France. [3]Physics for Medicine Paris – Inserm, ESPCI Paris-PSL, CNRS – Paris, France. [4]Fudan University, Department of neonatology, Children's Hospital of Fudan University, Shanghai, China. [5]INRAE, Université Paris-Saclay, AgroParisTech, UMR 1319 Micalis Institute, 78352 Jouy-en-Josas, France.

Background: Worldwide, 15 million preterm births occur each year and are often associated with maternal/fetal infections—inflammation that triggers neuroinflammation. This neuroinflammation is mediated by microglia, which release cytotoxic molecules that prevent the differentiation of oligodendrocytes, resulting in hypomyelination. Some preterm infants may experience lifelong motor and/or cognitive impairments associated with periventricular white matter injury (PWMI). Clinicians consider three types of delivery: spontaneous vaginal delivery, vaginal delivery after medical induction, and delivery by cesarean section (C-section). Accumulating evidence suggests that preterm infants delivered by C-section are at higher risk of neurodevelopmental disorders (NDD) compared with vaginally delivered infants. The main difference between vaginal delivery and C-section is the colonization of gut microbiota. When C-section is performed, the vertical transmission of the mother's vaginal microbiota is bypassed. Children born by C-section present a reduced microbiota diversity. Data on the possible additive effect of mode of delivery (vaginal versus C-section) on PWMI are currently lacking in the literature.

Aims: Therefore, our group was interested in comparing the possible additive effects of (i) mifepristone (RU-486)-induced vaginal preterm birth or (ii) cesarean surgical preterm birth with perinatal inflammation.

Methods: We developed two double-hit models: induction of preterm labor by (i) subcutaneous injection of RU-486 or (ii) perinatal inflammation induced by C-section combined with intraperitoneal injection of interleukin 1beta (IL-1beta) in the first week of life of the offspring.

Results: Consistent with literature data, exposure to RU-486 (crossing the placenta) prevented microglial reactivity associated with exposure to IL-1beta. On the other hand, this is not enough to prevent hypomyelination that occurs later. Thus, early birth alone did not exacerbate the effects of inflammation. We were able to demonstrate a reduction in microbiota diversity in a C-section mouse model, which was exacerbated when the offspring were exposed to IL-1beta. This reduction was accompanied by a more pronounced neuroinflammation phenomenon mediated by microglia. The long-term consequence of the combined effects of C-section and inflammation is more pronounced PWMI, leading to deficits in functional brain connectivity associated with NDD-like behaviors.

Conclusion: There are currently no recommendations regarding the mode of preterm delivery. In a similar inflammatory state, our data suggest that vaginal induction of preterm labor is less harmful than induction of preterm labor via C-section surgery due to gut microbiota miscolonization.

10. Modelling interactions between neuron and astrocyte through a tripartite synapse.

Whilrex Garcia D., Talou J., Jacquir S.
Université Paris-Saclay, CNRS, Institut des Neurosciences Paris-Saclay, 91400, Saclay

Gaining a comprehensive understanding of brain computations continues to be a key challenge in modern neuroscience, even with the notable advancements over the past decades. Employing a computational model is an outstanding and indispensable approach to studying this complex phenomenon, as it comprises a signi cant part of the investigation. Utilizing such models is also pivotal to understanding neurological disorders. This led to the development of numerous neuronal models in order to apprehend brain dynamics at multiple scales and complex- ities, ranging from highly detailed multi-compartmental models, like the Hodgkin-Huxley model, to simpler models, such as the leaky integrate-and- re model. However, most of the existing neuronal models do not consider the potential in uence of glial cells, speci cally astrocytes, on neuronal physiology. This is despite the emerging pieces of evidence suggesting their crucial role in regulating neural network activity and plasticity. In addition, recent works have demonstrated that astrocytes play a critical role and are implicated in some pathologies such as epilepsy and Alzheimer's disease. For instance, when astrocytes change the excitability of neurons and the strength of their connections, this can lead to a pattern of synchronized ring among neurons, which has been associated with epilepsy. Conversely, when impaired astrocytes cause some neu- rotransmitters that typically inhibit neural activity, such as Gamma-aminobutyric acid (GABA) to be released in larger amounts, this can result in a portion of the network's neurons becoming completely quiet, which been linked to Alzheimer's. In the literature, there are some works focused on the modeling of the interactions between neurons and glial cells but these approaches were sophisticated, mimicking the complexity of the physiological phenomenon. In our work, our aim is to propose a model of tripartite synapse, which is a model of synaptic transmission that includes the pre-synaptic neuron, the post-synaptic neuron, and the astrocyte, which will be simple but will include the main astrocytic calcium dynamics. We de ned the tripartite synapse model based on the Adaptive Exponential Integrate-and-Fire (AdEx) neuron model and a sim- pli ed scheme of the astrocyte model proposed previously by Postnov. Our preliminary results show that our model is able to reproduce the main dynamics of synaptic transmission due to the astrocytic calcium dynamics at the level of the tripartite synapse.
Keywords: neuron, astrocyte, tripartite synapse, calcium dynamics, gliotransmission.

11. Beneficial effect of the environmental enrichment on transgenic mouse model of ftd-als (fusopathy).

Burgard A.[1], Grgurina I.[1], Paiva I.[1], Tzeplaeff L.[1], Cosquer B.[1], Le Gras S.[2], Isik A.[1], Decraene C.[1], Cassel J-C.[1], Dupuis L.[3], Merienne K.[1], Boutillier A-L[1].

[1]Laboratory for Cognitive and Adaptive Neuroscience – UMR7364/CNRS - University of Strasbourg, France. [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France, 3Unité U1118 Inserm - CRBS, Strasbourg, France.

The Fused in Sarcoma (FUS) is ubiquitously expressed in different tissues, including the brain, and is able to bind to RNA and DNA. FUS protein aggregation is found in amyotrophic lateral sclerosis (ALS), the mutation of this gene leads to predominant motor alteration. It is also found in Frontotemporal Dementia (FTD) that involves more behavioral and language impairments associated with left temporal lobe atrophy. Enriched environment (EE) housing can enhance synaptic plasticity, improve memory function and ameliorate disease phenotypes in animal models for age-associated neurodegenerative diseases. In this study we tested the effect of (EE) on a transgenic FUS mouse model of DFT/ALS, with evaluations on behavioral and transcriptomic read-outs.

We used the heterozygous FUS∆NLS mouse model expressing a FUS truncated protein without Nuclear Localization Signal (NLS). These mice present memory dysfunctions, as lack of precision in spatial memory formation and object memory deficits. Mice were housed in EE (Marlau cages, 10-12 mice/cage) versus in Standard Environment (SE, 2 mice/cage), for 4 months after weaning and until tests, at 5 months of age. Mice underwent actometry (activity during day and night), and hippocampo-dependent tasks such as spatial memory in the Morris Water Maze (MWM) and Object in Place (OIP). A parallel cohort was raised for RNAseq experiments performed on bulk hippocampal tissue.

We found that in the MWM task, EE-housed FUS mice of both sexes (males and females) showed a significant targeted performance compared to SE-housed ones. In the OIP task, only males significantly performed correctly, and EE-housed FUS mice were significantly better than SE-housed mice. EE had no significant effect on WT mice. Transcriptomic data revealed that EE had a common effect of both FUS and WT mice by activating Immediate early genes (Fosb, Arc, Nr4a1…) as well as the Bdnf gene. By contrast, multiple pathways related to neuronal/synaptic plasticity were dysregulated by EE only in FUS mice. Several genes involved in extracellular matrix (Cadm1, Col19a2…) were down-regulated in FUS mice.

Our findings demonstrate a beneficial effect of EE housing on FUS mice at the behavioral level. At the transcriptomic level, EE increased neuronal activity-associated gene transcription in both genotypes, but selectively impacted neuronal/synaptic plasticity genes in the FUS hippocampi. Further studies are needed to understand how this relates to behavioral performance.

12. Unconventional secretion of misfolded SOD1 and toxicity spreading: a novel therapeutic strategy for ALS

Gosset P., Néel E., Labatut M., Lappeman S., Brugioti V., Challuau D., Ait Lahcen Y., Scamps F., Mezghrani A., Raoul C.

The Institute for Neurosciences of Montpellier, Inserm UMR 1051, Univ Montpellier, Saint Eloi Hospital, Montpellier, France.

OBJECTIVES: Amyotrophic lateral sclerosis (ALS) is characterized by the selective loss of motoneurons leading to paralysis and death. Among the familial forms of the disease (10%), the first gene identified codes for an ubiquitous protein, superoxide dismutase type 1 (SOD1). Transgenic mice expressing mutated human forms of SOD1 faithfully summarize the main features of the disease. Insufficient degradation of these aberrant proteins induces a gain in intracellular toxic function in motoneurons. However, numerous studies have shown that the loss of motor functions is due to a combination of deleterious non-cell autonomous mechanisms encountered in many cell types, involving the spread of toxic molecules such as mutant SOD1 in a prion-like fashion. Our objective is to understand how ALS-causing protein can be secreted and be therapeutically targeted to stop disease progression. METHODS & RESULTS: Through the description of unconventional secretion mediated by USP19, we study the secretion of SOD1G93A mutant using cell culture system and expression of functional mutants. The analyzis of UPS19 expression pattern is done at different disease stages by immunofluorescence and biochemistry in SOD1G93A mice. Besides, innovative technologies are validated to silence efficiently USP19 in vitro. Our data show that the USP19 promotes the secretion of SOD1G93A initiating its loading at the endoplasmic reticulum membrane. We found that USP19 is predominantly expressed in oligodendrocytes and show differential expression levels in ALS mice. CONCLUSION: These results provide new knowledge on the proteinopathy aspect of the ALS and pave the way for the preclinical evaluation of a targeted intervention in ALS mice.

13. VEGF modulates microglia activity in Alzheimer's disease models

De Gea P., Benkeder S., Bouvet P., Chounlamountri N., Meissirel C.
MeLiS laboratory, Institut NeuroMyoGène, Claude Bernard University Lyon, France

Alzheimer's disease (AD) is the most common neurodegenerative disorder associated with progressive memory loss and cognitive decline, leading to dementia. One of the first pathological processes is the accumulation of extracellular amyloid-beta (Aβ) peptide aggregates in the brain, which induces synaptic toxicity and later contributes to the formation of amyloid plaques. Evidences indicate that microglia, the resident immune cells of the brain, play a key role in the clearance of toxic Aβ aggregates. However, with disease progression, microglial clearance function via phagocytosis is compromised.
Our team showed that VEGF (Vascular Endothelial Growth Factor) can counteract synaptic toxicity in AD models and further colocalises with amyloid plaques. In addition, VEGF has been reported to increase chemoattraction of microglia towards plaques and to modulate α-secretase activity, which is responsible for the cleavage of Aβ receptors in microglia. Altogether, these findings suggest a potential role of VEGF in Aβ phagocytosis by microglia in AD.
First, we studied VEGF effect on microglial phagocytic ability by characterising kinetics of both Aβ oligomers and fibrils internalisation and degradation using a combination of flow cytometry and immunocytochemistry. Furthermore, we took advantage of aged AD mice (APP/PS1) characterized by an accumulation of Aβ plaques in their brain and used brain cryostat sections cultured with primary microglia to evaluate VEGF impact on Aβ plaque clearance by microglia. Finally, we characterised underlying molecular mechanisms by assessing VEGF effect on α-secretase activity and downstream signalling pathways overtime.
Our results show that VEGF increases microglial phagocytic function for Aβ oligomers but not fibrils. This functional effect was accompanied by a transient increase in α-secretase cleavage activity induced by VEGF. Altogether, our findings point out the early contribution of the VEGF pathway in Aβ clearance by microglia.

14. Uncovering the effect of the apoe4 risk factor on cell-type specific epigenomic signatures in dementia with lewy bodies (dlb).

Grgurina I.[1], Paiva I.[1], Cosquer B.[1], Le Gras S.[2], Isik A.[1], Decraene C.[1], Bellande T.[3], Ternier G.[4], Cassel J-C.[1], Merienne K.[1], Giacobini P.[4], Melki R.[3], Boutillier A-L.[1]

[1]CNRS - University of Strasbourg, Laboratory for Cognitive and Adaptive Neuroscience (LNCA), Strasbourg, France. [2]INSERM-CNRS-Unistra, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch-Graffenstaden, France. [3]CNRS-CEA, Laboratory of Neurodegenerative Diseases, Institut Francois Jacob (MIRCen), University Paris-Saclay, Fontenay Aux-Roses Cedex, France. [4]INSERM, Lille Neuroscience & Cognition, Lille, France.

Dementia with Lewy Bodies (DLB), the second most common dementia after Alzheimer's Disease (AD), is characterized by pathogenic deposition of α-synuclein-laden Lewy bodies within vulnerable neurons. Sporadic cases are most prevalent with the e4 allele of apolipoprotein (APOe4) being their strongest risk factor, as in AD. Recently, astrocytic APOe3, but not e4, was shown to positively influence neuronal epigenome upon learning by modulating histone acetylation. Since epigenetic mechanisms regulate cell-specific information processing and neuroplasticity in the hippocampus, a structure particularly vulnerable to e4, we investigated its APOe4-induced epigenomic/transcriptomic dysregulations specifically in astrocytes and neurons. The genome-wide epigenomic distribution of two histone marks H3K27ac (marking active transcription) and H3K27me3 (marking transcriptional repression) was assessed by novel CUT&Tag method followed by deep sequencing, on magnetically enriched fractions of hippocampal neurons and astrocytes from ApoE4 and ApoE3 (control) KI murine models. Same methodology was conducted on the aforementioned models with stereotaxic injections of pathological α-synuclein preformed fibrils (PFF) or PBS, mimicking DLB-characteristic neuropathology. In the presence of e4 alone, the astrocytic epigenome revealed strong deprivation of the lipid metabolism/apolipoprotein binding pathways, that was also corroborated at the bulk transcriptomic level. The neuronal epigenome showed hyperacetylation of energy metabolism genes, a compensatory mechanism that may be due to the failure of astrocyte metabolic support. In the combined presence of e4 and α-synuclein, both cell types showed epigenomic and genomic alterations in pathways associated with synaptic function, as well as metabolic pathways. This demonstrates that in the presence of synucleinopathy, e4 retains the molecular signature associated with lipid dysregulation, and further gives rise to synaptic/cognitive dysregulations, as observed in patients. Our study shows for the first time that APOe4 causes cell-type specific epigenetic deregulations of the synaptic/metabolic pathways, thus giving potential to new therapeutics that could restore these dysregulated pathways and potentially brain plasticity and memory in patients.

15. Functional and molecular characterization of the Olig2-AS, an astrocyte subtype

Clémence Debacq, Alice Davy, David Ohayon
Molecular, Cellular and Developmental Biology department, Center of Integrative Biology (CBI), Toulouse, France.

Astrocytes are the most numerous glial cells of the central nervous system. They are involved in essential functions such as synaptic development and regulation or the blood brain barrier formation. Long considered as a homogeneous population, many newer studies highlight their intrinsic diversity in terms of molecular identity or function. As knowledge about astrocytes has never been higher in rodents, a lot is yet to be discovered in humans (Oberheim et al., 2009; Thomsen et al., 2016). Progress in in vitro stem cell models, such as human induced pluripotent stem cells (hiPSC), allows to approach human astrocytes molecular and functional heterogeneity and how they may diverge from rodents (Krencik et al., 2011). In 2019, the team uncovered the existence of an astrocyte subtype in the mice adult spinal cord, named Olig2-AS, that can be distinguished from others by the expression of Olig2, a transcription factor currently used as a hallmark of the oligodendroglial population (Ohayon et al., 2019). Using a double transgenic mice model that allows us to visualize this astrocyte subtype, we studied the distribution of Olig2-AS in the adult mice brain. Results show a preferential enrichment of Olig2+ astrocytes in the upper layers of a given cerebral cortical area compared to deep layers, as well as a higher distribution in the somatosensory cortex compared to the motor cortex. To have a first insight into the presence of this subtype in Humans, we cultivated astrocytes derived from hiPSC and performed a molecular characterization. We showed that a subtype of mature human astrocytes is Olig2+ in this in vitro model. Based on these results, the transcriptome of Olig2-AS done in the spinal cord of adult mice (Ohayon et al., 2021) and recent studies highlighting the higher synaptic coverage of upper layer cortical astrocytes (Lanjakornsiripan et al., 2018), we hypothesize that Olig2-AS are involved in the synaptic regulation of upper layers' cortical neurons. By performing a morphological and molecular study comparing Olig2-AS and non Olig2-AS in both in-vivo and in-vitro models, as well as co-culturing human astrocytes and neurons derived from hiPSC, we will be able to approach the Olig2-AS function.

16. Characterization of astrocyte reactivity in a model of encephalopathy of prematurity

Heydari Olya A. [1], Hua J., Nicolas E., Faivre V. [1], Gressens P.[1], Van Steenwinckel J.[1].
[1]NeuroDiderot UMR114. Hôpital Robert Debré, INSERM, Université Paris Cité, Paris, France.

Premature birth caused by maternal infection represent an increased risk factor of brain lesions affecting both developing gray and white matter, known as encephalopathy of prematurity (EoP), and long-term neurodevelopmental disorders. It has been suggested that the set-up of a pro-inflammatory environment with the secretion of cytokines and chemokines might initiate an inappropriate inflammatory response driven by reactive microglia and astrocytes, which participate to neurodevelopmental disruption. Astrocytes, located at the interface between the brain parenchyma and the blood brain barrier, preserve homeostasis. They also participate in the inflammatory response and go through morphological and functional changes called astrogliosis. However, little is known about astrocytic reactivity during perinatal inflammation. Our team has developed a mouse model of EoP based on systemic injections of IL-1beta (a pro-inflammatory cytokine) reproducing the deficits seen in premature infants. Using this model, we aim to precisely characterize astrocyte reactivity in EoP. Astrocyte subpopulations are highlighted using flow cytometry, emphasizing their heterogeneity. Bulk RNA sequencing of purified astrocytes showed a transcriptomic signature of astrocytes during perinatal inflammation. Analysis of A1/A2 phenotypes by quantitative RT-PCR revealed a pro-inflammatory phenotype of the astrocytic response along time. Significant morphological changes of GFAP+ astrocytes in the subventricular zone have been shown by immunohistochemistry. Functional differences have been studied by quantitative RT-PCR and revealed a decrease of synaptogenesis factors secreted by astrocytes. This in-depth characterization of astrocytes will pave the way for designing new strategies to restore the homeostatic functions of astrocytes and protect the brain of preterm infants.

17. Mechanisms controlling neuroblasts migration and reprogramming during myelin repair

M. Falque, K. Magalon, P. Durbec.
IBDM, CNRS UMR 7288, Marseille, France.

Spontaneous myelin regeneration is observed in multiple sclerosis patients but is not fully characterized. Following myelin damage in the adult rodent brain, a self-repair process is observed via the formation of new myelinating oligodendrocytes (OLG). They arise from two sources: progenitors disseminated in cerebral parenchyma and stem cells located in the subventricular zone. Under physiological conditions, stem cells mainly generate neuroblasts (NB) that migrate along the rostral migratory stream adopting a collective migration mode ("in chains") to integrate the olfactory bulb and differentiate into interneurons. Under demyelinating conditions in mice, some NB leave their migration path and migrate ectopically toward the lesion as isolated cells (Cayre et al. 2013). This change in migratory behavior is associated with NB reprogramming, which generate OLG and participate in myelin repair (Jablonska et al. 2010 - El Waly et al. 2018).

In the team we previously showed that this cell fate change proceeds directly, forming a transient cellular state co-expressing markers of both neuronal and OLG identities. Using transcriptomic analysis, we showed that this fate reprogramming necessitates regulating several key processes, including fate determination transcriptional programs, cell migration and extracellular matrix modification, suggesting an essential role for the environment surrounding the cell in this process (El Waly & Bertet et al. 2022). Tissue stiffness is known to have a significant role in guiding migration during developmental and regenerative processes (Shellard et al. 2021) and data have demonstrated that OLG differentiation is impacted by mechanical forces (Lourenco et al. 2016 - Urbanski et al. 2016). In this context, we asked whether a change in mechanical properties of the tissue/matrix stiffness might control NB behavior.

We demonstrated that the modification of matrix mechanical properties in vitro influences the migration mode of NB, from collective (chain migration) to isolated cells and that soft matrix promotes NB reprogramming into OLG. With atomic force microscopy experiments on ex vivo fresh brain slices, we evaluated the mechanical properties of brain tissue during development and in different injury models (demyelination versus non demyelinating lesions, such as ischemia). We showed that injuries lead to corpus callosum mechanical properties modifications and that stiffness is correlated with myelin content in highly myelinating structures. Altogether our work suggests that the mechanical properties of the environment regulate NB migration mode and reprogramming into OLG.

18. Roles of astrocytes in neurovascular unit protection after systemic perinatal infections and juvenile mild traumatic brain injury (jmTBI)

Hippauf L. [1], Clement T.[1], Delahaye-Duriez A. [2,3], Van Steenwinckel J. [2], Dubois C. [1], Fournier M.L. [1], Aussudre J. [1], Blugeon C. [4], Lemoine S.[4], Faivre V.[2], Boccazzi M.[2], Konsman J.P. [1], Gressens P. [2], Badaut J. [1,5].

[1]CNRS UMR5236, University of Bordeaux, Bordeaux, France. [2]UMR 1141 Inserm, University Paris Diderot, Paris, France. [3]Service Histologie-Embryologie-Cytogénétique, Hôpital Jean Verdier, AP-HP, Université Sorbonne Paris Nord, Bondy, France, [4]Plateforme Génomique, Institut de Biologie de l'Ecole Normale Superieure (IBENS), Paris, France, [5]Department of Physiology, Basic Sciences, Loma Linda University School of Medicine, Loma Linda, CA, USA.

Mild Traumatic brain injuries (mTBIs) are the most common form of TBIs and represent a major health hazard, especially in children. The outcomes vary between individuals and possibly depend on the previous exposure to previous life events such as immune system and inflammatory stimulation during an infection. We therefore investigated whether previous life events such as a neonatal systemic infection can impact the outcome of later traumatic injuries and identified the neuroinflammatory processes at play.

To test this, we used a double hit model combining a neonatal peripheral inflammation and a juvenile mTBI (double hit model). We tested whether the outcome of a double hit would impact further the behavior and blood-brain interface outcomes compared to a single hit. We investigated the cellular and molecular mechanisms with a focus on the neurovascular unit (astrocytes and microglia) as part on the neuroinflammation.

Post-Natal-Day (PND) 1 to 5, mice were injected with saline or IL1b to induce a chronic exposure to circulating cytokines (first hit). At PND 17 mice were impacted according to the Closed-Head-Injury Longterm-Disorder (CHILD) model (second hit), or underwent a sham procedure. Behavioural outcomes were assessed with the open field, foot fault and light/dark box tests at 1,7 and 30 days after the time of the jmTBI. Changes in neurons (NeuN and NF200 staining), vasculature (IgG and fibronectin staining) and blood-brain barrier properties were investigated using immunohistochemistry. Microglia and astrocytes were isolated with MACS technology followed by RNAseq analysis. Astrocyte phenotypes were confirmed using immunohistochemistry for GFAP, vimentin and VEGF expression.

We found that a double hit did not modify the behavioral outcomes or neuronal staining (NeuN/NF200) when compared to single hits. After a single hit IgG and fibronectin stainings were significantly increased, suggesting an increase of BBB permeability and a priming of the brain to protect against vascular dysfunction after a second hit (jmTBI).

We next analysed the consequences on astrocytes and microglia with RNAseq analysis. We found that astrocytes were marked by significantly dysregulated genes after double hit (n=3195 genes dysregulated after double hit) compared to microglia (n=376 genes dysregulated) which suggests that astrocyte could have a role in priming and vascular protection after IL1b injection.

We further focused on astrocytes, looking at GFAP and vimentin staining, which were increased with the number of hits. Furthermore, RNAseq analysis showed upregulation of extracellular matrix genes such as Dcn, Col1a, VEGF. This is in relation with the observed increase of VEGF expression in astrocytes after double hit. We observed that the astrocytic changes were accompanied by transient overexpression of VEGF, which is associated with brain vasculature alterations.

Our study suggests a priming effect of a previous life event. This priming appears to protect the brain from further damage trough vascular protection, rather than worsening the outcomes.

19. Breaking the code of myelination: The Drosopus chimera, an evolutional perspective from invertebrates to vertebrates.

Frère N.[1], K. Mannioui 1, Georgios Tsoumpekos 1, Natalia Mora Garcia 1, M. Tovelill Baldenius 2, M. E. Bechler 3, B. Hassan 1, N. Sol Foulon 1, C. Lubetzki 1, C. Klämbt 2, B. Zalc 1

[1] Sorbonne Université, Inserm, CNRS, Institut du Cerveau et de la Moelle Épinière, GH Pitié-Salpêtrière, 75013 Paris, France. [2] Institut für Neuro- und Verhaltensbiologie, Universität Münster, Badestraße 9, 48149 Münster, Germany. [3] Cell and Developmental Biology; Neuroscience and Physiology SUNY Upstate Medical University Syracuse, NY 13210, USA.

Myelin is a key constituent of nervous system of all Gnathostomes. In contrast axons generally have only a single glial wrap (or a few throughout aging), with the exception of rare arthropods, annelids, or insects invertebrates. These glial wraps, are missing the principal characteristics of myelin, that distinguish invertebrates' glial wraps from compact myelin in vertebrates, such as the lipid composition, the presence of highly conserved proteins or the level of compaction of myelin.

The objective of our project is to break the molecular code, sufficient and necessary, for a glial cell to wrap spirally around an axon and formed a compact myelin sheath. Assuming that evolution of myelin is based on processes and mechanisms that also operate in "primitive" invertebrate organisms, one might postulate that Drosophila glial cells are capable of recognizing vertebrate axons. To decipher the mechanisms governing interactions between neuron and glia and to identify the genetic and molecular basis of wrapping, we undertook to create an invertebrate (Drosophila melanogaster,)/vertebrate (Xenopus laevis) chimera (the Drosopus chimera) two species that can be raised and maintained at the same environmental temperature (20-22°C).

In a first set of experiments, we set up conditions to observe either Xenopus or Drosophila glial cells wrapping around synthetic microfibers of different diameter. To facilitate the manipulation of the Drosopus chimera, we developed neuron-glial co-culture conditions in which glial cells of both species can survive and wrap around axons. Finally, to test in vivo the possibility for Drosophila glial cells to wrap around a demyelinated Xenopus axon, we used as donor Drosophila transgenic line in which, under the control of nrv2, glial cells are marked with mCherry and APEX2 reporters for immunofluorescence and transmission electron microscopy analysis, respectively. The recipient host was the Tg(mbp:gfpntr) Xenopus transgenic line, in which demyelination is achieved by conditional ablation of myelinating oligodendrocytes following treatment with metronidazole. In this favorable demyelinated environment, Drosophila glial cells transplanted into the Xenopus ventricle recipient, survived, migrated and even wrapped up to at least 7 times around recipient axons, generating a Drosophila myelin sheath around Xenopus axons. Presently, to improve the previous protocol and better live monitor the wrapping process we are developing experimental conditions to graft Drosophila glial cell into demyelinated Xenopus optic nerve.

We anticipate that our experimental strategy will permit to elucidate the nature of intrinsic and extrinsic axo-glial interactions and open the door to cues to repair MS lesions that otherwise do not remyelinate.

20. Microglia-astrocyte crosstalk increases morphine metabolism during neuroinflammation

Hovhannisyan V.[1], Berkati A-K.[1], Gabel F.[1], Andry V.[1,2], Goumon Y.[1,2].

[1]University of Strasbourg, INCI CNRS UPR 3212, Strasbourg, France, [2]SMPMS-INCI (plateforme de Spectrométrie de Masse des Petites Molécules de Strasbourg, INCI CNRS UPR 3212, Strasbourg, France.

Morphine remains the gold standard painkiller drug used in the clinic to relieve severe pain. Once administrated, morphine is metabolized in the liver and the brain by UDP-glucuronosyltransferases (UGTs) leading mainly to the formation of the pronociceptive metabolite morphine-3-glucuronide (M3G). For this reason, the metabolic M3G/morphine ratio reflects the pronociceptive/antinociceptive balance. Little is known concerning morphine metabolism in the central nervous system (CNS), with only a few data suggesting the role of microglia and astrocytes in this enzymatic process. For this reason, we have developed a primary glial cell culture model to study morphine metabolism in control and lipopolysaccharide (LPS) induced inflammatory conditions. Our results indicate a basal metabolism by microglia and astrocytes and important synergy between these cells in inflammatory conditions leading to an increase in morphine metabolism (i.e M3G formation quantified by mass spectrometry). To characterize a possible crosstalk between microglia and astrocytes, we cultured microglia with or without LPS in order to obtain microglial conditioned medium (MCM) and incubated purified astrocytes with these media and results show that MCM LPS increased astrocytic metabolism of morphine. A vice versa effect was also seen when microglia were incubated with conditioned medium from astrocytes stimulated by LPS, showing a total interaction between these two cell types. To go further, we found that combination of TNFα, IL1α and C1q (TIC, shown to induce neurotoxic reactive A1 astrocytes) were able to fully mimic LPS effect. To investigate how TIC induces the increase of morphine metabolism in glial cells, we re-analysed RNAseq datasets published by Ben Barres laboratory and found that TIC increased the expression of UGTs in astrocytes through the Aryl Hydrocarbon Receptor (AhR). Inhibiting specifically this transcription factor with StemRegenin-1 (SR1) allowed us to decrease M3G formation under inflammatory conditions. Considering the importance of metabolism in the effects of molecules such as morphine that mainly act in the CNS, these results pave the way forward a major implication of neuroinflammation and glial cells in these processes. More precisely, under conditions where morphine is ineffective such as neuropathic pain, characterized with a huge central neuroinflammation and glial cell reactivity.

21. Development of an approach to isolate microglia and astrocytes from adult mouse brain without gene activation

Valentin Garcia[1], Grégoire Stym-Popper[2*], Carole Escartin[3], Guillaume Dorothee[4], Hélène Hirbec[1]*

[1]IGF, CNRS, INSERM, Univ. Montpellier, Montpellier, France. [2]Institut Cochin, INSERM, Sorbonne Université, Paris, France. [3]CEA, CNRS, Université Paris-Saclay, Paris, France. [4]CRSA, INSERM, Sorbonne Université, Paris, France.

* Both authors contributed equally to the work

Astrocytes and microglia play key roles both in brain physiology and in the neuroinflammatory processes associated with central nervous system (CNS) pathologies. However, understanding the specific roles and diversity of these different cell types requires conducting experiments on isolated cells population rather than a heterogeneous mixture of cells.

Recent studies revealed that functional changes in astrocytes and microglia are not independent, but rather rely on crosstalk between these two cell types that need to be further characterized. Single-cell RNA sequencing approaches (scRNA-seq) can allow to decipher inter-cellular crosstalk, but this requires microglia and astrocytes to be isolated simultaneously from the same sample.

Several protocols have been described for the isolation of microglia. In most cases, they are based on mechanical dissociation coupled or not with enzymatic dissociation. In contrast, only few protocols have been described for astrocytes. Yet, to our knowledge, there are no published method allowing the simultaneous isolation of these two cell types.

In this study, our objective was to develop a protocol that allows the simultaneous isolation of microglial and astrocytic cells. Three criteria were considered for the protocol validation: yield, purity and absence of activation.

We first compared two approaches combining mechanical and enzymatic dissociation. More specifically, we compared dissociation at 37°C using the ABDK kit, (Miltenyi) and dissociation at 4°C using the cold-active Bacillus licheniformis protease. For each condition, we also evaluated the effect of actinomycin D, an inhibitor of transcription.

Following dissociation steps, we then compared two methods to purify microglia and astrocytes: FACS (Fluorescence activated cell sorting) and MACS (Magnetic activated cell sorting) using anti-CD11b and anti-ACSA2 antibodies to purify microglia and astrocytes, respectively.

For each approach, we compared the number of cells obtained and their purity. We also assessed their activation level by measuring the expression of Immediate Early Genes (IEGs) by qPCR.

Our optimized mechanical and enzymatic dissociation approach allow the simultaneous isolation of microglial and astrocytic cells from adult mouse brain. Our results show that the resulting cell suspensions are compatible with RNA-seq and scRNA-seq approaches.

22. Developmental cell death of lineage-related interneurons and oligodendroglia impacts prefrontal cortex function

Khelfaoui H[1], Ibaceta-González C[1], Poilbout C[1], Angulo MC[1,2].

[1] Université Paris Cité, Institute of Psychiatry and Neuroscience of Paris (IPNP), Paris, France. [2] GHU PARIS Psychiatrie & Neurosciences, F-75014 Paris, France.

Many neurological and psychiatric disorders have developmental origins, thus emphasizing the importance of the proper unfolding of brain development to ensure correct functioning. A major biological process of cortical development is the early postnatal programmed cell death (PCD) of excess neurons and glial cells. Among both populations, lineage-related interneurons and oligodendrocyte precursor cells (OPCs) die within the same early postnatal period and maintain a tight relationship throughout development and beyond. However, the extent to which their PCD impacts the establishment and function of cortical circuits remains elusive. Using a mouse model of PCD impairment in which the pro-apoptotic factor BAX was genetically inactivated in lineage-related subpopulations of cortical interneurons and OPCs, we investigate the impact of PCD on myelination, cortical architecture, circuit function and behavior. We have used patch-clamp recordings on acute slices to analyze the excitation/inhibition balance and spontaneous activity of pyramidal neurons of the medial prefrontal cortex (mPFC) at different developmental stages. Our preliminary results uncovered a hyper-inhibition leading to an excitation/inhibition imbalance in the mPFC of PCD-impaired juvenile and adult mice. This defect in inhibition is accompanied by a prefrontal-dependent cognitive flexibility impairment as revealed by a two-week Barnes maze test. We are currently performing immunohistochemical analyses in control and mutant mice to assess differences in the cell densities of interneurons and oligodendroglia as well as myelination in the mPFC. Our current and future work will contribute to a better understanding of the neuron-glia relationship, their PCD and its importance for cortical development on one hand, and uncovering possible lines of inquiry concerning the etiology of neurodevelopmental disorders on the other.

Keywords: prefrontal cortex, interneurons, OPCs.

23. Metabolism as an origin of sexual dimorphism in morphine-induced analgesia but not in the setting of analgesic tolerance in mice

Goumon Y.[1,2], Gabel F.[1], Hovhannisyan V.[1], Berkati A-K.[1], Andry V.[1,2].

[1]INCI CNRS UPR 3212 & University of Strasbourg, Strasbourg, France. [2]SMPMS (plateforme de Spectrométrie de Masse des Petites Molecules de Strasbourg), INCI CNRS UPR 3212, Strasbourg, France.

Aims- In humans and rodents, sex influences morphine analgesia. In the liver and brain, morphine is metabolized into proalgic morphine-3-glucuronide (M3G). We have hypothesized that sexual dimorphism in morphine metabolism and differential metabolic modulation during the setting of analgesic tolerance might contribute to behavioural differences. Thus, we have studied if differences in peripheral and central morphine metabolism exist after acute and chronic morphine treatments in male and female mice.

Methods- Sexual dimorphism in morphine analgesia and tolerance were studied using the tail immersion test. Morphine and M3G metabolic kinetics in the blood after acute and chronic morphine treatments were determined using LC-MS/MS. Morphine and M3G were also quantified in several central nervous system (CNS) regions.

Results – Our results indicate that female mice display weaker morphine analgesia and faster tolerance setting compared to males. In addition, female mice have higher concentrations of proalgic M3G in the blood and several pain-related CNS regions (PAG, amygdala…) than male mice. At the opposite, lower concentrations of morphine were found in these regions. These major differences reflect a major imbalance in the pronociceptive/antinociceptive balance within the CNS.

Conclusion- Sex differences in morphine analgesic effects were mainly attributable to morphine central metabolism in pain-related CNS regions, consistent with weaker morphine analgesic effects in females. However, the implication of morphine metabolism in analgesic tolerance appeared to be limited.

24. Serglycin at the Glia Limitans, a key player of neuro-inflammation pathophysiology

Laisné M.[1], Veyrieres E.[1], Mora P.[1,2], Rouault P.[1], Cornuault L.[1], Bourgignon C.[1], Foussard N.[1], Gadeau A-P[1], Renault M-A.[1], Chapouly C.[1]

[1]Inserm U1034, Biology of Cardiovascular Diseases, Bordeaux, France. [2]University hospital of Bordeaux, Bordeaux, France

During neuropathology, notably multiple sclerosis (MS), Blood Brain Barrier breakdown leads to parenchymal inflammatory infiltration. Recently, we highlighted the capacity of astrocyte to communicate with other neurovascular unit cells, producing pro-inflammatory and pro-permeability factors.

We performed an RNA sequencing (RNAseq) on quiescent versus reactive human astrocytes (hRA) and identify serglycin (SRGN) as highly expressed by hRA. We confirmed these results in vivo in human MS lesions and in Experimental Auto-immune Encephalomyelitis mice model. Our goal is to unravel the contribution of SRGN at the Glia Limitans in neuro-inflammatory condition. Our hypothesis is that SRGN modulates astrogliosis and immune cell infiltration during MS neuro inflammation. We treated hRA in vitro with SRGN siRNA (siSRGN) to investigate the impact of SRGN at a cellular and molecular level.

We observed a decreased protein level of maker for reactive astrocytes (Vimentin), pro-inflammatory factor (IL-6, IL-8) in siSRGN treated hRA compared to positive control. Moreover, using immunofluorescence, we observed a reorganization of the cytoskeleton in hRA treated with siSRGN compared to positive control. Finally, with a scratch test assay, we studied the migration of hRA for 24 hours and observe that siSRGN treatment slow down the hRA migration process compared to negative and positive control. To determine the potential signaling pathway or interaction involved in these modulations, we performed an RNAseq hRA treated with siSRGN vs positive control. We identified Legumain (or AEP), as downregulated in absence of SRGN, as a potential actor of SRGN-mediated cellular behavior in astrocytes.

Collectively, these data suggest that SRGN upregulation in reactive astrocytes promotes cellular and molecular changes at the glia limitans under neuroinflammatory condition. Identify the actors underlying these changes could be a great interest.

25. Astrocyte distribution and morphology is altered in a mouse model with abnormal vascular development

Guille N.[1,2], Alvear-Perez R.[1,2], Philippe Mailly [2], Monnet H.[2], Cohen-Salmon M.[1,]2, Boulay A-C[1,2].

[1]Physiology and Physiopathology of the Gliovascular Unit Research Group, Labex Memolife, PSL Research University, Paris, France. [2]Center for Interdisciplinary Research in Biology (CIRB), College de France, CNRS Unité Mixte de Recherche 724, INSERM Unité 1050, Paris, France

Astrocytes are glial cells playing essential roles in the regulation of brain functions. In the cortex, they are produced by neural stem cells during the first two postnatal weeks: a dynamic phase of proliferation and spatial dispersion is followed by a maturation phase with morphological complexification and volume increase[1]. Astrocytes acquire their mature characteristics through a combination of an intrinsic repertoire and interactions with their environment, in particular blood vessels, which are produced earlier during brain development. However, this environmental induction of astrocyte network formation has been poorly studied until now.

Here, we characterized the development of astrocytes in the cortex of PdgfβRet mice, which present an altered expression of the Pdgfβ protein due to loss of proteoglycan binding motif (Ret), resulting in deficient mural cell recruitment and altered vasculature development.

We show an altered astrocyte distribution, with a clustering around blood vessels, both in mature brains and during postnatal development. We further studied astrocyte morphology in adult brains using AAV-induced or transgenic expression of Fluorescent proteins in PdgfβRet cortical astrocytes. We found that astrocytes have an altered morphology: they are smaller and display less primary processes arising from the soma. Altogether, our results suggest that the Pdgfβ pathway which compromises the embryonic development of the vascular system strongly influences postnatal astrocytes distribution and morphological maturation.

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26. Cytoskeleton abnormalities triggered by toxic cug rna repeats in dm1 astrocytes

Magneron P., Lallemant L., Correa L., Gourdon G., Gomes-Pereira M.
Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, 75013 Paris, France.

Myotonic dystrophy type 1 (DM1) is a multisystemic disorder caused by the abnormal expansion of a non-coding trinucleotide DNA repeats in the 3'UTR of the DMPK gene. Although the muscular symptoms of DM1 are well studied, the dysfunction of the central nervous system (CNS) is also prevalent and can contribute significantly to neurological impairment, characterised by cognitive and memory deficits, as well as emotional disturbance. DM1 pathogenesis is primarily driven by the accumulation of toxic RNA, with mutant DMPK transcripts that contain expanded CUG repeats aggregating in the cell nucleus and forming RNA foci. RNA foci are deleterious because they interfere with key RNA
binding proteins that regulate RNA processing, including alternative splicing. Despite our increasing understanding of muscle disease pathogenesis, the molecular mechanisms underlying brain disease are not fully understood. In particular we do not know the cell types and molecular pathways primarily dysregulated in the brain.
We have previously generated a transgenic mouse model of DM1, carrying a large CTG repeat expansion. The DMSXL mice display relevant behaviour phenotypes, impaired synaptic plasticity and increased neuronal firing in specific brain regions. Given the pronounced accumulation of toxic RNA foci in astrocytes, it is our hypothesis that astrocytes are affected by DM1 and contribute to brain pathology through unresolved mechanisms. This view is corroborated by the astrocyte hypotrophy and misorientation that we detected in DMSXL mouse brains. Using global RNA sequencing and phophoproteomics, we found multiple abnormalities in actin cytoskeleton-related transcripts and proteins. Interestingly, DMSXL astrocytes express RNA isoforms typical of immature astroglia, suggesting impaired astrocyte maturation.
To assess the impact of such molecular abnormalities on cytoskeleton biology, we are investigating the structure and dynamics of actin microfilaments in DMSXL astrocytes, using high resolution microscopy and biochemical assays. We collected evidence of microfilament misorganisation and destabilisation, demonstrated by a lower ratio of filamentous actin (F-actin) to globular actin (G-actin) in primary DMSXL astrocytes. The molecular evidence of astrocyte cytoskeleton defects along with the morphological abnormalities of astrocytes in vivo and in culture indicate the critical impact of toxic CUG RNA foci on astrocytes biology, which may ultimately affect the support provided to neighbouring neurons at the tripartite synapse and contribute to altered neurotransmission. We will use live cell imaging techniques to further investigate astrocyte cytoskeleton phenotypes in DM1, and to decipher the underlying mechanisms and functional consequences.
Our work suggests that DM1 brain disease is not solely mediated by neuronal defects, involving an important glial component. Our findings will reveal new disease intermediates that may offer windows for therapeutic intervention.
Keywords: Astrocyte; RNA biology; Cytoskeleton; Myotonic dystrophy; Transgenic mouse model

27. Do neurotransmitters regulate astrocyte-derived extracellular vesicle secretion and miRNA content?

Hekking R.[1#], Gassama Y.[2#], Franco L.[2], Oliveira A.[1], Barry-Carroll L.[3], Delpech J-C.[3], Rouglan V. [1], Leste-Lasserre T.[1], Leger C.[1], Ciofi P.[1], Avignone E.[1], Favereaux A.[2], Panatier A.[1*]*

[1]Neurocentre Magendie, University of Bordeaux, INSERM U1215, Bordeaux Cedex, France. [2]Interdisciplinary Institute for Neuroscience, University of Bordeaux, CNRS UMR5297, Bordeaux Cedex, France. [3]Nutritrion et Neurobiologie Intégrative, University of Bordeaux, INRAE 1286, Bordeaux Cedex, France.

[#]These authors contributed to work equally

[*]These authors supervised the work equally

Cerebral function depends on the transmission of chemical signals between neuronal elements at synapses. Over the last two decades, accumulating data have shown that this fundamental process is regulated by astrocytes. Among other functions, astrocytes participate in the clearance of ions and neurotransmitters. In addition, these glial cells release gliotransmitters to regulate synaptic functions such as basal synaptic transmission and long-term synaptic plasticity. Clearance and gliotransmission are not the only ways through which astrocytes influence neuronal communication. One interesting but under-investigated pathway would be through the release of extracellular vesicles (EVs). These vesicles are 30-1000 nm in diameter and contain proteins, lipids and nucleic acids. To understand the role of EVs during synaptic transmission, we first investigated whether acute stimulation of astrocytes with neurotransmitters can impact the quantity, size, and content of secreted EVs. We combine calcium imaging, EV quantification techniques, electronic microscopy and RNAseq analysis to address whether exposing primary mouse astrocyte cultures to 50 µM ATP, Glutamate or GABA for 30 min is sufficient to modify the number of EVs released and their miRNA content.

28. Long term impairment of cognitive function and neural network activity associated with structural changes in myelin after a transient episode of demyelination in adult mouse.

Mercier O.[1], Quilichini P.[2], Magalon K.[1], Gil F.[1], Antoine G.[2], Cayre M.[3], Durbec P.[1]

[1]Aix-Marseille Université - CNRS, IBDM, Marseille, France. [2]Aix-Marseille Université - INSERM, INS, Marseille, France. [3]Aix-Marseille Université - CNRS, LNC, Marseille, France

In the central nervous system, the myelin sheath is essential for cerebral activity by allowing saltatory conduction of action potentials and metabolic support to neurons. In the adult brain, activity-dependent myelin plasticity is required for proper learning and memory consolidation. In this context, myelin loss, alteration or even subtle structural modifications can compromise network activity, leading to functional impairment as in multiple sclerosis (MS). In this pathology, sensory motor deficiencies have been widely studied, but the cognitive consequences of MS are less addressed, although half of the patients are facing it.

A spontaneous myelin repair process is possible in MS but heterogeneous between patients, and sometimes leads to functional recovery, often more visible at the motor than at the cognitive level. In rodents, remyelination is very robust. In the cuprizone mouse model, a massive brain demyelination is followed by a spontaneous remyelination. However, the reformed myelin, although functional, does not have the same morphological characteristics as the myelin initially formed during development, and this can have an impact on the activity of neural networks.

In this context, we used the cuprizone model in mouse to analyze the cognitive, structural and functional long-term effects of transient demyelination. Our results show that an episode of demyelination induces cognitive impairment maintained on the long-term despite remyelination, such as deficits of spatial working memory, social memory, cognitive flexibility and behavioral hyperactivity. These data suggest that the remyelination process could be incomplete or imperfect in grey matter structures associated with these modalities. Indeed, these deficits are correlated with a reduction in myelin content in the medial prefrontal cortex (mPFC) and hippocampus (HPC), as well as structural myelin modifications. In vivo electrophysiological recordings show that the demyelination episode alters the synchronization of HPC-mPFC activities, which is crucial for memory processes. Altogether our data indicate that the myelin repair process following transient demyelination does not allow the complete recovery of initial myelin properties in cortical structures, and these subtle modifications alter network features leading to prolonged cognitive deficits in mice.

29. Role of astrocyte JAK-STAT pathway in regulating mouse socio-sexual behavior

Lakomy T., Dubet A., Guillermier M., Auregan G., Cambon K., Dhenain M., Escartin C., Ben Haïm L.

Université Paris-Saclay, CEA, CNRS, MIRCen, Laboratoire des Maladies Neurodégénératives, 92265, Fontenay-aux-Roses, France

The JAK-STAT pathway is a ubiquitous signaling cascade which regulates the expression of genes involved in a variety of cellular functions. In astrocytes, the JAK-STAT pathway has exclusively been studied in pathological conditions, as it is a master regulator of cytokine-induced neuroinflammation. Astrocytes influence various physiological behaviors in a brain-region specific manner, through the expression of distinct receptors and signaling molecules. However, whether astrocytes also signal through the JAK-STAT pathway to regulate physiological behaviors remains unknown.

Accumulating evidence indicates that basal cytokine signaling plays a role in physiological behaviors such as social interactions. Because astrocytes regulate mouse behavior and that the JAK-STAT pathway is central for cytokine signaling in these cells, we hypothesize that local astrocytes might be involved in cytokine-mediated regulation of social behavior. Our preliminary results show that social interaction with a novel mouse activates the pathway in the mouse ventral hippocampus (vHPC). Furthermore, we found that expressing a constitutively active form of JAK2 specifically in vHPC astrocytes selectively promotes sociability. This project aims at deciphering the effect of local astrocyte JAK-STAT pathway activation on brain networks involved in mouse social behavior through viral mediated targeting of astrocytes, ex vivo electrophysiology, functional brain imaging and behavioral analysis. This exciting project suggests an unexpected role for astrocyte JAK-STAT pathway as a dual regulator of cytokine signaling and social behavior in mice.

30. Protective role of microglia and T lymphocytes in a mouse model of temporal lobe epilepsy

Moreno-Montano M.[1], Nobili P.[1], Martins L.[1], Virenque A.[2], Dardalhon V.[3], Hirbec H.[1], Garcia V.[1], Mateos-Languerak J.[4], Marchi N.[1], Audinat E.[1].

[1]Inst. Génomique Fonctionnelle, Montpellier, France; [2]HiLIFE/ Neurosci. center, Helsinki Univ., Helsinki, Finland. [3]Inst. de génétique moléculaire de Montpellier, Montpellier, France; [4]Inst. de génétique humaine, Montpellier, France.

It is generally assumed that seizure recurrence causes lesions in regions distant from the primary epileptic focus, and these lesions in turn worsen epilepsy. However, the hypothesis of secondary seizure dependent damage has not been proven. Clinical data obtained on postsurgical epileptic brain tissue, showed that areas of seizure propagation did not show any lesions. Although this evidence argues against the hypothesis of seizure-induced damage, non-resected areas of seizure propagation may undergo functional modifications. Therefore, we suppose the existence of endogenous protective mechanisms that limit the harmful effects of network hyperexcitability, thus preserving the tissue from damage.

Accumulated data suggest that immune cells develop a dual profile, pro- and anti inflammatory, and that the balance between these two states determines the evolution of the disease. Few data showed that the deleterious pro-inflammatory response that accompanies epileptogenesis, is partly counteracted by an endogenous anti-inflammatory component involving microglia and T cells. Our working hypothesis is that the interaction between microglia and T cells is responsible for the protective effect observed in areas of seizure propagation. To test this hypothesis, we worked on a robust model of temporal lobe epilepsy (TLE), the most frequent form of focal epilepsy in adults. We have observed that unilateral intra-cortical Kaïnate injection in CD3KO mice (lacking T cells) and in WT injected with anti CD25 antibodies (mice depleted of T regulatory cells, Tregs), leads to the appearance of injury biomarkers in contralateral areas to which the seizures spread from the epileptic focus. This damage was never observed in non-immunodeficient epileptic animals. The aggravation of hippocampal injury was accompanied by a loss of arginase-1, a marker of anti-inflammatory microglia, and an increase of seizure frequency. These observations suggest that, among the large family of T cells, Tregs are responsible for driving a microglial anti-inflammatory phenotype that appears to be essential not only to reduce seizures but also to protect propagating zones from seizure-induced lesions. Preliminary analysis at 5 days post-KA revealed a minor deregulation within 700 glial genes in the contralateral hippocampus of mice lacking Tregs. Pursuing this research would allow us to validate new pro- and anti-inflammatory biomarkers on our mouse model, that could subsequently be applied to humans and identify new therapeutic targets.

31. Oligodendroglial ADAM10, an architect of re/myelination of the central nervous system

Lavaud M., Simon A., Ríos-Concepción G., Meffre D., Jafarian-Tehrani M.
Université Paris Cité, INSERM, UMR-S1124, T3S, Team: "Myelination and Nervous System Pathologies", Paris, France.

Oligodendrocytes, the myelinating glial cells of the central nervous system (CNS), envelop the axons with their membrane extensions and form the myelin sheath. Loss of oligodendrocytes and/or myelin (demyelination) occurs in traumatic brain injury or pathologies such as multiple sclerosis. Since, there is no specific cure for demyelination; we are interested in enhancing myelin endogenous repair. We focus our work on A Desintegrin and Metalloprotease (ADAM) 10, the main α-secretase of the CNS, that generates the neuroprotective soluble fragment sAPPα via the cleavage of the Amyloid Precursor Protein (APP). Previous results from the team have shown that the pharmacological activation of ADAM10 has a protective effect against demyelination and enhanced remyelination ex vivo and in vivo. To investigate the role of oligodendroglial ADAM10 in the de/re/myelination processes, we have generated a novel mouse strain, KOOLA10, in which we invalidate ADAM10 in oligodendrocyte at different important time points for oligodendrogenesis or myelination. Our findings showed that the maturation of OPCs primary cultures deficient for ADAM10 are delayed and that myelin sheaths are thinner in the cerebellum of adult KOOLA10 mice. Moreover, we observed a worsen loss of myelinated axons in organotypic slices of cerebellum obtained from KOOLA10 mice. Taken together our results suggest that ADAM10 may be an architect of re/myelination due to its role from OPC maturation to myelin ultrastructure making it an interesting candidate in the study and treatment of demyelinating diseases.

32. The ribosomal-associated protein rack1 represses kir4.1 translation in astrocytes and influences neuronal activity

Oudart M.[1], Avila-Gutierrez K.[1], Moch C.[2], Dossi E.[1], Milior G.[1], Boulay A-C.[1], Gaudey M.[1], Moulard J.[1], Lombard B.[3], Loew D.[3], Bemelmans A-P.[4], Rouach N.[1], Chapat C.[2], Cohen-Salmon M.[1]

[1]Center for Interdisciplinary Research in Biology (CIRB), Collège de France, CNRS, INSERM, Université PSL, Paris, France. [2]Laboratoire de Biochimie, Ecole Polytechnique, CNRS, Université Paris-Saclay, Palaiseau, France. [3]CurieCoreTech Spectrométrie de Masse Protéomique, Institut Curie, Université PSL, Paris, France. [4]CEA, Institut de Biologie François Jacob, Molecular Imaging Research Center (MIRCen), CNRS, CEA, Université Paris-Sud, Université Paris-Saclay, Fontenay-aux-Roses, France

Post-transcriptional regulations are essential mechanisms that determine the composition of the translatome. In astrocytes, abundant glial cells in the brain, these mechanisms have been poorly studied. Astrocytes are morphologically, molecularly and functionally polarized. They display long processes contacting both synapses and blood vessels. Perisynaptic astrocytic processes (PAP) form the tripartite synapse with the neuronal compartments and participate in gliotransmission, neurotransmitters recycling and ion homeostasis among others. Perivascular astrocytic processes (PvAP) contact all brain vessels and regulate vascular functions such as blood brain barrier integrity or the cerebral blood flow[1].

To advance our understanding on translation mechanisms in astrocytes, we identified proteins associated with astrocytic polysomes to study their role in translation2. We combined an astrocyte specific translating ribosome affinity purification (TRAP) approach[2,3] with mass spectrometry (MS). Among identified proteins, we focused on the ribosomal protein Receptor for activated C kinase 1 (RACK1), a crucial factor in translational regulation.

We showed that RACK1-associated ribosomes were preferentially associated with astrocytic specific mRNAs such as Kcnj10, coding for the critical potassium channel Kir4.1 and enriched in PAPs and PvAPs. To further study RACK1's role, we developed a transgenic mouse model in which RACK1 is specifically depleted from astrocytes in the adult stage. These mice displayed higher levels of Kir4.1 in the cortex and hippocampus as well as in the PAPs and in some PvAPs, compared to control mice.

We showed that this elevation of Kir4.1 resulted in higher astrocytic volumes and astrocytic inward potassium currents. It also modified neuronal network activity attenuating burst frequency and increasing burst duration.

Finally, we found that RACK1 repressed Kcnj10 translation by targeting the second part of its 5'UTR.

Our present results show that RACK1-associated ribosomes bind specifically to Kcnj10 mRNAs and regulate their translation in astrocytes. In a previous analysis, we showed that RACK1 polysomal mRNAs are enriched in PAPs and PvAPs compared to the soma 3. Thus, RACK1 may also control Kcnj10 local translation. Altogether, this post-transcriptional mechanism may control astrocytic potassium homeostasis at the neuroglial and gliovascular interface, which is crucial for neuronal and vascular functions in the brain.

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33. Role of astrocytes in maintaining energy balance and consequences of their adaptation to a high calorie diet

Léger C.[1], Lecoin L.[2], Castel J.[1] , Ansoult A.[1] , Franco C.[3] , Li D.[3] , Rouach N.[2] , Luquet S.[1] , Martin C.[1]

[1]Université Paris Cité, Unité de Biologie Fonctionnelle et Adaptative, Paris, France. [2]Collège de France, Centre Interdisciplinaire de recherche en biologie, Paris, France. [3]Sorbonne Université, Neurosciences Paris Seine, Paris, France

An increasing number of studies aim to characterize the function of a subset of glial cells, the astrocytes, in the context of energy homeostasis and their potential contribution in the development of obesity (García-Caceres et al., 2019). Hypothalamic astrocytes, are ideally positioned to participate in the integration of hormones and metabolites transported by the bloodstream which regulate energy metabolism and food intake.

In the brain, hypothalamus plays a fundamental role in body weight regulation and directly regulates feeding and metabolism (García-Caceres et al., 2019). Neurons located in the paraventricular nucleus (PVN) of the hypothalamus regulate the Autonomic Nervous System (ANS), body metabolism and feeding behavior. PVN neurons are known to control energy balance, their activity is tightly dependent on adequate delivery of energy substrates provided by astrocytes. Obesity as well as high energy food intake were shown to trigger inflammatory-like response in hypothalamic astrocytes in association with dysregulation of neurometabolic coupling.

However, the functional involvement of hypothalamic astrocytes-neuron communication in the central regulation of energy balance and the development of obesity remains unresolved.

The work of our team (Chao et al., 2022) shows that astrocyte function and astrocyte-neuron communication within PVN of the hypothalamus, participate in the central control of food intake, energy expenditure, and glucose metabolism.

Using genetic and molecular tools, we further obtained data on how changes in astrocyte-neuron communication within the hypothalamus impairs glucose homeostasis and energy expenditure. In particular, we focused on the role of specific hemichannel, called Pannexin in lean or obese mice.

34. Characterization of astrocyte activity modulation using functional neuroimaging

Pereira M.[1], Vidal B.[3], Valdebenito M.[2], Vidal L.[1], Nestor L.[5], De Bundel D.[5], Smolders I.[5], Droguerre M.[3], Zimmer L.[1,2,4]

[1] Université Claude Bernard Lyon 1, Lyon Neuroscience Research Center, Lyon, France. [2] CERMEP-Imaging Platform, Lyon, France. [3] Theranexus, Lyon, France. [4] Hospices Civils de Lyon, Lyon, France. [5] Department of Pharmaceutical Chemistry, Drug Analysis and Drug Information, Research Group Experimental Pharmacology, Center for Neurosciences (C4N), Vrije Universiteit Brussel (VUB), Laarbeeklaan 103, 1090, Brussels, Belgium.

Exploration of neuroglial interactions represents an important challenge in new therapeutic strategies research. Regarded as a major actor of synaptic transmission, astrocytes are currently becoming a pharmacological target of interest. Given their critical importance in CNS diseases, it is necessary to develop new preclinical tools and approaches allowing the study of neuron-astrocyte networks. In this context, we aim to identify and characterize the contribution of astrocytes in neurovascular and neurometabolic coupling using neuroimaging in vivo techniques in rodent. To do so, we studied lesional and chemogenetic models using functional ultrasound imaging (fUS) and 18F-FDG positron emission tomography (PET). Lesional models involved stereotaxic injections of astrocyte specific gliotoxins (L-alpha Aminoadipic acid and fluorocitrate) in the visual cortex of rats before fUS imaging at different timepoints (1h and 48h post-injection). For chemogenetics, astrocytic DREADDs receptors (hM3Dq) in the visual cortex were activated in mice by CNO injection during fUS and microPET neuroimaging. To control for possible unspecific effects of CNO, an effect-dose study was performed beforehand in wild-type mice. We show that the gliotoxins L-alpha aminoadipic acid (L
AAA) and fluorocitrate (FC) impact the fUS signal differently in rats, with few changes following L AAA but a strong perturbation of the shape of the response to sustained visual stimuli following FC injection (see figure below). The preliminary results of the chemogenetic study suggest a higher interindividual variability for response to visual stimuli in DREADDs compared to control mice, although the sample size is currently too low to draw any conclusion.
Impact of early disruption of parvalbumin interneuron-OPC interactions on prefrontal-dependent cognitive processes.

35. Delta like 4 at the Glia Limitans, a key player of neuro-inflammation pathophysiology

Mora P., Laisne M., Rouault P., Bourguignon C., Renault Ma., Chapouly C.

Introduction: During neuro-inflammation, astrocytes undergo morphological and molecular changes named “astrogliosis” and drive the conversion from acute inflammatory injury to chronic neurodegenerative state. To characterize astrocyte signature during astrogliosis, a RNA sequencing on reactive astrocytes was performed and identified delta-like 4 (Dll4) as highly expressed during astrogliosis.

Aims: Our goal is to unravel the contribution of Dll4-Notch1 signaling to astrogliosis and neurovascular unit (NVU) disruption.

Methods: We induced neuro-inflammation in both conditional astrocytic Dll4 KO (Dll4ACKO) mice vs controls and in C57BL/6 mice treated with an anti-DLL4 monoclonal antibody vs IgG. Lesion size, inflammation, demyelination and clinical disability were measured. In parallel, an in vitro analysis has been performed on human reactive astrocytes KO for DLL4 vs control.

Results: In vivo, both Dll4ACKO and anti-DLL4 mAb treated mice exhibited a milder pathology and astrogliosis than controls. It was correlated to the decreased expression of IL-6 and pro-permeability factors. In vitro, we demonstrated that the Dll4-Notch1 juxtacrine signaling in reactive astrocytes directly controls IL-6 transcriptional level and that blocking IL-6 receptor decreases astrogliosis and pro-permeability factor expression.

Discussion: Collectively, these data suggest that the Dll4-Notch1 signaling drives astrogliosis during neuro-inflammation via IL-6 up regulation promoting NVU disruption and pathology severity. Thus, DLL4 might be a new therapeutic target in neuro-inflammatory disorders.

36. Impact of early disruption of parvalbumin interneuron-OPC interactions on prefrontal-dependent cognitive processes.

Plaisier F.[1,2], Ibaceta C.[1], Poilbout C.[1], Angulo M.C.[1,3].

[1]Université Paris Cité, Institute of Psychiatry and Neuroscience of Paris, INSERM U1266, 102-108 Rue de la Santé, Paris, France. [2]Inovarion, 251 rue Saint Jacques, 75005 Paris, France. [3]GHU PARIS psychiatrie & neurosciences, F-75014 Paris, France.

GABAergic inhibitory interneurons have a prominent role in the activity of cortical networks and function. They act as important regulators of the excitatory output and confer dynamic modulation to neuronal circuits and information processing. We have previously shown that parvalbumin (PV)-expressing interneurons, the largest proportion of GABAergic neurons of the cerebral cortex, represent the major transient synaptic input of oligodendrocyte precursor cells (OPCs) during postnatal development (Orduz et al., 2015 & 2019). PV interneurons are also known to play an essential role in the proper functioning of cortical circuits by controlling cortical oscillations and the functional connectivity between multiple brain areas for the accomplishment of high-order cognitive tasks. Interestingly, the myelination of these neurons did not attract attention until recently. How the myelination of these neurons can influence cortical inhibition remains still poorly understood. Using a mouse model in which the γ2 subunit of GABA-A receptors was genetically inactivated in OPCs (γ2f/f mouse line; Balia et al., 2017), we recently demonstrated that the disruption of PV interneuron-OPC GABAergic synapses, prior to myelination onset, resulted in severe PV interneuron myelination defects, impacting inhibitory circuits of the somatosensory cortex as well as a whisker-based texture discrimination behavior (Benamer et al., 2020).

Here, we hypothesize that PV interneuron myelination defects impair the function of the medial prefrontal cortex (mPFC), an area implicated in diverse neurodevelopmental disorders such as schizophrenia. We used a fear conditioning task and found that γ2f/f mice presented fear extinction learning deficits. Since the infralimbic (IL) region of the mPFC has been involved in this process, we evaluated the excitation-inhibition (E-I) balance of its layers 2/3 pyramidal neurons by recording postsynaptic currents in acute brain slices. We found a significant imbalance of the E-I ratio caused by a decreased inhibition. However, no changes in either cell density or distribution of IL PV interneurons were observed. Taken together, our results show that early postnatal disruption of PV interneuron-OPC synaptic interactions causes deficits in cortical inhibitory circuit function and cognitive processes.

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37. Characterization of appnl-f mice, a relevant model to study alzheimer's disease early stages

Prieur M.[1], Hemonnot A-L.[1], Garcia V.[1], Thanawat P.[1], Leportier M.[1], Perrier V.[2], Vignon A.[2], Hirbec H.[1].

[1]IGF, CNRS, INSERM, Univ. Montpellier, Montpellier, France. [2]INM, INSERM, Univ. Montpellier, France

With over 1.2 million people affected in France, Alzheimer's disease (AD) is a major public health problem. This progressive neurodegenerative disease is characterized by cognitive deficits, including memory and behavioral disorders. At the tissue level, it is characterized by two main histological alterations: amyloid plaques consisting of aggregated amyloid-β peptide (Aβ) and neurofibrillary tangles caused by the aggregation of hyperphosphorylated TAU protein. Neuroinflammation is another important feature of AD. The early stages of AD represent an interesting phase to target to slow disease progression, but they are still ill-characterized.

The accumulation of Aβ peptides, or amyloidosis, is an early feature of AD that occurs several years before the cognitive symptoms. Thus, Aβ-based animal models represent useful tools for studying the early phases of the disease. The main mouse models used to study AD are transgenic mice, which are based on the overexpression of human forms of APP and/or PSEN1/2 carrying mutations identified in AD patients. Such models lead to the overexpression of both APP and Aβ; their phenotype is thus biased by the overexpression of other APP degradation fragments. To overcome this problem, Dr. Saido's team has developed Knock-In models, including the APPNL-F mice [1]. In this model APP is humanized, carries the Swedish (NL) and Iberian (F) mutations, but is expressed under the control of its endogenous promotor, leading to the overproduction of Aβ peptide while APP expression remains at physiological levels. Yet, information is lacking regarding the kinetics of the apparition of the AD-like alterations in this model.

To better characterize this more pathophysiological model and determine the optimal window to study the early stages of the disease, we used a battery of behavioral, histological, and molecular approaches and characterized alterations in 3-, 6-, 9- and 12-month-old mice. Cognitive impairment was assessed by the Barnes Maze and Hamlet tests. The number of Aβ-plaques, and the astrocytic and microglial reaction were evaluated by immunohistochemical quantification. Finally, neuroinflammation was analyzed by qPCR. In both males and females, we evaluated the impact of genotype (WT, NLF/+ and NLF/NLF) on AD key features.

Our results identify the onset of mild cognitive impairment, amyloidosis and glial reaction, and specify the age, sex and genotype that are the most relevant in this model to study the early phase of AD.

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38. Molecular characterization of reactive astrocytes through different omics: transcriptomics and proteomics insights

Riquelme-Pérez M.[1,2], Abjean L.[1], Ben Haim L.[1*], Carrillo-de Sauvage M-A.[1*], Derbois C.[2], Hantraye P.[1], Brouillet E.[1], Olaso R.[2], Deleuze J-F.[2], Redeker V.[1,3], Bonnet E.[2], Brohard-Julien S.[2], Escartin C.[1]*

[1]Université Paris-Saclay, CEA, CNRS, MIRCen, Laboratoire des Maladies Neurodégénératives, 92265, Fontenay-aux-Roses, France. [2]Centre National de Recherche en Génomique Humaine (CNRGH), Institut de Biologie François Jacob, CEA, Université Paris-Saclay, Evry, France. [3] Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France.

* These authors contributed equally to this work.

Astrocytes undergo significant morphological and functional changes in disease. However, the specific molecular changes triggered in these reactive cells and their upstream regulators are yet to be fully characterized. The Janus kinase (JAK)2/signal transducer and activator of transcription (STAT)3 signaling pathway is a central cascade controlling the reactive response of astrocytes, including in neurodegenerative diseases such as Huntington disease, which affects mainly striatal neurons. Hence, we aim to define the JAK2-STAT3-dependent molecular changes in mouse striatum astrocytes.

To activate the JAK2-STAT3 pathway, we designed an “AAV-JAK2ca” targeting astrocytes and encoding a constitutive active form of JAK2. We induced astrocyte reactivity through this pathway selectively in WT and HD mice to characterize the subsequent glial reactivity. Several approaches were involved. In a first set of studies, a microarray analysis on acutely-sorted striatal astrocytes was performed. WT mice were injected with an AAV-GFP (control) or an AAV-JAK2ca. We also sorted injected astrocytes from the striatum of HD mice to perform RNA sequencing (RNA-seq) and differential expression analysis between HD-GFP and HD-JAK2ca astrocytes. Finally, we carried out a proteomics analysis of WT-GFP and WT-JAK2ca astrocytes, and immunohistological validation of candidate proteins.

Histological analysis demonstrated that the activation of JAK2-STAT3 cascade in WT astrocytes is sufficient to induce morphological changes (increased soma surface and branching index) and the over-expression of GFAP and Vimentin, fundamental astrocyte reactivity markers. Microarray analysis of the WT sorted astrocytes showed that JAK2 activation induces extensive transcriptional changes, with 689 differentially expressed genes between JAK2--reactive astrocytes and GFP

astrocytes, including an induction of genes linked to inflammation, cytokine signaling and immune reaction. The JAK2-STAT3 pathway also changed the expression of proteostasis, transport and energy metabolism genes. Interestingly, many of these functions were also confirmed by RNA sequencing analysis of astrocytes isolated from the striatum of a HD murine model when activating JAK2-STAT3 pathway (Abjean et al. 2022, Brain). 269 differential expressed genes were reported between HD

JAK2ca and HD-GFP samples. Last, by label-free mass spectrometry, 87 proteins were found differentially abundant between JAK2ca- and GFP-astrocytes. They were involved in cell adhesion, cytoskeleton and oxidative phosphorylation, revealing only limited overlap between mRNA and protein changes. Ongoing experiments aim to validate the differential expression of candidate genes/proteins in reactive astrocytes on mouse models of Huntington disease and in human brains.

Our multi-omics analysis of the JAK2-STAT3 signature identifies coordinated morphological and molecular changes in striatal reactive astrocytes, which helps define a specific reactive state, which could be linked to the beneficial effects of JAK2- dependent reactive astrocytes that we observed in Huntington disease models. A finer knowledge on the molecular signature controlled by specific astrocyte signaling cascades will help outline the different reactivity states seen in many brain diseases.

39. Microglia-enriched human midbrain organoids for studying Parkinson's disease

Winterberg H.[1], Heneine J.[1], Galet B.[1], Muscat A.[1], Dusanter C.[1], Lachal P.[1], Smeyers J.[1], Latouche M.[1], Ravassard P.[1], Corti O.[1]

[1]Paris Brain Institute, ICM, Paris, F-75013 France. Inserm, U1127, Paris, F-75013 France. CNRS, UMR 7225, Paris, F-75013 France. Sorbonne Université, Paris, F-75013 France

Microglia, the resident immune cells of the central nervous system, are suspected to be key players in the pathogenesis of Parkinson's disease (PD). PD patients display signs of microglial activation and increased proinflammatory cytokine levels. The precise interplay between neuronal and microglial cells, and how it affects the vulnerable dopaminergic neurons in PD, remains to be determined. Brain organoids generated from iPSCs provide a platform for studying cellular and molecular mechanisms underlying disease in a context resembling the human-brain. However, region-specific cerebral organoids lack microglial cells, which originate from yolk sac progenitors. In this study, we incorporated human microglia-like cells differentiated from peripheral blood-derived monocytes (MDMi) into human midbrain-specific organoids (hMOs) and investigated their long-term integration and impact on dopamine neuron maturation and organization within the hMOs. As early as 30 days in vitro, hMOs expressed dopaminergic markers, including tyrosine hydroxylase (TH), the FOXA2 transcription factor and the dopamine transporter (DAT). Peripheral blood monocytes from healthy donors were cultured for two weeks in the presence of IL-34 and GM-CSF to generate MDMi (Sellgren et al., 2017). At this stage the cells expressed the myeloid markers IBA1 and CD11b, and the microglia-specific marker P2RY12. When incubated with hMOs, the MDMi integrated into the 3D structures, acquiring ramified morphologies. In current work, we explore how MDMi interact with their 3D environment. As a perspective, these studies will be extended to investigate how mutations responsible for familial forms of PD affect neuroimmune interactions in hMO incorporating patient-derived microglia-like cells.

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AMOYAL	William
ANGULO	Maria Cecilia
AOUADI	Khouaila
AUBERT	Antoine
AUBRY	Anne-Sophie
AUDINAT	Etienne
AVIGNONE	Elena
AVILA-GUTIERREZ	Katia-Raquel
BALLOUT	Nissrine
BANCEL VEGA	Roxane
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