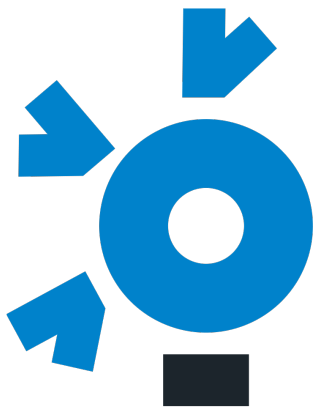




NCBio
ERA CHAIR

D3.3

Neural Cell Biology Group's annual activity report 2



**Funded by
the European Union**

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Report on Neural Cell Biology Group's annual activity report 2

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Abbreviations and Acronyms

Abbreviation Acronym	Definition
D	Deliverable
IBMC/i3S	Institute for Molecular and Cell Biology/Institute for Research and Innovation in Health
M	Month
NCBio	Neural Cell Biology
PNND	Program in Neurobiology and Neurological Disorders
SBG	Synapse Biology Group
WP	Work package

Index

Abbreviations and Acronyms.....	2
Executive summary.....	4
1. Research Activities.....	5
2. Dissemination and Communication Activities	7
2.1 Organization of International Seminars	7
2.2 Public Engagement.....	10
2.2.1 World Cancer Day 16 February 2023	10
2.2.2 Brain Awareness Week 13-19 March 2023.....	10
2.2.3 World Brain Day 22 July 2023.....	11
3. NCBio Stakeholder Hub	12
4. Achievements	13
4.1. Recruitment.....	13
4.2. Publications (Open Access).....	13
4.3. Participation in (inter)national conferences and courses.....	14
4.4. Scientific Service.....	16
5. Education and Capacity Building.....	16
ANNEX 1.....	18
ANNEX 2.....	19
ANNEX 3.....	20
ANNEX 4.....	21

Executive summary

The year of 2023 has been marked by several achievements by the Synapse Biology Group (SBG). First, the SBG is now complete (last hired member will be joining in January 2024) and fully integrated at the IBMC/i3S. Second, the recruited staff was able to kick start their experiments in the laboratory and have already generated encouraging preliminary results: the SBG is validating proximity labelling systems that will be crucial to identify the surface proteome of tripartite synapses and astrocyte-secreted factors across the brain.

The SBG has continued to publish in high-impact journals and two of those works were featured as front covers in *Essays in Biochemistry* and *EMBO Molecular Medicine*.

The IBMC/i3S has leveraged on the ERA Chair Holder's strong academic and industrial network to bring international, renowned scientists and top R&D leaders to give talks as part of the NCBio Seminar Series and by sponsoring their participation in IBMC/i3S events organized by members of the Neurobiology and Neurologic Disorders integrative program (PNND) that share common research interests (e.g., 6th Ph.D. Day, i3S Neuro Day).

This year was also marked by the launch of the NCBio Stakeholder Hub, a one-day event that brought together patient associations, clinicians, health industry and researchers from the PNND to talk about their needs and expectations that will ultimately shape the continuity of hub activities.

Finally, the ERA Chair Holder was appointed Vice-Coordinator of the PNND and was recently invited to be part of the editorial board for the *GLIA* journal, adding further visibility to the IBMC/i3S at international level.

1. Research Activities

Assessing the role of astrocyte heterogeneity in differential synapse formation.

The goal of the SBG is to adopt a bottom-up approach based on the identification of key proteins involved in inhibitory synapse formation and maturation. We will focus on astrocytes (the most abundant glial cell in the mammalian brain) and combine different proteomics methods with genetics to identify the surface proteome of tripartite synapses and astrocyte-secreted factors across brain regions. The background and rationale of the research strategy has been previously alluded in deliverable D3.1 Neural Cell Biology Research and Innovation.

We are currently implementing biotin-based proximity labeling technologies to enable streptavidin-based recovery of proteins for identification by mass spectrometry. Our preliminary results show that **we are able to detect biotinylated proteins at cell-cell contact sites** using cell culture-based systems (Figure 1). Once we validate our proximity labeling systems, we will move forward to *in vivo* labeling of the astrocyte surface proteome at tripartite synapses.

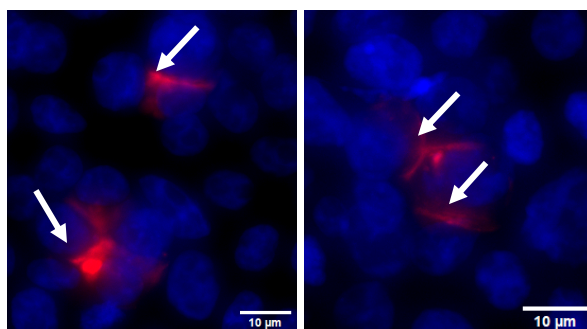


Figure 1. Biotinylation at cell-cell contact sites. Red indicates biotinylation. Blue indicates nuclei. Arrows indicate cell-cell interfaces. Scale bars, 10 μ m.

An orthogonal approach to identifying key proteins involved in (inhibitory) synapse formation and maturation is by looking at its secretome. We are currently developing endoplasmic reticulum (ER)-resident systems to selectively label proteins in the astrocyte secretory pathway. To date, we validated our biotin ligase plasmid constructs by confirming biotin ligase localization to the ER (Figure 2) and by confirming its functional activity in cell culture-based systems (Figure 3). Together, **these preliminary results provide the proof-of-concept for ER-based biotinylation.**



Figure 2. Biotin ligase co-localizes with the ER-resident protein calnexin. Green indicates calnexin. Magenta indicates v5 epitope tag (biotin ligase). Blue indicates nuclei. Arrowheads depict co-localization between v5 and calnexin. Scale bars: 25 μ m.

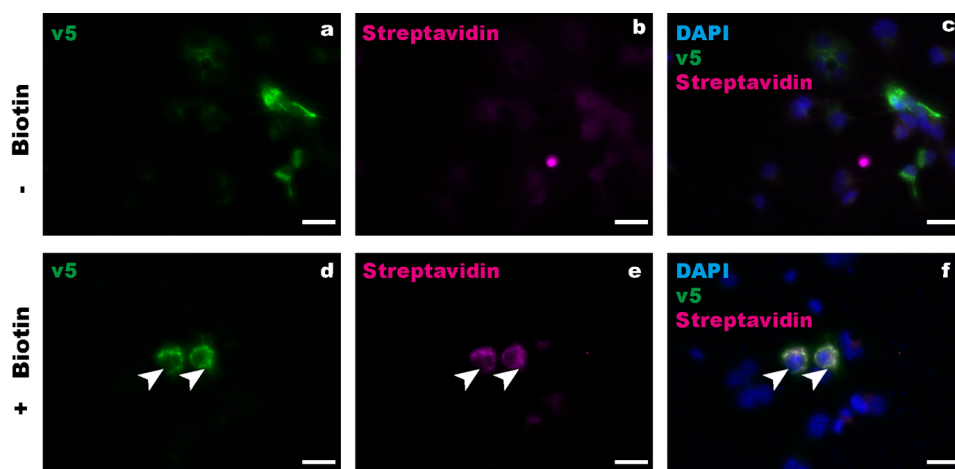


Figure 3. ER-resident biotin ligase biotinylates proteins in cell culture-based systems. Green indicates v5 epitope tag (biotin ligase). Magenta indicates streptavidin. Blue indicates nuclei. Arrowheads represent transfected cells that have biotinylated proteins. Scale bars, 25 μ m.

We are currently determining whether biotinylated proteins are secreted into the medium of cultured cell lines by pulling down biotinylated proteins from the media (using streptavidin magnetic beads) for further identification by mass spectrometry.

While these initial biotinylation experiments were carried out in cultured cell lines due to their ease of use, we plan to carry out biotinylation experiments in primary astrocyte cultures that have been used for experiments aimed at identifying astrocyte-secreted synaptogenic proteins.

We developed a protocol for purifying and growing primary astrocyte cultures from mouse (Figure 4) and rat brains (data not shown). Concretely, we isolated astrocytes from cortex and hippocampus and optimized conditions (e.g., orbital shaking, digestion conditions) to yield maximum purity of astrocytes in culture. We validated the purity of our primary astrocyte cultures by immunofluorescence and by RT-PCR, using cell type-specific markers for astrocytes but also for other cell types including oligodendrocytes, neurons and microglia (Figure 4). **With our protocol, we were able to obtain 81-98% pure astrocyte cultures from mouse brain**, encouraging us to move forward to testing our proximity labelling systems in primary astrocyte cultures.

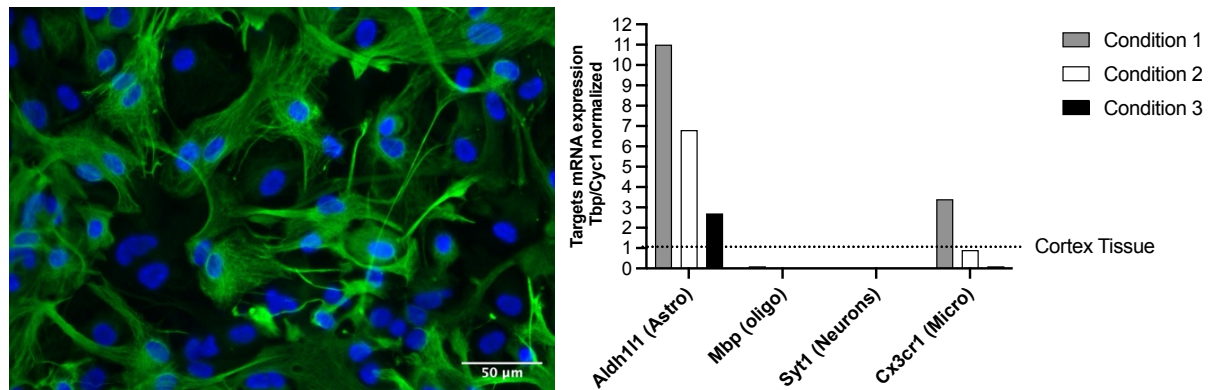


Figure 4. Primary culture of astrocytes isolated from mouse brain. **Left:** Immunofluorescence imaging of astrocytes isolated from mouse cortex. DAPI was used to stain for cell nuclei (blue) and GFAP was used as a marker for astrocytes (green). Scale bar, 50 μ m. **Right:** RT-PCR of cell type-specific markers to assess purity of primary culture: Aldh1l1 was used as a marker for astrocytes; Mbp was used as a marker for oligodendrocytes; Syt1 was used as a marker for neurons and Cx3cr1 was used as a marker for microglia. Conditions 1-3 indicate passage number of primary astrocyte cultures.

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2. Dissemination and Communication Activities

2.1 Organization of International Seminars

We continue to capitalize on the ERA Chair Holder's network of international contacts to expose the i3S research community to high-profile speakers, by inviting two speakers for the NCBio Seminar series and one for a Satellite Seminar. We maximize their presence at the i3S by 1) inviting them to interact with Ph.D. candidates in a relaxed, informal lunch; 2) give a career seminar and by 3) organizing individual meetings with researchers who are looking to collaborate and/or find scientific input into their projects. All the seminars organized and/or sponsored by the NCBio ERA Chair can be found at <https://ncbio.i3s.up.pt/outputs/scientific-outputs/>.



Figure 5. Left: Prof. Dr. Adrian Liston. **Middle:** Dr. Liston giving a talk for the NCBio Seminar series in a full auditorium. **Right:** Dr. Liston giving a career seminar.

On February 17, 2023 we invited Prof. Dr. Adrian Liston (University of Cambridge) for the **NCBio Seminar Series** entitled “Regulatory T cells in the tissues”. Dr. Adrian Liston is a Senior Group Leader at the Babraham Institute and a Senior Research Fellow at Churchill College, University of Cambridge. He co-authored several publications with Dr. Holt and is one of the co-founders of the spin-off company Aila Biotech (<https://www.ailabiotech.com/>). During his talk, Dr. Liston explained how immune function in the brain is essential for its correct development and function and how uncontrolled inflammation is a driving force behind CNS pathology (acute traumas and during chronic neurodegenerative diseases).

On May 17, 2023 we invited Dr. Nachiket Kashikar—CEO and co-founder of the European Spatial Biology Center (ESBC)—for a **Satellite Seminar** entitled “Spatial Omics at ESBC - enabling new scientific discoveries”. The aim of his talk was to introduce and offer his expertise on end-to-end spatial omics services to the i3S community. Additionally, we invited Dr. Kashikar for a career session with young scientists to share his experience and perspective of transitioning from academia to industry.

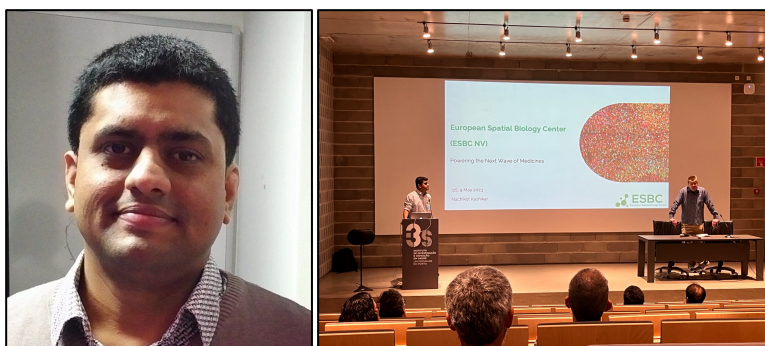


Figure 6. Left: Dr. Nachiket Kashikar. **Right:** Dr. Kashikar introducing the European Spatial Biology Center to the i3S community.

On June 16, 2023, we invited Prof. Dr. Bassem Hassan to give a talk as part of the **NCBio Seminar series**. Dr. Hassan is the Scientific Director and Deputy General Director of the Paris Brain Institute, where he also leads the Brain Development Group.

Dr. Bassem gave his talk entitled “Noise, variation and robustness in neuronal circuit development” where he showcased his team’s work on uncovering the neural basis of individuality.



Figure 7. Left: Prof. Dr. Bassem Hassan. **Right:** Dr. Hassan giving his NCBio Seminar in a full auditorium the i3S.

On October 4, 2023 we sponsored the **i3S Ph.D. Day “Brilliant Ph.D.s, brighter future: Shaping Science together!”** (<https://www.i3s.up.pt/event.php?v=273>) organized by the Ph.D. Student Community of the i3S by securing the participation of Prof. Dr. Benedikt Berninger, Professor of Developmental Neurobiology at King’s College London and a renowned specialist in neurogenesis of the mammalian brain. The title of his talk was “Engineering neurogenesis in the postnatal brain by in vivo lineage reprogramming: cellular context matters!”.



Figure 8. Left: Poster of the 6th Ph.D. Day, organized by the students of the Molecular and Cellular Biology Doctoral Program. **Right:** Keynote presentation of Prof. Dr. Benedikt Berninger.

On November 10, 2023 the NCBio project sponsored the participation of Prof. Dr. Pierre Vanderhaegen in the **i3S Neuro Day**, an annual meeting dedicated to general neurobiology themes within the Neurobiology and Neurologic Disorder program of the i3S. Dr. Vanderhaegen has done significant contributions to the field of development of the human cerebral cortex, continuously publishing in top-tier journals like Science, Nature and Cell and is the recipient of several prestigious grants, including two ERC Advanced grants (2013 and 2021).

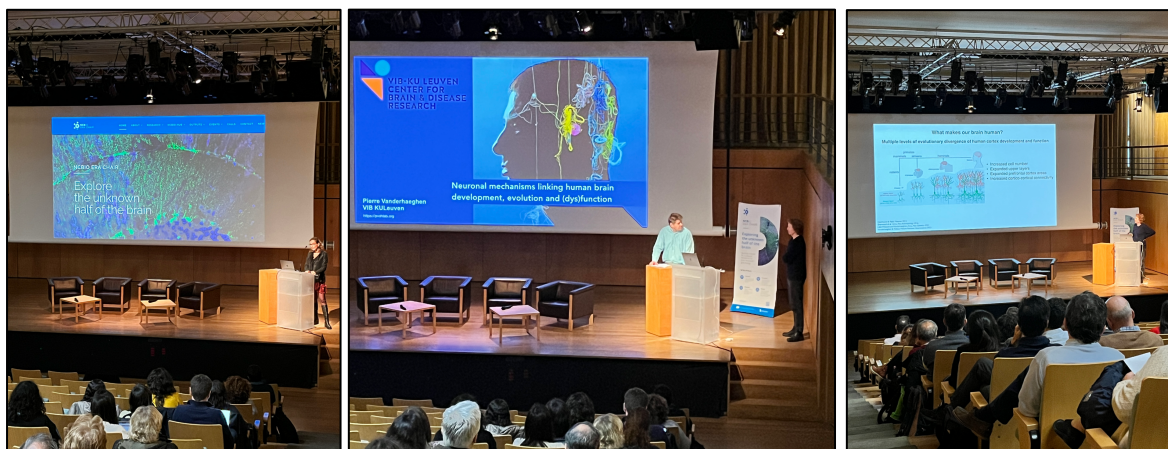


Figure 9. **Left:** The Project Coordinator of NCBio, Dr. Mónica Sousa, introducing the NCBio project and the ERA Chair Holder. **Middle:** ERA Chair Holder Dr. Matthew Holt introducing Prof. Dr. Pierre Vanderhaeghen. **Right:** Prof. Dr. Pierre Vanderhaeghen giving his talk during the i3S NeuroDay.

2.2 Public Engagement

2.2.1 World Cancer Day | 16 February 2023

Prior to joining the SBG, Dr. Simone Bessa (Senior Research Technician of the SBG) worked extensively in oncology research and was invited by the Lousada Secondary School to give a talk as part of the school's initiative to raise awareness for World Cancer Day.

Link to event: <http://www.aelousada.net/index.php/destaques/2499-dia-mundial-do-cancro-2023>



Figure 10. Dr. Simone Bessa gives a talk about cancer at the Lousada Secondary School.

2.2.2 Brain Awareness Week | 13-19 March 2023

To celebrate Brain Awareness Week, Dr. Matthew Holt challenged the Ciência Viva Science Clubs and 7th, 8th and 9th graders to become neuroscientists and discover the unknown side of the brain by producing a zine (mini magazine) about glial cells. Their original works were displayed at the i3S and the students were invited to do hands-on activities organized by several research groups.

A short video featuring Dr. Holt was produced to launch the challenge and can be found here: <https://www.youtube.com/watch?v=-i2KmYB3U58&themeRefresh=1>. Details of the competition can be found on the website of i3S's educational program "Ciência et al" (https://cienciaetal.i3s.up.pt/?page_id=8304, Portuguese only). The event was also documented on NCBio's website (<https://ncbio.i3s.up.pt/baw-2023/>).



Figure 11. The ERA Chair Holder (Dr. Holt) inviting students to explore the unknown side of the brain.



Figure 12. Celebration of the Brain Awareness Week at the i3S.

2.2.3 World Brain Day | 22 July 2023

Dr. Olga Sin (Project Manager) was featured in the i3S' social media (Instagram and Twitter/X) to promote World Brain Day. In her video, she talked about the "wonders of the brain", describing the role glial cells and how important their diversity and function are for keeping the brain healthy.

Link to Instagram: <https://www.instagram.com/p/CvAKTpotqsm/>

Link to X/Twitter: https://x.com/i3S_UPorto/status/1683413436493967360

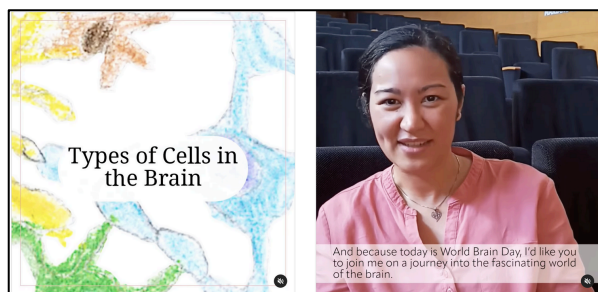


Figure 13. Snapshot of the video posted on IBMC/i3S' Instagram.

3. NCBio Stakeholder Hub

The NCBio Stakeholder Hub aims to facilitate cross-talk between IBMC/i3S basic research, local hospitals and clinics, the health industry (including pharma) and patient associations.

The NCBio Stakeholder Hub was officially launched June 20, 2023 (month 30) at the IBMC/i3S. Briefly, the ERA Chair Holder invited all 19 stakeholders who pledged their support to the NCBio ERA Chair candidacy to meet face-to-face and to share their needs and expectations as being part of the hub. The event was organized into two sessions, one dedicated to "Research and Clinics" and a second session that looked "Towards Innovation and Entrepreneurship". Each session had pitch-talks and a round table which counted with the participation of the stakeholders as well as the group leaders of the PNND and external guests, including patients and their caretakers. A dedicated report has been written and can be found in deliverable D5.1 "Report on NCBio Stakeholder Hub".



Figure 14. Family photo during the kick-off meeting of the NCBio Stakeholder Hub.

4. Achievements

4.1. Recruitment

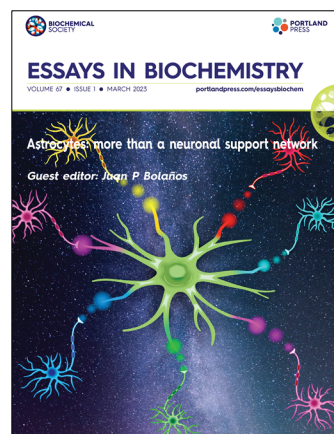
The SBG has successfully finished the recruitment of its staff. Dr. Stéphanie Castaldo was appointed **Senior Laboratory Technician** (following the departure of Dr. Mobina Alemi for re-allocation to another country). The group recruited **one Postdoctoral Researcher**, Dr. Maria Joana Pinto and **two Ph.D. students**, Mr. João Guimarães and Ms. Rafaela Seixas. Further information on the recruitment process can be found in deliverable D2.2 "Report on ERA Chair Team Recruitment". Additionally, the SBG welcomed **three students for M.Sc. internships** in the lab: Ms. Luísa Florido, a student of the Master degree in Neurobiology of the University of Porto; Mr. Carlos Pinto, a student of the Master degree in Applications in Biotechnology and Synthetic Biology of the University of Porto and Ms. Julia Oster, a student of the Master degree in Molecular Biotechnology of the University of Heidelberg. The expansion of the Research Team is proudly reflected on the ERA Chair's (<https://ncbio.i3s.up.pt/research-team/>).

4.2. Publications (Open Access)

The SBG continues to publish in several high-impact scientific journals and a summary of each work is listed below. All articles are deposited in the Open Repository of the University of Porto, the national open access repository structure (*Repositório Científico de Acesso Aberto de Portugal* – RCAAP) and OpenAIRE. The articles are listed below and † indicates senior authorship by Dr. Holt.

1. **Holt†**, *Astrocyte heterogeneity and interactions with local neural circuits*, *Essays in Biochemistry* 67, 93–106 (2023).

This review article highlights the role of astrocytes as active players in all aspects of the synaptic life cycle and discusses the importance of their heterogeneity in supporting local circuits. This review was featured as front page of *Essays in Biochemistry*.



Link to article:

<https://portlandpress.com/essaysbiochem/article/67/1/93/232536/Astrocyte-heterogeneity-and-interactions-with>

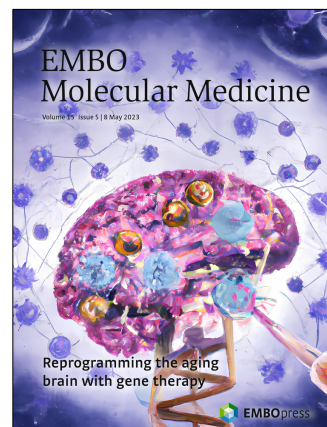
2. Viana, J.F., Machado, J.L., Abreu, D.S., Veiga, A., Barsanti, S., Tavares, G., Martins, M., Sardinha, V.M., Guerra-Gomes, S., Domingos, C., Pauletti, A., Wahis, J., Liu, C., Cali, C., Henneberger, C., **Holt, M.G.**, Oliveira, J.F. *Astrocyte structural heterogeneity in the mouse hippocampus*. *Glia* 71, 1667–1682 (2023).

This article describes a tridimensional reconstruction of astrocytes across the mouse hippocampus and confirms the presence of astrocyte heterogeneity, which appears to follow layer-specific cues and depend on the neuro-glial environment.

Link to article: <https://onlinelibrary.wiley.com/doi/10.1002/glia.24362>

3. Lemaitre, P., Tareen, S.H.K., Pasciuto, E., Mascali, L., Martirosyan, A., Callaerts-Vegh, Z., Poovathingal, S., Dooley, J., **Holt, M.G.†**, Yshii, L., Liston, A. *Molecular and cognitive signatures of ageing partially restored through synthetic delivery of IL2 to the brain*. EMBO Molecular Medicine 15, e16805 (2023).

In this article, the authors test a brain-specific IL2 delivery system for the prevention of neurological decline in aging mice and propose immune modulation as a potential path to preserving cognitive function for healthy aging. This work was featured as front page of EMBO Molecular Medicine.



Link to article: <https://www.embopress.org/doi/full/10.15252/emmm.202216805>

4. Pereira, M. J., Ayana, R., **Holt, M.G.†**, Arckens, L. *Chemogenetic manipulation of astrocyte activity at the synapse—a gateway to manage brain disease*. Frontiers in Cell and Developmental Biology 11, (2023).

This article introduces genetically engineered GPCRs as a means to manage brain disorders via modulation of astrocyte activity.

Link to article:

https://www.frontiersin.org/articles/10.3389/fcell.2023.1193130/full?utm_source=Email_to_authors&utm_medium=Email&utm_content=T1_11.5e1_author&utm_campaign=Email_publication&field=journalName=Frontiers_in_Cell_and_Developmental_Biology&id=1193130

4.3. Participation in (inter)national conferences and courses

Dr. Matthew Holt, ERA Chair Holder and Group Leader

- | | |
|----------|---|
| 14/03/23 | M.Sc. Lecture: "Understanding brain diversity: Adventures with single cell technologies", ICVS, Braga |
| 31/03/23 | Lecture: "Astrocytes: master regulators of inhibition and plasticity", University of Coimbra |
| 27/04/23 | Bioengineering Hub, Round table: "NeuroBingo – Challenges in neurodegeneration", i3S, Porto |

- 3-5/05/23 FENS Regional Meeting, Talk: "Blood-brain barrier crossing viral vector systems: unique opportunities for minimally invasive, brain wide gene delivery", Algarve
Link: <https://fensrm2023algarve.pt/scientific-programme/>
- 11-14/06/23 Member of the ATIP-Avenir Fellowship Selection Committee
- 8-11/06/23 16th European Meeting on Glial Cells in Health and Disease, Berlin
(<https://glia2023.eu/>)
Posters:
- The astrocyte $\alpha 1A$ -adrenoreceptor is an essential component of the neuromodulatory system in mouse visual cortex. Wahis, J., Akkaya, C., Kirunda, A.M., Mak, A., Zeise, K., Verhaert, J., Gasparyan, H., Hovhannisyan, S., Arckens, L., Holt, M.G.
- Structural analysis of astrocytes in different experimental conditions. Barsanti, S., Machado, J.L., Viana, J.F., Veiga, A., Abreu, D.S., Wahis, J., Holt, M., Oliveira, J.F.
- Astrocyte structural heterogeneity in the mouse hippocampus. Machado, J.L., Viana, J.F., Abreu, D.S., Barsanti, S., Martins, M., Tavares, G.P., Veiga, A.M., Sardinha, V.A.M., Domingos, C., Pauletti, A., Wahis, J., Liu, C., Cali, C., Henneberger, C., Holt, M., Oliveira, J.F.P.
- 5-6/08/23 ISN Satellite Meeting Astrocytic Control of Brain Circuits and Behavior, Talk: "Astrocyte heterogeneity and interactions with local neural circuits", Braga
Link: <https://icvs.uminho.pt/event/isn-satellite-workshop-astrocytic-control-of-brain-circuits-and-behavior/>
- 08/23 Speaker: "The emerging concept of astrocyte heterogeneity: Consequences for CNS function.", ICVS, Braga
- 18-20/09/23 Single-Domain Antibodies Conference, Talk: "AAV-based delivery of anti-BACE1 VHH alleviates pathology in an Alzheimer Disease mouse model", Paris
Link: <https://www.nanobodies2023.conferences-pasteur.org/programme>
- 11/23 Astrocytes and APOE mini-symposium, Talk: "The emerging concept of astrocyte heterogeneity: Consequences for CNS function", KU Leuven

Dr. Olga Sin, Project Manager

- 17/04/23 Norte Portugal Regional Coordination and Development Commission (CCDR-N), Norte Smart Specialization 2021-2027: Challenges and Priorities for a more Innovative Norte, Bragança
Link: <https://www.ccdr-n.pt/noticia/outros-destaques/ccdr-norte-apresenta-estrategia-de-especializacao-inteligente-do-norte-ate-2027-17-de-abril-em-braganca>
- 23/05/23 2nd National Meeting of Clinical Research and Biomedical Innovation, University of Coimbra
Link: <https://aicib.pt/2023/04/10/2o-encontro-nacional-de-investigacao-clinica-e-inovacao-biomedica-2/>
- 25/05/23 Worldwide Accelerators Rally, Matosinhos
Link: <https://thenextbigidea.pt/warm-2023-acelerou-projetos-e-startups-ligadas-a-saude/>

- 25/09/23 The Future of Science Management in Portugal: Annual Meeting of the Platform of Interface to Science (PIC)
- 29-30/09-23 Global Health Forum, Estoril Congress Center Cascais
Link: <https://globalhealth-forum.com/#home>

Dr. Stéphanie Castaldo, Senior Research Technician

Sep-Oct 2023 Participation in the course: Building and Leading Research Teams, i3S, Porto

4.4. Scientific Service

On October 7, 2022 Dr. Holt officially became part of **editorial board for the GLIA journal**, adding visibility to the IBMC/i3S.

Link: <https://onlinelibrary.wiley.com/page/journal/10981136/homepage/editorialboard.html>

As of 2023, Dr. Holt was nominated **Vice-Coordinator of the Neurobiology & Neurologic Disorders Program** of the IBMC/i3S.

Link: <https://www.i3s.up.pt/integrative-programs.php#neuro>

5. Education and Capacity Building

On March 13, 2023 Dr. Holt was invited by the i3S Ph.D. student community to participate in **"The Story Behind the PI"**, an informal meeting between the group leader and young scientists where they hear about the group leader's life stories and experiences in science, as well as the lessons learned along the way inside and outside academia into becoming a group leader (<https://www.i3s.up.pt/event.php?v=260>). The event occurred in an informal setting and counted with 15 students. Dr. Holt briefly resumed his academic career path, and the bulk of the event was followed by Q&A by the students.



Figure 15. Dr. Holt sharing his experience in becoming a principal investigator (PI) in the "Story Behind the PI".

As part of our commitment to increase the i3S' building capacity, we sponsored the training event **"Course Building and Leading Research Teams"** in collaboration with the i3S Career Development Unit. This training aims to give researchers the tools to effectively build, manage and inspire a research group in the academic enterprise. It will count with a Consultancy Service in Work Psychology from the Faculty of Psychology and Educational Sciences of the University of Porto. The course is organized in 4 sessions totaling 28 hours and will cover the following topics: 1) current work context and skills for the future; 2) culture and alignment: vision, mission and objectives; 3) Leadership; 4) team management; 5) communication and 6) negotiation and problem solving.

Link: <https://www.i3s.up.pt/training-detail.php?v=272>



Our Senior Research Technician, Dr. Stéphanie Castaldo, was nominated by the SBG to attend this course.

ANNEX 1

Essays in Biochemistry (2023) **67** 93–106
<https://doi.org/10.1042/EBC20220136>





Review Article

Astrocyte heterogeneity and interactions with local neural circuits

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OPEN  ACCESS

Astrocytes are ubiquitous within the central nervous system (CNS). These cells possess many individual processes which extend out into the neuropil, where they interact with a variety of other cell types, including neurons at synapses. Astrocytes are now known to be active players in all aspects of the synaptic life cycle, including synapse formation and elimination, synapse maturation, maintenance of synaptic homeostasis and modulation of synaptic transmission. Traditionally, astrocytes have been studied as a homogeneous group of cells. However, recent studies have uncovered a surprising degree of heterogeneity in their development and function, suggesting that astrocytes may be matched to neurons to support local circuits. Hence, a better understanding of astrocyte heterogeneity and its implications are needed to understand brain function.

Introduction

Astrocytes comprise the largest class of glial cells in the mammalian brain and are essential for central nervous system development and function [1]. Differences between astrocytes have been reported. Classically, astrocytes have been classified based on gross morphology into protoplasmic (gray matter) astrocytes and fibrous (white matter) astrocytes. In addition, there is known variability in the expression of marker proteins, such as glial fibrillary acidic protein (GFAP) and excitatory amino acid transporter 1 (EAAT1/GLAST), as well as in astrocyte numbers across brain regions. Finally, various types of specialist astrocytes have been reported in retina (Müller glia) and cerebellum (Bergmann glia). Despite these obvious differences, however, astrocytes have traditionally been viewed as a largely homogeneous cell type [2].

Recent advances in tools to specifically label and manipulate astrocytes are beginning to change this view [3]. It is now widely acknowledged that astrocytes interact with a wide variety of neuronal types [4,5] and are key players at all stages of the synaptic life cycle, including synapse formation and elimination, synapse maturation, maintenance of synaptic homeostasis and modulation of synaptic transmission [6]. This diversity of functions raises the rather obvious question of whether they are performed by all astrocytes, as presupposed, or whether functional subsets of astrocytes exist.

Evidence is now accumulating that astrocytes show profound differences in developmental origin, molecular profile, physiology and functional outputs, which combine to produce a previously unsuspected level of heterogeneity both between and within brain regions. The goal of this mini-review is 3-fold. First, I aim to discuss recent advances in our understanding of astrocyte heterogeneity: to do this, I will concentrate the discussion on protoplasmic astrocytes, principally in mouse cortex, where we arguably have the most complete picture of the astrocyte life-cycle from birth through to adulthood – although other examples will be used when instructive. Readers interested in other astrocyte types are, therefore, directed to recent review articles, which cover aspects of their development and function [7,8]. Second, I aim to discuss the evidence for a central role of neurons in determining local (protoplasmic) astrocyte

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93

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ANNEX 2

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RESEARCH ARTICLE

Astrocyte structural heterogeneity in the mouse hippocampus

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Abstract

Astrocytes are integral components of brain circuits, where they sense, process, and respond to surrounding activity, maintaining homeostasis and regulating synaptic transmission, the sum of which results in behavior modulation. These interactions are possible due to their complex morphology, composed of a tree-like structure of processes to cover defined territories ramifying in a mesh-like system of fine leaflets unresolved by conventional optic microscopy. While recent reports devoted more attention to leaflets and their dynamic interactions with synapses, our knowledge about the tree-like "backbone" structure in physiological conditions is incomplete. Recent transcriptomic studies described astrocyte molecular diversity, suggesting structural heterogeneity in regions such as the hippocampus, which is crucial for cognitive and emotional behaviors. In this study, we carried out the structural analysis of astrocytes across the hippocampal subfields of *Cornu Ammonis* area 1 (CA1) and dentate gyrus in the dorsoventral axis. We found that astrocytes display heterogeneity across the hippocampal subfields, which is conserved along the dorsoventral axis. We further found that astrocytes appear to contribute in an exocytosis-dependent manner to a signaling loop that maintains the backbone structure. These findings reveal

João Filipe Viana and João Luís Machado contributed equally to this study.

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ANNEX 3

Article

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Molecular Medicine

Molecular and cognitive signatures of ageing partially restored through synthetic delivery of IL2 to the brain

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Abstract

Cognitive decline is a common pathological outcome during aging, with an ill-defined molecular and cellular basis. In recent years, the concept of inflammaging, defined as a low-grade inflammation increasing with age, has emerged. Infiltrating T cells accumulate in the brain with age and may contribute to the amplification of inflammatory cascades and disruptions to the neurogenic niche observed with age. Recently, a small resident population of regulatory T cells has been identified in the brain, and the capacity of IL2-mediated expansion of this population to counter neuroinflammatory disease has been demonstrated. Here, we test a brain-specific IL2 delivery system for the prevention of neurological decline in aging mice. We identify the molecular hallmarks of aging in the brain glial compartments and identify partial restoration of this signature through IL2 treatment. At a behavioral level, brain IL2 delivery prevented the age-induced defect in spatial learning, without improving the general decline in motor skill or arousal. These results identify immune modulation as a potential path to preserving cognitive function for healthy aging.

Keywords Aging; Brain; Gene Therapy; Interleukin 2; Regulatory T cells
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Introduction

Aging is an irreversible process associated with physical deterioration and hence especially noticeable in organs containing mainly postmitotic cells, such as the brain. The progressive decline in memory, orientation, attention, motivation, and cognition seen in humans is thought to stem largely from aging-related processes (Hof & Morrison, 2004). Although cognitive aging research generally focuses on neurons, which are widely held to be the computational units of the brain, there is an increasing realization that reciprocal interactions between neurons and non-neuronal cells (collectively referred to as glia) play key roles in all aspects of nervous system function. These include the regulation of synapse formation and function by astrocytes, as well as myelination and provision of trophic support to neurons by oligodendrocytes. Once thought of as solely immune cells, which protect the CNS against injury and disease, there is increasing evidence that the same systems used by microglia to remove invading pathogens from the brain have also been co-opted to remove weak and/or inappropriate neuronal synapses, establishing precise patterns of neuronal connectivity in the brain (Allen & Lyons, 2018). It should not be surprising, therefore, that aging-related cognitive decline has been associated with several molecular processes, including chronic inflammation, impaired autophagy, macromolecular damage, and mitochondrial dysfunction and senescence (Hou *et al*, 2019), which impact the function of all CNS cell types (Jurk *et al*, 2012; Bussian *et al*, 2018; Chinta *et al*, 2018; Ogrodnik *et al*, 2019).

In addition to the shared cellular phenotypes of aging, unique phenotypes emerge among the individual glial cell types with aging.

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Chemogenetic manipulation of astrocyte activity at the synapse— a gateway to manage brain disease

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Astrocytes are the major glial cell type in the central nervous system (CNS). Initially regarded as supportive cells, it is now recognized that this highly heterogeneous cell population is an indispensable modulator of brain development and function. Astrocytes secrete neuroactive molecules that regulate synapse formation and maturation. They also express hundreds of G protein-coupled receptors (GPCRs) that, once activated by neurotransmitters, trigger intracellular signalling pathways that can trigger the release of gliotransmitters which, in turn, modulate synaptic transmission and neuroplasticity. Considering this, it is not surprising that astrocytic dysfunction, leading to synaptic impairment, is consistently described as a factor in brain diseases, whether they emerge early or late in life due to genetic or environmental factors. Here, we provide an overview of the literature showing that activation of genetically engineered GPCRs, known as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), to specifically modulate astrocyte activity partially mimics endogenous signalling pathways in astrocytes and improves neuronal function and behavior in normal animals and disease models. Therefore, we propose that expressing these genetically engineered GPCRs in astrocytes could be a promising strategy to explore (new) signalling pathways which can be used to manage brain disorders. The precise molecular, functional and behavioral effects of this type of manipulation, however, differ depending on the DREADD receptor used, targeted brain region and timing of the intervention, between healthy and disease conditions. This is likely a reflection of regional and disease/disease progression-associated astrocyte heterogeneity. Therefore, a thorough investigation of the effects of such astrocyte manipulation(s) must be conducted considering the specific cellular and molecular environment characteristic of each disease and disease stage before this has therapeutic applicability.

KEYWORDS
astrocytes, synaptogenesis, synaptic plasticity, heterogeneity, DREADDs, CNS disease

Introduction

The central nervous system (CNS) is a highly diverse cellular environment, where neurons are surrounded by a multitude of cell types, including astrocytes, microglia, oligodendrocytes, and ependymal cells, which are collectively known as glial cells. For many years, brain function and behavioral output were thought to depend exclusively on