

NCBio
ERA CHAIR

D3.4 Neural Cell Biology Group's annual activity report 3



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Neural Cell Biology Group's annual activity report 3

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Abbreviations and Acronyms

Abbreviation Acronym	Definition
ASD	Autism Spectrum Disorders
APPDA-Norte	Portuguese Association for Developmental Disorders and Autism Norte
BBB	Blood-Brain Barrier
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

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Executive Summary

The ERA Chair Team (also known as the Synapse Biology Group) has done considerable progress in the last 10 months (from month 36 until month 45).

As we continued our focus on the role of astrocyte heterogeneity in differential synapse formation, we invested heavily in biotin-based proximity labeling technologies to capture proteins for subsequent identification using mass-spectrometry. We have successfully developed ER-resident systems allowing selective labeling of proteins in the astrocyte secretory pathway, and systems expressed at the cell surface for local biotinylation of astrocytic plasma membrane proteins (section 1).

During the reporting period, the ERA Chair Team published 4 original research articles, 1 review article and 2 book chapters (section 4.2).

The ERA Chair Team has also successfully won three fellowships. Dr. Maria Joana Pinto (postdoctoral researcher) was awarded €50,000.00 for an Exploratory Research Project by the Portuguese Foundation for Science and Technology (FCT) and both our Ph.D. candidates Ms. Rafaela Seixas and Mr. João Guimarães were awarded FCT Ph.D. Fellowships. More details on section 4.4.

We have continued bringing high-profile, international experts in the glia and neuro fields. The NCBio ERA Chair Seminar Series has been very popular in terms of capacity building and knowledge transfer to the research community of the IBMC/i3S. Examples include Prof. Dr. Wim Annaert (KU Leuven, Belgium), Prof. Dr. Keith Murai (McGill University, Canada) and Prof. Dr. Nils Brose (Max Planck Institute for Multidisciplinary Sciences, Germany), who won an ERC Advanced grant and two ERC Synergy grants. The popularity of these seminars has spread to others Portuguese research institutes, namely the Center for Neuroscience and Cell Biology (CNC-UC, University of Coimbra) and the Life and Health Sciences Research Institute (ICVS, University of Minho). In fact, talks are currently underway with Dr. Cristina Marquez (ERA Chair Holder at CNC-UC) to expand this initiative and make these seminars bilateral. More details on section 2.1.

Finally, the ERA Chair Team has been actively engaged in outreach activities. Examples include participation in a neuroscience podcast, the Brain Awareness Week 2024 and the European Researchers' Night. We currently have two lab members as ambassadors for the IBMC/i3S educational program "Ciência et al" (section 2.2).

1. Research Activities

Assessing the role of astrocyte heterogeneity in differential synapse formation.

During the specified work period, the Synapse Biology Group continued to pursue its interest in astrocyte heterogeneity and how it affects local circuit formation and function (Batiuk et al., 2020; Bayraktar et al., 2020; Holt, 2023). The principal work completed during this period was a major bioinformatics study integrating data from multiple single cell sequencing studies to generate the first hierarchical classification for astrocyte subtypes throughout the brain (in preparation).

In a recent review article (Holt, 2023), we proposed that heterogeneity likely occurs at multiple cellular levels, which impact function. At the synaptic level, this likely occurs at the level of individual astrocyte peripheral processes, involving both secreted pro-synaptogenic proteins and cell adhesion molecules (Holt, 2023). To fully investigate the mechanisms underlying differential synapse formation and function, the group has invested heavily in biotin-based proximity labeling technologies, allowing subsequent streptavidin-based recovery of proteins for identification using mass-spectrometry. To date, we have developed ER-resident systems allowing selective labeling of proteins in the astrocyte secretory pathway (Figure 1), as well as systems expressed at the cell surface for local biotinylation of astrocytic plasma membrane proteins (Figure 2). These experiments are ongoing with multiple independent repeats necessary to ensure we achieve full proteomic coverage.

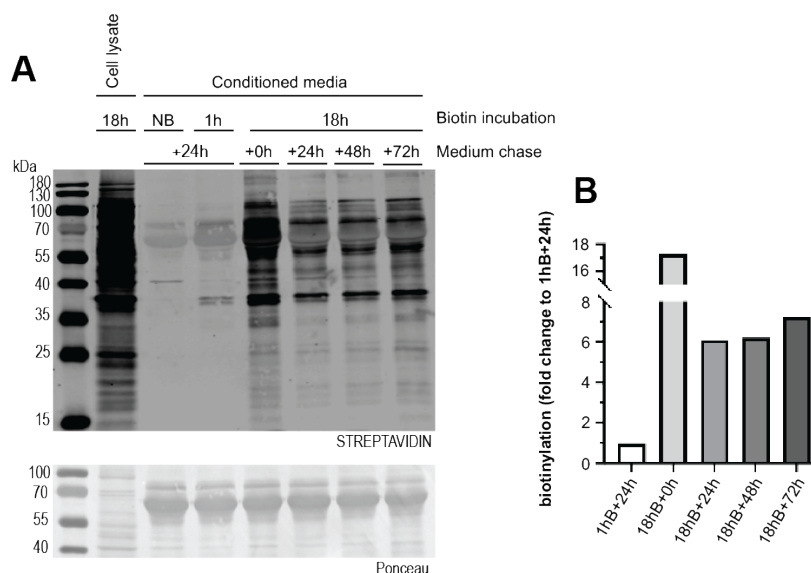


Figure 1. Biotinylation of secreted proteins using an ER-resident biotin ligase. (A) C8D1A cells were transfected with ER-directed biotin ligase. The day after transfection, cells underwent a 18 h pulse of 100 μ M biotin, after which lysates and medium were collected. Other sets of cells were left to secrete protein into the medium for either 24, 48 or 72 h after this pulse. Conditioned media were collected. Other cells were used to collect the conditioned medium from a no-biotin (negative) control and following a 1 h biotin pulse followed by a 24 h chase. Protein was extracted from lysates, and media samples were concentrated. Samples were run on an SDS-PAGE, transferred to a membrane, and blotted with streptavidin-647. **(B)** Quantification of the streptavidin signal. Streptavidin signal was normalized with Ponceau staining, and results are shown as a fold change of the 1-hour pulse and 24 h chase condition.

Cell Surface Biotin Ligase

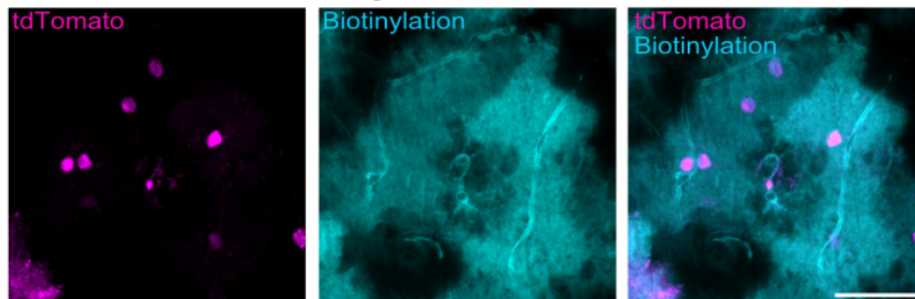


Figure 2. Plasma membrane expression of biotin ligase allows the biotinylation of cell surface proteins, as revealed by streptavidin-647 staining. Scale bar, 50 μm .

In addition, during the reporting period, we have been active implementing workflows necessary for the follow-up characterization of proteins identified in our mass-spectrometry experiments. These include an *in vitro* synapse assembly assay to screen the synaptogenic potential of secreted proteins, endogenous tagging methodologies to facilitate protein localization studies and CRISPR-Cas9 based loss-of-function approaches to allow functional characterization.

References

- Batiuk, M. Y., Martirosyan, A., Wahis, J., de Vin, F., Marneffe, C., Kusserow, C., . . . Holt, M. G. (2020). Identification of region-specific astrocyte subtypes at single cell resolution. *Nat Commun*, *11*(1), 1220. doi:10.1038/s41467-019-14198-8
- Bayraktar, O. A., Bartels, T., Holmqvist, S., Kleshchevnikov, V., Martirosyan, A., Polioudakis, D., . . . Rowitch, D. H. (2020). Astrocyte layers in the mammalian cerebral cortex revealed by a single-cell in situ transcriptomic map. *Nat Neurosci*, *23*(4), 500-509. doi:10.1038/s41593-020-0602-1
- Holt, M. G. (2023). Astrocyte heterogeneity and interactions with local neural circuits. *Essays Biochem*, *67*(1), 93-106. doi:10.1042/EBC20220136

2. Dissemination and Communication Activities

2.1 Organization of International Seminars

In month 36, Dr. Holt invited Dr. Vanessa Coelho-Santos to give a Satellite Seminar at the IBMC/i3S. Dr. Coelho-Santos is a young group leader from the University of Coimbra who has recently started her own research group and whom Dr. Holt has been mentoring informally (e.g., consultation during the preparation of an ERC Starting grant application).

In 2024, Dr. Holt hosted Prof. Dr. Wim Annaert, an expert in Alzheimer's disease from KU Leuven (Belgium), Prof. Dr. Keith Murai, an expert in astrocyte biology from McGill University (Canada) and Prof. Dr. Nils Brose, expert in neuronal development and synaptogenesis and director at the Max Planck Institute for Multidisciplinary Sciences (Germany) who has won an ERC Advanced grant and two ERC Synergy grants.

Table 1. Summary of speakers invited by Dr. Holt to the IBMC/i3S during the reporting period.

Month	Speaker	Expertise	Event
36	Vanessa Coelho-Santos University of Coimbra (PT)	Neurogliovascular system	Satellite Seminar
39	Wim Annaert KU Leuven (BE)	Alzheimer's disease	NCBio ERA Chair Seminar
40	Keith Murai McGill University Health Centre (CA)	Astrocyte biology	NCBio ERA Chair Seminar
45	Nils Brose MPI for Multidisciplinary Sciences (DE)	Neurodevelopment	NCBio ERA Chair Seminar



Figure 3. (Inter)national, high-profile speakers invited by Dr. Holt for the ERA Chair Seminar Series or Satellite Talks. Top left: Talk by Dr. Vanessa Coelho-Santos. Top right: Talk by Prof. Dr. Wim Annaert. Bottom left: Prof. Dr. Keith Murai. Bottom right: Talk by Prof. Dr. Nils Brose.

To maximize capacity building in the IBMC/i3S community, all guest speakers were set up in **one-to-one meetings with researchers** who wished to discuss and get feedback on their individual projects. Additionally, a lunch with the guest speakers was organized exclusively for young scientists (Ph.D. candidates and post-docs) to give them the time and space to ask about career advice in an informal setting.

The NCBio ERA Chair Seminar series are having an impact beyond the IBMC/i3S and shaping the research landscape in the center-northern region of Portugal. Dr. Holt has taken the initiative to **live stream the seminars** (upon prior consent from the guest speakers) to other Portuguese research institutes, namely the CNC-UC (University of Coimbra) and the ICVS (University of Minho). The caliber of guest speakers brought by Dr. Holt means that we frequently include researchers from other institutes and universities (CNC-UC, ICVS, Faculty of Medicine of the University of Porto) who travel to IBMC/i3S for one-to-one sessions with guest speakers. Examples include Dr. João Oliveira (ICVS, U. Minho) and Dr. Patrícia Monteiro (Faculdade de Medicina, U. Porto). In fact, talks are currently underway with Dr. Cristina Marquez (ERA Chair Holder at CNC-UC) to expand this initiative and make these seminars bilateral.

2.2 Public Engagement

2.2.1 Brain Gain

In month 39, Dr. Pinto was invited to the popular science podcast "Brain Gain", where she talked about microglia, one of the glial cell types in the brain (<https://braingain.pt/online2024/>).



Figure 4. Poster for the Podcast Brain Gain where Dr. Pinto talked about microglia (Portuguese only).

2.2.2 Brain Awareness Week

In month 39, the ERA Chair Team celebrated the 2024 Brain Awareness Week by inviting young adults with autism spectrum disorders (ASD) from the Portuguese Association for Developmental Disorders and Autism (APPDA-Norte) for a guided tour at the IBMC/i3S. Our guests looked at brain slices from different transgenic model organisms under the fluorescence microscope and learned about how they are used in research (<https://ncbio.i3s.up.pt/brain-awareness-week-2024-celebrating-different-minds/>). This event also involved the contribution of other research groups

of the IBMC/i3S, namely the Genetics of Cognitive Dysfunction Group and Cell Division and Genomic Instability Group.



Figure 5. Celebrating BAW 2024 with young adults with ASD from the APPDA-Norte association.

2.2.3 European Researchers' Night

In month 45, the ERA Chair Team participated in the European Researchers' Night held at IBMC/i3S (Figure 29). The team set up a stall entitled "Guardians of the Brain" and organized several interactive and educational activities that included memory games and looking at "fluorescent" astrocytes and neurons in brain slices under the microscope. The event was featured on the University of Porto's website: <https://noticias.up.pt/foto-da-semana/a-noite-no-laboratorio/>.



Figure 6. Participation of the ERA Chair Team in the 2024 European Researchers' Night at IBMC/i3S.

2.2.2 Ambassadors for Ciência et al Educational Program at IBMC/i3S

Dr. Ana Eufrásio (Postdoc) and Ms. Rafaela Seixas (Ph.D. student) became ambassadors for the 'Ciência et al' educational program at IBMC/i3S, through which they introduce their scientific

research to elementary and high school students. More information can be found at https://cienciaetal.i3s.up.pt/?page_id=1810.

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.

3.1 Collaboration with NCBio stakeholder: APPDA-Norte

Given Dr. Holt's specific research interests on the relationship between astrocyte-mediated synapse formation and ASD (as alluded in section 1), Dr. Holt invited the APPDA-Norte to become part of the NCBio Stakeholder Hub (month 34). This partnership already resulted in **three joint grant applications** to the laCaixa Foundation (2023 and 2024 calls) and the FCT's Scientific Research and Technology Development (SR&TD) Projects Call in all Scientific Domains 2023 (results to be announced). This partnership was further reinforced by inviting APPDA-Norte to celebrate the 2024 Brain Awareness Week with a guided tour at the IBMC/i3S (section 2.2.2).

3.2 Promoting collaborations with the pharma industry

We have been working with the IBMC/i3S's Coordinator for Translational Research & Industry Partnerships to survey projects within the Program in Neurobiology and Neurological Disorders (PNND) that have the potential to **attract investment from pharma companies** and/or to form the basis of joint application for competitive grant funding. A preliminary survey has already been made to identify IBMC/i3S research groups whose research interests match pharma companies with relevant R&D pipelines in Portugal.

3.3 Organization of the first Portuguese Workshop in Blood-Brain Barrier-Crossing Strategies

We are capitalizing on Dr. Holt's affiliation to Aila Biotech (which was seen as a very strong positive in the last audit by the European Commission) to set up a **national working group dedicated to delivery systems that cross the blood-brain barrier (BBB)**. Dr. Holt is collaborating with ViraVector—a national research infrastructure specializing in viral vector-based technologies that is located in Coimbra—to bring experts in AAVs, nanoparticles, nanobodies and other technologies that deliver drugs/biologics across the BBB. The workshop will take place in month 46 and the expectation is to form a working group to apply for a COST Action (Horizon Europe).

4. Achievements

4.1 Recruitment

Currently, the ERA Chair Team is composed by:

- 1 Project Manager (Dr. Olga Sin);
- 1 Senior Research Technician (Dr. Simone Bessa);
- 2 Post-Doctoral Researchers (Dr. Maria Pinto and Dr. Ana Eufrásio);
- 2 Ph.D. Students (Mr. João Guimarães and Ms. Rafaela Seixas).

During the reporting period, Dr. Stéphanie Castaldo resigned from her position as Senior Laboratory Technician due to relocation to another part of the country. Dr. Ana Eufrásio was hired as a postdoctoral researcher and joined the group in month 44. A full description of the Research Team is proudly reflected on the ERA Chair's (<https://ncbio.i3s.up.pt/research-team/>).

Additionally, the ERA Chair Team frequently welcomed (inter)national students for internships or stays in the lab. An overview of the students visiting the lab is shown in the Table 2.

Table 2. Overview of students who performed/are performing internships in the SBG.

Student	Degree	Start Month	End Month
Luísa Florido	M.Sc. in Neurobiology, U. Porto	32	45
Carlos Pinto	M.Sc. in Applications in Biotechnology and Synthetic Biology, U. Porto	33	45
Julia Oster	M.Sc. in in Molecular Biotechnology of the University of Heidelberg	35	39
Domenico Natale	Arenberg Doctoral School, KU Leuven	38	-
Ahmed Obaid	M.Sc. in Molecular Life Sciences, University of Hamburg	42	45
Helia Naderi	M.Sc. in Biomedical Sciences, KU Leuven	43	-

Ms. Florido and Mr. Pinto successfully graduated their master thesis with a score of 18 (out of 20). Ms. Oster was given a score of 1 (out of 6, German grade equivalent to "Excellent") for her internship report and has returned to Germany to continue her master degree studies.

4.2 Publications (Open Access)

During the reporting period (month 36 until month 45), 4 original research articles, 1 review article and 2 book chapters have been published. 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.

- Martirosyan, A., Ansari, R., Pestana, F., Hebestreit, K., Gasparyan, H., Aleksanyan, R., Hnatova, S., Poovathingal, S., Marneffe, C., Thal, D.R., Kottick, A., Hanson-Smith, V.J., Guelfi, S., Plumbly, W., Belgard, T.G., Metzakopian, E., **Holt, M.G.†** *Unravelling cell type-specific responses to Parkinson's Disease at single cell resolution*. *Mol Neurodegeneration* 19, 7 (2024).

Link to article:

<https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/s13024-023-00699-0>

- Hendrix, E., Vyver, M.V., **Holt, M.G.**, Smolders, I. *Regulatory T cells as a possible new target in epilepsy?* *Epilepsia* 00: 1–11 (2024).

Link to article: <https://doi.org/10.1111/epi.18038>

- Bielefeld, P., Martirosyan, A., Apresyan, A., Meerhoff, G., Pestana, F., Poovathingal, S., Reijners, N., Koning, W., Clement, R.A., Van de Veen, I., Toledo, E., Durá, I., Hovhannisyan, S., Nilges, B., Bogdoll, A., Kashikar, N., Lucassen, P.J., Belgard, T.G., Encinas, J.M., **Holt, M.G.†**, Fitzsimons, C.P. *Traumatic brain injury promotes neurogenesis at the cost of astroglialogenesis in the adult hippocampus of male mice*. *Nat Commun* 15, 5222 (2024).

Link to article: <https://doi.org/10.1038/s41467-024-49299-6>

- Wahis J., Akkaya, C., Kirunda, A.M., Mak, A., Zeise, K., Verhaert, J., Gasparyan, H., Hovhannisyan, S., **Holt M.G.†** *The astrocyte $\alpha 1A$ -adrenoreceptor is a key component of the neuromodulatory system in mouse visual cortex*. *Glia*, 1–19 (2024).

Link to article: <https://onlinelibrary.wiley.com/doi/10.1002/glia.24591>

- Zeng, H., Hendriks, L.E.L., Belderbos, J., Brandts, L., Compter, I., Dubois, L., **Holt, M.G.**, Houben, R., Schagen, S., Zhang, X., Prezzemolo, T., De Ruyscher, D., *Association of Serum Biomarkers with Neurocognitive Decline After PCI in Small Cell Lung Cancer: An Exploratory Study of the Phase III NCT01780675 Trial*, *Clinical Lung Cancer*, Volume 25, Issue 7, 653 - 659.e1

Link to article: <https://doi.org/10.1016/j.clcc.2024.08.008>

Book chapters

- Natale, D., **Holt, M.G.†** "Retro-orbital delivery of AAVs for CNS wide astrocyte targeting" in "Astrocytes" (Ed: Barbara Di-Benedetto), Springer Protocols, Humana Press, 2024.
- Pestana, F., Belgard, T.G., Voet, T., **Holt, M.G.†** "Studying the roles of astrocytes at synapses with single cell transcriptomics" in "New Technologies for Glutamate Interaction: Neurons and Glia" (Ed: Maria Kukley), Springer Protocols, Humana Press, 2024.

4.3 Participation in (inter)national conferences and courses

The ERA Chair Team participated in the (inter)national meetings listed below in Table 3. Every talk and poster included the NCBio brand as well as the EU flag and funding statement acknowledging the source of funding. The information below is also given on the NCBio website (<https://ncbio.i3s.up.pt/outputs/scientific-outputs/>).

Table 3. List of (inter)national conferences attended by members of the ERA Chair Team.

Month	Name of event	Location	Presentation	Presenter
38	54th Meeting of the Portuguese Society of Pharmacology	Lisbon, PT	Talk	R. Seixas
38	Invited talk at the Institut du Cerveau et de la Moelle Épinrière	Paris, FR	Talk	M. Holt

39	Gordon Research Conference: Antibody Biology and Engineering	Barga, IT	Talk	M. Holt
40	VIII Portuguese Glial Network	Braga, IT	Poster	D. Natale
40	Invited talk at the University of Padova	Padova, IT	Talk	M. Holt
40	International Astrocyte Summer School	Bertinoro, IT	Talk	M. Holt
41	V Symposium of Biochemistry	Braga, PT	Talk	R. Seixas
41	IJUP 2024, 17th edition	Porto, PT	Poster	L. Florido
43	Sissa (International School for Advanced Studies)	Trieste, IT	Talk	M. Holt
45	2024 Annual IBMC/i3S Neuro Day	Porto, PT	Talk	D. Natale

The ERA Chair Team members have been attending training courses as part of their individual capacity building plan to address gaps in knowledge and competences. A summary of the training courses attended by members of the ERA Chair Team is summarized in Table 4.

Table 4. Training courses or workshops attended by ERA Chair Team members during the reporting period.

Month(s)	Training Course/Workshop	ERA Chair Team Member(s)
36	Principles of High-Throughput Proteomics Research; CIIMAR and Escola Superior de Tecnologia da Saúde do Porto, PT	Luísa Florido (M.Sc. student), João Guimarães (Ph.D. student)
37-38	Advanced Course on Synapse Assembly, Function and Plasticity; Universidade de Coimbra, PT	Rafaela Seixas (Ph.D. student)
39	Health-NCP-Net 3.0 Workshop on "Unlocking Success: Insider Insights for Winning Horizon Europe Health Proposals" (online)	Olga Sin (Project Manager)
40	International Astrocyte School Bertinoro, IT	Domenico Natale (external Ph.D. student)
41	Introduction to Python and Machine Learning for the Biosciences; IBMC/i3S, PT	João Guimarães (Ph.D. student)
42	LifeSaver Technology Transfer Workshops; IBMC/i3S, PT	Olga Sin (Project Manager)
43	Mass Spectrometry-based Proteomics; IBMC/i3S, PT	Rafaela Seixas (Ph.D. student)
43	16 th European Summer School "Advanced Proteomics"; Bressanone, IT	Domenico Natale (external Ph.D. student)

4.4 Application to Funding

The ERA Chair Team has been actively applying to competitive European funding programs to secure the sustainability of the team.

Dr. Holt applied to the laCaixa Health Call 2023 (scored just below interview cut off and will submit again to the 2024 call), to the FCT's Scientific Research and Technology Development (SR&TD) Projects Call in all Scientific Domains 2023 (results to be announced).

Dr. Pinto (post-doctoral researcher) was awarded **€50,000.00 for an Exploratory Research Project** (Fundação para a Ciência e Tecnologia).

Mr. Guimarães applied to the Boehringer Ingelheim Fonds Ph.D. program (scored below threshold). Mr. Guimarães and Ms. Seixas applied to the **FCT's individual Ph.D. Fellowships** and **were both successfully awarded this fellowship**.

5. Scientific Service

In month 41, Dr. Holt was invited to serve in the Neuroscience Committee for the CNRS/ATIP Avenir Fellowship Selection.

During the reporting period, Dr. Holt was frequently invited to review manuscripts for peer-reviewed scientific journals including Molecular Therapy (Nucleic Acids), Nature Communications, Nature Neuroscience and Glia.

Dr. Holt served as evaluator for several funding agencies including the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation); the Agence nationale de la recherche (ANR, French National Research Agency); the ZonMw Dementie (Dutch Dementia Research Programme), and the Schweizerischer Nationalfonds (SNF, Swiss National Funds).

Finally, Dr. Holt is currently serving as **Vice-Coordinator of the Neurobiology & Neurologic Disorders Program** of the IBMC/i3S and is a **member of the institute's Scientific Management Board**.

6. Education and Capacity Building

Dr. Holt contributed to re-designing neuroscience modules in the Molecular and Cellular Biology (MCB) and the Neuroscience (PDN) doctoral programs of the University of Porto. Specifically, the neuroscience modules now **integrate more lectures dedicated to glia biology**, including:

- General considerations on the glial system: types, morphology, ultra-structure and basic mechanisms
- Neuron-glial interaction
- Astrocytes and the tripartite synapse
- Glial cells and myelination
- Microglia and neuroinflammation.

Dr. Holt's lecture on "**Astrocytes and the Tripartite Synapse**" received very positive feedback from the director of the PDN, Dr. Vasco Galhardo (group leader at the IBMC/i3S and Faculty of Medicine at U. Porto).

In 2024, Dr. Holt was invited to give lectures at the M.Sc. Molecular Genetics master program (University of Minho) and act as faculty for the Synapse Assembly, Structure, and Function Ph.D. course (University of Coimbra) and the annual International Astrocyte School (Bertinoro, Italy).

In addition to lecturing duties, Dr. Holt **mentors young scientists**. Dr. Holt is mentor to Ph.D. students Mr. Guimarães and Ms. Seixas and to M.Sc. students Ms. Naderi and Mr. Ribeiro. Until month 45, Dr. Holt was also mentor to M.Sc. students Ms. Florido, Mr. Pinto and Mr. Obaid. Dr. Holt was also mentor to Dr. Clive Jabangwe, whom he helped prepare for his postdoctoral career at the University of Nottingham (UK).

Dr. Holt is currently part of several thesis committees, including 1) Ms. Sarah Silver, a Ph.D. candidate of the Molecular and Cellular Biotechnology Applied to Health Sciences (BiotechHealth) Doctoral Program at ICBAS in Dr. Paulo Aguiar's lab (Neuroengineering and Computational Neuroscience Group); 2) Ms. Georgia Athanasopoulou, a Ph.D. candidate in Dr. Ana Paula Pêgo's lab (NanoBiomaterials for Targeted Therapies group); 3) Mr. Natale, 4) Mr. Francisco Pestana and 5) Ms. Maria Pereira, all Ph.D. candidates from KU Leuven (Belgium); 6) Ms. Ashley Bomin Lee (Copenhagen); 7) Ms. Sara Pinto (Institute for Molecular Medicine, Lisbon); 8) Giulia Favetta (University of Padova, Italy).

Dr. Holt already served as evaluator in the thesis committee of Ms. Eva Carvalho from Dr. Ana Paula Pêgo's lab. He currently co-supervises Ms. Evelin Hendrix with Prof. Dr. Ilse Smolders (expert in epilepsy at Vrije Universiteit Brussel, Belgium).