NCBIO
ERA CHAIR

D3.2 Neural Cell Biology Group's annual activity report 1

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Neural Cell Biology Group's annual activity report 1

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Coordinator	Mónica Sousa (IBMC)
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Abbreviations and Acronyms

Abbreviation Acronym	Definition
AAV	Adeno-associated virus
CNS	Central nervous system
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

Executive Summary

Work package 3 (WP3) was designed to enable the scientific installation and integration of the ERA Chair Holder (Dr. Matthew Holt) and his team, as well as develop and implement the R&I strategy of the new Neural Cell Biology (NCBio) group to build up the R&I capacity of IBMC/i3S. The overall objective of WP3 is to create an inspiring, attractive, and competitive environment, characterized by the development of stimulating research excellence, with a high added-value at IBMC/i3S. The scientific work of the ERA Chair research group will aim to change the R&I landscape of the Program in Neurobiology and Neurologic Disorders (PNND) area of IBMC/i3S, increase its international and national visibility and attractiveness.

This report details the installation and integration of Dr. Matthew Holt and his newly formed research group—officially designated **Synapse Biology group**—at the IBMC/i3S.

1. Activities

1.1 Organization of International Meetings

Dr. Matthew Holt contributed to the glia community of the IBMC/i3S (Drs. João Relvas, Paulo Aguiar, Teresa Summavielle and Ana Pêgo) by sponsoring and organizing the **Portuguese Glia meeting**, held in Porto in October 2022. This meeting capitalized on the ERA Chair's scientific network within the glia field to bring together experts from across Europe to discuss glial cell development and function (Annex 1). A similar meeting (**i3S Neuro Day**) was organized around general neurobiology themes in November 2022 for the benefit of the entire Neurobiology and Neurologic Disorder program (Annex 2).

1.2 Evaluation Committees

Dr. Matthew Holt has been frequently invited to participate as an evaluator in several international degree-awarding committees listed below.

Ph.D. committees:

1. Nick Benfey, Montreal Neurological Institute in McGill University, Canada
2. Félicia Jeannelle, Laboratoire National de Santé, Luxembourg
3. Katarina Dittlau, Katholieke Universiteit Leuven, Belgium
4. Jasper Janssens, Katholieke Universiteit Leuven, Belgium
5. Hannah Walgrave, Katholieke Universiteit Leuven, Belgium

M.Sc. committees:

6. Hayk Gasparyan, Yerevan State University, Armenia
7. Sargis Hovhannisyan, Yerevan State University, Armenia

B.Sc. committees:

8. Razmik Aleksanyan, Yerevan State University, Armenia

2. Achievements

2.1 Recruitment

A pivotal aspect for the integration of Dr. Matthew Holt at the IBMC/i3S was the formation of the NCBio research group, officially designated as **Synapse Biology group**. Dr. Olga Sin was appointed as the **Project Manager** (contract initiated on month 20). Dr. Olga Sin has a strong background in Neuroscience with proven record of neuroscience research, grant funding and outreach. This was followed by the recruitment of **two Senior Laboratory Technicians**, Drs. Mobina Alemi (contract initiated on month 24) and Simone Bessa Garcia (contract initiated on month 25). Mobina Alemi is a neuroscientist by training with a strong background with *in vivo* mouse work; Simone Bessa is an oncologist with a strong background in molecular biology.

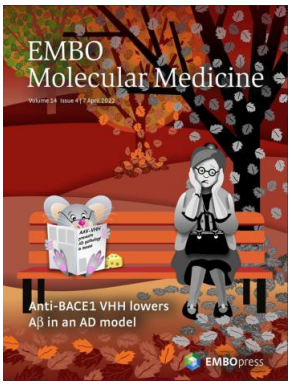
2.2 Publication (Open Access)

The research performed by the Synapse Biology group has been published in several high-impact scientific journals and a summary of each work is listed below.

2022:

1. Marino, M., et al., *AAV-mediated delivery of an anti-BACE1 VHH alleviates pathology in an Alzheimer's disease model.* EMBO Mol Med, 2022. **14**(4): p. e09824, doi:10.15252/emmm.201809824

Link to article: https://www.embopress.org/doi/pdf/10.15252/emmm.201809824



This article describes the successful delivery of a nanobody (VHH) into the central nervous system (CNS) that lowers the load of amyloid-beta in a model for Alzheimer's disease. The innovation lies in using an adeno-associated-virus (AAV) that is able to cross the blood brain barrier into the CNS. This work was featured as front page of EMBO Molecular Medicine. First page of article attached as Annex 3.

2. Marino, M. and M.G. Holt, *AAV Vector-Mediated Antibody Delivery (A-MAD) in the Central Nervous System.* Front Neurol, 2022. **13**: p. 870799, doi:10.3389/fneur.2022.870799

Link to article:
https://www.frontiersin.org/journals/neurology/articles/10.3389/fneur.2022.870799/full

This review article discusses the latest knowledge on the design and exploitation of blood- brain barrier crossing viral vector systems for antibody delivery in the CNS. First page of article attached as Annex 4.

3. Yshii, L., et al., *Astrocyte-targeted gene delivery of interleukin 2 specifically increases brain-resident regulatory T cell numbers and protects against pathological neuroinflammation.* Nat Immunol, 2022. **23**(6): p. 878-891, doi:10.1038/s41590-022-01208-z

Link to article: https://www.nature.com/articles/s41590-022-01208-z

This article describes another successful AAV-based therapeutics for gene delivery. Specifically, it describes how traumatic brain injury, stroke, and autoimmunity can be treated by boosting local production of IL-2, a biologic that lowers neuroinflammation in the CNS. First page of article attached as Annex 5.

4. Shinmyo, Y., et al., *Localized astrogenesis regulates gyrification of the cerebral cortex.* Sci Adv, 2022. **8**(10): p. eabi5209, doi:10.1126/sciadv.abi5209

Link to article: https://www.science.org/doi/10.1126/sciadv.abi5209

This article focuses on the cellular mechanisms and the mechanical principle of gyrification in the mammalian brain and highlight astrogenesis as a major event for gyrification. First page of article attached as Annex 6.

5. Abdelfattah, A.S., et al., *Neurophotonic tools for microscopic measurements and manipulation: status report.* Neurophotonics, 2022. **9**(Suppl 1): p. 013001, doi:10.1117/1.NPh.9.S1.013001

Link to article: https://www.spiedigitallibrary.org/journals/neurophotonics/volume-9/issue-S1/013001/Neurophotonic-tools-for-microscopic-measurements-and-manipulation-status-report/10.1117/1.NPh.9.S1.013001.full

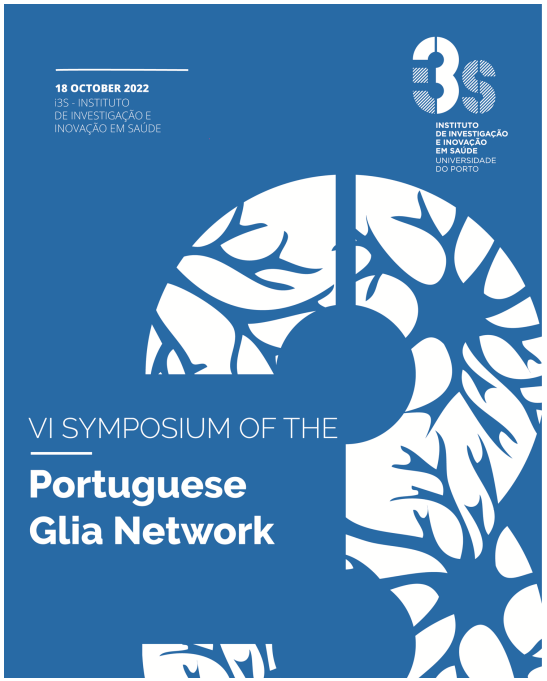
This review gives an update of the current state-of-the-art tools for studying brain activity with high temporal resolution in animal models. First page of article attached as Annex 7.

2.3 Talks at International Conferences

The Synapse Biology group showcased its scientific work at the Belgian Neuroscience Society Meeting (Brussels, 9th May, see Annex 8) and the Dutch Neuroscience Meeting (Tiel, 17th June). Dr. Matthew Holt was invited to shared his experience as an ERA Chair at the Science Europe Workshop on Widening Participation and Spreading (online, 24th May, https://www.scienceeurope.org/news/first-workshop-brain-circulation/).

Annex 1

ABSTRACT BOOK



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Ana Rita Matias, i3S, Porto, Portugal (ana.matias@i3s.up.pt)

WELCOME

Dear Students and Colleagues,

This year's speakers have been chosen to cover a range of topics from the molecular determinants of glial identity and development, their physiological roles in controlling circuit function and, ultimately, behavior. We will showcase researchers from national research institutes, including João Oliveira and Luísa Pinto (ICVS, Universidade do Minho) and be joined by top European researchers, including Karine Loulier (Institut des Neurosciences de Montpellier, France), Alex Charlet (University of Strasbourg Institute for Advanced Study, France), Nathalie Rouach (Centre Interdisciplinaire de Recherche en Biologie, Paris, France), Mick Hastings (MRC Laboratory of Molecular Biology, Cambridge, UK) and Klaus Armin Nave (Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany), giving plenty of opportunities for discussion of cutting-edge glial research and networking.

This is an excellent opportunity for junior scientists to showcase their work to leaders in the field, which is why we selected four abstracts from junior scientists for oral presentations! Moreover, awards will be given to the best oral and poster presentations, so stay tuned!

We look forward to seeing you all in October at the i3S!

Matthew Holt and João Relvas
Co-Chairs

VENUE

Address
i3S – Instituto de Investigação e Inovação em Saúde
Universidade do Porto
Rua Alfredo Allen 208
4200-135 Porto

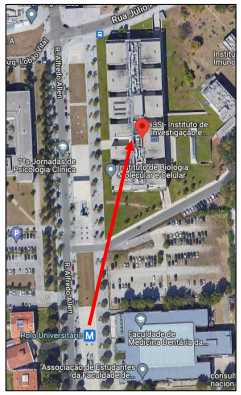
GPS: 41° 10' 30.008" N, 8° 36' 12.488" W

How to get to i3S
The subway (Ⓜ) is the easiest way to get to the i3S because there is a subway stop (Pólo Universitário) just next to it (<1 min walk).

From the Francisco Sá Carneiro airport
- Subway: take line E (direction: Estádio do Dragão) and get out at Trindade station. Change to line D (direction: Hospital São João) and get out at Pólo Universitário station.

From Campanhã train station
- Subway: all lines are possible (A, B, C, E and F). Get out at Trindade station and then change to line D (direction: Hospital São João) and get out at Pólo Universitário station.

From São Bento train station:
- Subway: take line D (direction: Hospital São João) and get out at Pólo Universitário station.



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Facebook: https://www.facebook.com/RedeGlial/

Instagram: @ptglianetwork

Twitter: @GlialRede

PROGRAMME

8:30-9:00 REGISTRATION

9:00 WELCOME (Auditório Mariano Gago)

9:15-11:00 SESSION I
Chairs: Luísa Pinto (ICVS, University of Minho, Portugal) and Teresa Summavielle (i3S, Porto, Portugal)

9:15 1ST KEYNOTE TALK
Molecular Heterogeneity of Astrocytes: Potential Implications for Development and Function
Matthew Holt (i3S, Porto, Portugal)

10:00 Diving into the Diversity of Cortical Astrocytes through the Prism of the Unsuspected Complexity of their Developmental origin
Karine Loulier (Institut des Neurosciences de Montpellier, Montpellier, France)

10:30 Oxytocin Receptor Mediated Modulation of Amygdala Astro-Neuronal Circuits
Alex Charlet (University of Strasbourg Institute for Advanced Study, Strasbourg, France)

11:00 COFFEE BREAK

11:30-16:30 SESSION II
Chair: Matthew Holt (i3S, Porto, Portugal)

11:30 Astrocytes: Guardians of Critical Period Plasticity in the Visual Cortex
Nathalie Rouach (Centre Interdisciplinaire de Recherche en Biologie, Paris, France)

12:00 2ND KEYNOTE TALK
Astrocytes and Circadian Time-Keeping: Star Clocks
Mick Hastings (MRC Laboratory of Molecular Biology, Cambridge, UK)

12:45 Selected Talk 1: Adenosine Receptors: the On-and-Off Switch of Astrocytic Cannabinoid Signaling
Joana Gonçalves-Ribeiro (iMM João Lobo Antunes, Lisbon, Portugal)

PROGRAMME

13:00 LUNCH AND POSTER SESSION

14:30 Selected Talk 2: Age and Sex-specific Proteome Plasticity of Brain Microglia
Joana Moreira (i3S, Porto, Portugal)

14:45 Selected Talk 3: Alterations in CNS Pathogenesis of the In Vivo Model of Multiple Sclerosis: Age Impact
Ana Rita Valente Ribeiro (iMed.ULisboa, University of Lisbon, Portugal)

15:00 The involvement of astrocyte calcium-dependent signaling in fear memory
João Oliveira (ICVS, University of Minho, Portugal)

15:30 Adult Astrogliogenesis: a Key Mechanism Underlying the Pathophysiology of Stress-Induced Depression
Luísa Pinto (ICVS, University of Minho, Portugal)

16:00 Selected Talk 4: Intrathecal Application of miR-124-Based Secretome to Prevent Disease Progression in the ALS Mice
Marta Alexandra Santos (iMed.ULisboa, University of Lisbon, Portugal)

16:15 Selected Talk 5: RhoA Regulates the Onset of CNS Myelination
Raquel Vale-Silva (i3S, Porto, Portugal)

16:30 COFFE BREAK AND POSTER SESSION

17:30-18:15 SESSION III
Chair: João Relvas (i3S, Porto, Portugal)

17:30 3RD KEYNOTE TALK
Novel Functions of Oligodendrocytes in Axonal Energy Metabolism and Neurodegenerative Disease
Klaus Armin Nave (Max Planck Institute for Multidisciplinary Sciences, Göttingen, Germany)

18:15 AWARDS FOR BEST ORAL AND POSTER PRESENTATIONS AND WRAP-UP

Annex 2

i3S NeuroDay

Program of Neurobiology and Neurologic Diseases (PNND) of the i3S

Date: Friday, 4th November 2022
Venue: Fundação Dr. António Cupertino De Miranda, Av. da Boavista 4245, 4100-140 Porto
Parking: free at the venue.

08:30-09:00 ARRIVAL AND COFFEE

09:00-09:15 WELCOME
João Relvas (Glial Cell Biology) and Matthew Holt (Synapse Biology Group)

SESSION I
Chairs: Mónica Sousa (Nerve Regeneration Group) and Matthew Holt (Synapse Biology Group)

09:15-10:00 Contingency signaling in axonal branching and CNS synapse formation: New molecular mechanisms underlying neuronal wiring in brain development.
Dietmar Schmucker (University of Bonn, Germany)

10.00-10:30 Molecular Heterogeneity of Astrocytes: Potential Implications for Development and Function
Matthew Holt (Synapse Biology Group)

10:30-11:00 Endosomal sorting in dendrites maintains neuronal polarity and synaptic function
Luís Ribeiro (University of Coimbra, Portugal)

11:00-11:30 COFFEE BREAK

SESSION II
Chairs: Teresa Summavielle (Addiction Biology Group) and Paulo Aguiar (Neuroengineering and Computational Neuroscience Group)

11:30-12:00 The dynamic nature of memory: heterosynaptic plasticity and the maintenance of memory
Rosalina Fonseca (NOVA University of Lisbon, Portugal)

12:00 -12:30 The impact of chronic stress on striatal brain circuits
Patrícia Monteiro (University of Minho, Braga,Portugal)

12:30-14:00 LUNCH

SESSION III
Chair: Ana Paula Pêgo (nanoBiomaterials for Targeted Therapies Group)

14:00-14:15 Tension-driven axon elongation triggers cytoskeleton and membrane remodelling
Sara Castro Sousa, Nerve Regeneration Group

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14:15-14:30 How ether-phospholipids modulate the way neurons talk
Tiago Silva, Neurolipid Biology Group

14:30-14:45 Probing potassium channel mechanism using an antibody sensor and live cell FRET
Carol Ann Harley, Structural Biochemistry Group

14:45-15:00 Dynein motors: critical for neurodevelopment and sensory functions
Tiago Dantas, UnIGENe

15:00-15:30 Circuit pruning in neuroimmune critical periods
João Peça (University of Coimbra, Portugal)

15:30-16:00 Future Challenges of the PNND with João Relvas and Paulo Aguiar

16:00-17:00 COFFEE BREAK

SESSION IV
Chairs: João Relvas (Glial Cell Biology Group) and Paulo Aguiar (Neuroengineering and Computational Neuroscience Group)

17:00–17:45 The numerous ways of glial cells to tune brain function
Frank Kirchhoff (University of Saarland, Homburg, Germany)

17:45-18:30 *(online)* Breaking sensitivity and specificity limits in functional and microstructural MRI
Noam Shemesh (Champalimaud Centre for the Unknown, Lisbon, Portugal)

18:30 Goodbyes

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Annex 3

Report

TRANSPARENT PROCESS OPEN ACCESS

EMBO Molecular Medicine

AAV-mediated delivery of an anti-BACE1 VHH alleviates pathology in an Alzheimer's disease model

Marika Marino[1,2,†], Lujia Zhou[1,2,†], Melvin Y Rincon[1,2,†], Zsuzsanna Callaerts-Vegh[3], Jens Verhaert[1,2], Jérôme Wahis[1,2], Eline Creemers[1,2,4], Lidia Yshii[1,2,5], Keimpe Wierda[1,2,4], Takashi Saito[6], Catherine Marneffe[1,2], Iryna Voytyuk[1,2], Yessica Wouters[1,2], Maarten Dewilde[1,2], Sandra I Duqué[1,2], Cécile Vincke[7], Yona Levites[8], Todd E Golde[8], Takaomi C Saido[9], Serge Muyldermans[7], Adrian Liston[1,5,10], Bart De Strooper[1,2,11,12] & Matthew G Holt[1,2,12,13,*]

Abstract

Single domain antibodies (VHHs) are potentially disruptive therapeutics, with important biological value for treatment of several diseases, including neurological disorders. However, VHHs have not been widely used in the central nervous system (CNS), largely because of their restricted blood–brain barrier (BBB) penetration. Here, we propose a gene transfer strategy based on BBB-crossing adeno-associated virus (AAV)-based vectors to deliver VHH directly into the CNS. As a proof-of-concept, we explored the potential of AAV-delivered VHH to inhibit BACE1, a well-characterized target in Alzheimer's disease. First, we generated a panel of VHHs targeting BACE1, one of which, VHH-B9, shows high selectivity for BACE1 and efficacy in lowering BACE1 activity *in vitro*. We further demonstrate that a single systemic dose of AAV-VHH-B9 produces positive long-term (12 months plus) effects on amyloid load, neuroinflammation, synaptic function, and cognitive performance, in the *App*$^{NL-G-F}$ Alzheimer's mouse model. These results constitute a novel therapeutic approach for neurodegenerative diseases, which is applicable to a range of CNS disease targets.

Keywords AAV; Alzheimer's disease; anti-BACE1; VHH
Subject Categories Genetics, Gene Therapy & Genetic Disease; Neuroscience

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EMBO Mol Med (2022) 14: e09824

Introduction

The high selectivity of monoclonal antibodies (mAbs) offers unique opportunities to target key proteins involved in the etiology of neurodegenerative conditions, such as Parkinson's disease and Alzheimer's disease (AD) (Zhou *et al*, 2011; Panza *et al*, 2014). However, their potential as central nervous system (CNS) therapeutics is largely limited by their inability to cross the blood–brain barrier (BBB) (Zafir-Lavie *et al*, 2018), comparatively poor biodistribution through the parenchyma (Freskgård & Urich, 2017), and short half-life (Wang *et al*, 2018). In addition, there is the potential for Fc receptor-mediated immunogenicity, mediated by microglia, which can cause vasogenic edema and cerebral microhemorrhage (Panza *et al*, 2014).

Single variable domain antibodies (VHHs) are increasingly seen as an alternative to mAbs for therapeutic use (Steeland *et al*, 2016; Bannas *et al*, 2017; Gomes *et al*, 2018; Jovčevska & Muyldermans, 2020). In fact, the VHH-based therapeutic Cablivi® (caplacizumab-

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11 UK Dementia Research institute at UCL, London, UK
12 Leuven Brain Institute, Leuven, Belgium
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Annex 4

frontiers | Frontiers in Neurology

REVIEW
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AAV Vector-Mediated Antibody Delivery (A-MAD) in the Central Nervous System

Marika Marino [1,2†] *and Matthew G. Holt* [1,2,3,4*†]

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In the last four decades, monoclonal antibodies and their derivatives have emerged as a powerful class of therapeutics, largely due to their exquisite targeting specificity. Several clinical areas, most notably oncology and autoimmune disorders, have seen the successful introduction of monoclonal-based therapeutics. However, their adoption for treatment of Central Nervous System diseases has been comparatively slow, largely due to issues of efficient delivery resulting from limited permeability of the Blood Brain Barrier. Nevertheless, CNS diseases are becoming increasingly prevalent as societies age, accounting for ∼6.5 million fatalities worldwide per year. Therefore, harnessing the full therapeutic potential of monoclonal antibodies (and their derivatives) in this clinical area has become a priority. Adeno-associated virus-based vectors (AAVs) are a potential solution to this problem. Preclinical studies have shown that AAV vector-mediated antibody delivery provides protection against a broad range of peripheral diseases, such as the human immunodeficiency virus (HIV), influenza and malaria. The parallel identification and optimization of AAV vector platforms which cross the Blood Brain Barrier with high efficiency, widely transducing the Central Nervous System and allowing high levels of local transgene production, has now opened a number of interesting scenarios for the development of AAV vector-mediated antibody delivery strategies to target Central Nervous System proteinopathies.

Keywords: AAV vectors, AAV vector-mediated antibody delivery (A-MAD), Monoclonal antibodies, Nanobodies (VHH), Central Nervous System, Blood Brain Barrier (BBB)

OPEN ACCESS

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INTRODUCTION

Antibodies, or Immunoglobulins (Ig), are glycoproteins produced by the immune system, characterized by their ability to recognize and bind a specific region (epitope) of an antigen with high specificity and (generally) high affinity, neutralizing potential pathogens. The basic structure of an Ig, determined by X-ray crystallography, is a tetramer of ∼150 kDa, formed by two identical pairs of heavy (50 kDa) and light polypeptide chains (25 kDa), joined by disulfide bonds. Heavy and light chains present highly variable complementarity determining regions (CDRs), which form the Fragment antigen binding (Fab) regions of the antibody, responsible for antigen recognition and binding. In contrast, the Fragment crystallizable (Fc) region binds to a variety of receptors on immune cells, to mediate antibody interaction

Annex 5

ARTICLES
https://doi.org/10.1038/s41590-022-01208-z

nature immunology

Check for updates

OPEN

Astrocyte-targeted gene delivery of interleukin 2 specifically increases brain-resident regulatory T cell numbers and protects against pathological neuroinflammation

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The ability of immune-modulating biologics to prevent and reverse pathology has transformed recent clinical practice. Full utility in the neuroinflammation space, however, requires identification of both effective targets for local immune modulation and a delivery system capable of crossing the blood–brain barrier. The recent identification and characterization of a small population of regulatory T (T_{reg}) cells resident in the brain presents one such potential therapeutic target. Here, we identified brain interleukin 2 (IL-2) levels as a limiting factor for brain-resident T_{reg} cells. We developed a gene-delivery approach for astrocytes, with a small-molecule on-switch to allow temporal control, and enhanced production in reactive astrocytes to spatially direct delivery to inflammatory sites. Mice with brain-specific IL-2 delivery were protected in traumatic brain injury, stroke and multiple sclerosis models, without impacting the peripheral immune system. These results validate brain-specific IL-2 gene delivery as effective protection against neuroinflammation, and provide a versatile platform for delivery of diverse biologics to neuroinflammatory patients.

Acute central nervous system (CNS) trauma is the leading cause of death and disability for people under the age of 45 years[1]. Although the causes of trauma are diverse, the common end result is substantial neuronal damage, or neuronal loss, in the affected region. This is thought to underlie the cognitive, sensorimotor function and personality changes typically seen in patients[1]. To date, drug treatments adopting a 'neuro-centric' approach have failed to deliver notable clinical benefits for the treatment of CNS injury[1,2], indicating that this approach is too narrow. Acute CNS injury is now recognized as triggering a multicellular response, involving CNS-resident immune cells (microglia and astroglia) alongside infiltration of peripheral immune cells to the brain parenchyma[3]. While there is evidence to support a neuroprotective effect of immune activation during the initial CNS response, prolonged activation invariably becomes neurotoxic[3,4]. The involvement of the immune system allows immune-modulating biologics to emerge as a key therapeutic option. However, adoption of immune-modulating biologics in the neuroinflammatory clinical space first requires

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Annex 6

SCIENCE ADVANCES | RESEARCH ARTICLE

DEVELOPMENTAL NEUROSCIENCE

Localized astrogenesis regulates gyrification of the cerebral cortex

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The development and evolution of mammalian higher cognition are represented by gyrification of the laminar cerebral cortex and astrocyte development, but their mechanisms and interrelationships remain unknown. Here, we show that localized astrogenesis plays an important role in gyri formation in the gyrencephalic cerebral cortex. In functional genetic experiments, we show that reducing astrocyte number prevents gyri formation in the ferret cortex, while increasing astrocyte number in mice, which do not have cortical folds, can induce gyrus-like protrusions. Morphometric analyses demonstrate that the vertical expansion of deep pallial regions achieved by localized astrogenesis is crucial for gyri formation. Furthermore, our findings suggest that localized astrogenesis by a positive feedback loop of FGF signaling is an important mechanism underlying cortical folding in gyrencephalic mammalian brains. Our findings reveal both the cellular mechanisms and the mechanical principle of gyrification in the mammalian brain.

INTRODUCTION

During mammalian evolution, the cerebral cortex has changed markedly, resulting in the acquisition of higher cognitive functions (*1*–*7*). Notable changes during evolution include the expansion and folding (i.e., gyrification) of the cerebral cortex. Because malformations of cortical folds in human patients, such as lissencephaly and polymicrogyria, are associated with severe intellectual disability and epilepsy, folding of the cerebral cortex is considered to be indispensable for higher brain functions (*8*–*12*). Therefore, investigation of molecular and cellular mechanisms underlying cortical folding is critical for understanding not only the evolution of the mammalian cerebral cortex but also the pathogenesis of human cortical malformations. However, our understanding of the mechanisms underlying cortical folding is still rudimentary.

In addition to neurons, glial cells have markedly increased with brain expansion during evolution (*13*). Astrocytes, one type of glial cell, play a range of crucial roles such as modulation of synaptic functions, maintenance of extracellular ion balance, and provision of nutrients to neurons (*14*–*18*). By contacting the nodes of Ranvier, astrocytes also participate in remodeling myelin structures, which influence the conduction velocities of axons (*19*). Furthermore, astrocyte dysfunction is involved in various neurological and neurodevelopmental diseases (*20*). A previous study showed that mice that have human astrocytes exhibited improved cognitive abilities, suggesting that astrocytes themselves have also changed during evolution (*21*). Thus, astrocytes are increasingly appreciated as important contributors to higher brain functions.

To investigate the mechanisms underlying the development and evolution of the cerebral cortex, carnivore ferrets have attracted attention as an important model animal (*22*–*26*). This is because ferrets have gyrencephalic brains, and genetic manipulation techniques for ferrets have been established (*27*–*30*). Using ferrets, previous studies have uncovered neuronal processes involved in the development and evolution of the cerebral cortex (*25*–*27*, *30*–*32*). Here, we propose a novel two-step model of gyrification of the cerebral cortex. We established genetic manipulation techniques for astrocytes in the cerebral cortex by combining in utero electroporation (IUE) and the *piggyBac* system and found that a marked expansion of astrocytes in restricted areas within gyri in the ferret cortex was mediated by a positive feedback loop driven by fibroblast growth factor (FGF) signaling. The overproduction of astrocytes by activation of FGF signaling induced gyrus-like protrusions in the mouse cerebral cortex. Furthermore, we found that localized astrogenesis is indispensable for cortical folding in the ferret brain via its role in the vertical expansion of the deep pallial regions. Our findings reveal both the cellular mechanisms and the mechanical principle of gyrification in the mammalian brain.

RESULTS

Ferret astrogenesis is regulated by FGF signaling in an autocrine manner

We previously showed that FGF signaling is crucial for folding of the cerebral cortex in ferrets (*31*, *33*). We noticed that activation of FGF signaling increased not only layer 2/3 neurons but also astrocytes (*33*). This finding raised the possibility that FGF signaling might directly control the number of astrocytes during development. To test this possibility, we activated FGF signaling during astrogenesis in the developing mouse cerebral cortex by combining IUE, the $ER^{T2}CreER^{T2}$/loxP system, and the *piggyBac* system (fig. S1A). The activation of FGF signaling during astrogenesis induced

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Annex 7

Neurophotonic tools for microscopic measurements and manipulation: status report

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Annex 8

14th Meeting of the Belgian Society for Neuroscience

May 9th, 2022, ULB Erasme Campus, Brussels

8:45 - 09:15 Registration and putting up (Building W)

09:15 – 11:55 Morning session (Building W – Auditorium Madeleine de Genst)

09:15 – 09:25 Introduction
09:25 – 10:25 Keynote **Andreas Lüthi (Friedrich Miescher Institute (FMI, Basel, Switzerland)**
10:25 – 10:55 **Wim Vandufel (KUL)**

10:55-11:25 Coffee Break + Poster Session (Atrium)

11:25 – 11:55 **Patricia Bonnavion (ULB)**

11:55 - 12:55 Parallel Session 1 (Auditorium Madeleine de Genst)

11:55 – 12:15 Selected abstracts
12:15 – 12:35 Selected abstracts
12:35 – 12:55 Selected abstracts

11:55 - 12:55 Parallel Session 2 (Auditorium Elisabeth Wollast)

11:55 – 12:15 Selected abstracts
12:15 – 12:35 Selected abstracts
12:35 – 12:55 Selected abstracts

12h55- 14h15 Lunch + Poster Session

14:15 – 16:55 Afternoon Session - Building F (Auditorium Claude)

14:15 – 15:15 Keynote **Sarah Garfinkel (Institute of Cognitive Neuroscience, London, UK)**
15:15 – 15:45 **Matthew Holt (KUL)**

15:45-16:15 Coffee Break + Poster Session (Atrium)

16:15 – 16:45 **Gilles Pourtois (UGent)**

16:45 - 17:45 Parallel Session 3 (Auditorium Claude)
16:45 – 17:05 Selected abstracts
17:05 – 17:25 Selected abstracts
17:25 – 17:45 Selected abstracts

16:45 - 17:45 Parallel Session 4 (Auditorium F2.103 A/B)

16:45 – 17:05 Selected abstracts
17:05 – 17:25 Selected abstracts
17:25 – 17:45 Selected abstracts

17:45 - 18:00 Award ceremony and Conclusion (Auditorium Claude)