Universidade do Minho
School of Medicine

ICVS/3B's
Associate Laboratory
University of Minho

Final Scientific Report

Bial Scientific Research Bursaries

Decoding the neuron-astrocyte dialogue that supports cognitive processing

Fellowship 037/18

João Filipe Oliveira, PhD

Braga, November 15th, 2023

Activities description

The present document reports the works carried out, and it sums up the findings and outputs of the research project **"Decoding the neuron-astrocyte dialogue that supports cognitive processing" - Grant 037/18** carried in the Life and Health Sciences Institute of the School of Medicine (ICVS), University of Minho.
This report summarizes the outputs of the extensive work performed to address the project goals.

Aims

The main research objective of this project was to dissect the spatial and temporal components of astrocytic control of hippocampal-cortical circuits that mediate cognitive performance.

Methods

As initially proposed, we used an innovative combination of novel viral vectors to manipulate astrocyte exocytosis in a spatially- and temporally-controlled manner. Using this approach, we could dissect the spatial and temporal properties of the astrocytic signals in HIP-PFC circuits that process different forms of learning and memory. Furthermore, we screened the effects of this manipulation by complementary functional, structural, and molecular evaluations of the HIP-PFC networks. We employed state-of-the-art behavior testing, in vivo electrophysiology, transcriptomics, morphological, immunohistochemical, and molecular studies.

Results and Discussion

These efforts led us to accomplish the main goals:

- Control and manipulation of astrocytes or neurons in a temporally controlled manner and evaluation of the electrophysiological and consequences to cognition.
- Identification and regional control of a calcium-dependent signaling pathway that controls synaptic structure and plasticity, leading to modulation of long-term memory

These main findings were translated into specific scientific achievements, detailed in the progress reports:
- Implementation of the viral vector approach to temporally control exocytosis in neurons and astrocytes
- Activation of miniSOGs fused to VAMP3 in neurons reduced neuronal activity dramatically
- Activation of miniSOGs fused to VAMP3 in astrocytes steadily increased neuronal activity in the theta range, as well as in faster frequencies
- The miniSOG approach allows targeting specifically astrocytic exocytosis
- Mice lacking astrocytic calcium elevations display enhanced performance in hippocampal-dependent cognitive tasks, that rely on the theta frequency oscillations
- Mice lacking astrocytic somatic calcium activity display a shift to an immature spine profile in hippocampal pyramidal neurons
- The activity of the Foxo1 transcription factor is upregulated in mice lacking astrocytic somatic calcium activity
- Spatially controlled overexpression of FOXO1 in CA1 astrocytes of the dorsal hippocampus causes a shift to an immature spine profile in hippocampal pyramidal neurons in C57BL/6J mice
- Spatially controlled overexpression of FOXO1 in CA1 astrocytes of the dorsal hippocampus leads to reduction of long-term depression in C57BL/6J mice

- Spatially controlled overexpression of FOXO1 in CA1 astrocytes of the dorsal hippocampus drives long-term fear memory enhancement in C57BL/6J mice

It is noteworthy that we have made a rigorous management of the available budget, employing talented researchers to perform specific techniques and implement state-of-the-art protocols. This effort ultimately resulted in an increased consistency of the data obtained, confirmed by the high-quality publications and theses produced (see Outputs).
In the first year, we faced several constraints due to COVID-19 regarding lab access, social distancing, and administrative procedures, namely at the project start. Nevertheless, we counted on the close support of the host institution to minimize the impact on the project. After a difficult period, the COVID-19 pandemic restrictions, the team, with the help of the host institution, managed to return the laboratory activities to normal.
During that period, we faced a deviation in the execution of the proposed plan since the approach employed to spatially block exocytosis in astrocytes failed to work correctly *in vivo* (detailed in the progress report).
We rapidly circumvented these issues by studying the mouse model that lacks Ca^{2+} intracellular elevations, a required control mechanism of exocytotic release. Studying this model, we found a surprising astrocyte Ca^{2+}-dependent enhancement of hippocampus-dependent learning and memory and regulation of structural plasticity in hippocampal pyramidal neurons. The exciting results triggered the pursuit of the molecular pathways underlying the observations reported. We found that these mice display alteration in the regulation of transcription factors, namely some involved in signaling release, structural changes in neurons, and cognitive function. The spatially restricted manipulation of this signaling pathway in astrocytes allowed for recapitulation of the structural and synaptic plasticity changes, as well as the modulation of long-term fear memory.

Conclusions and Recommendations

Astrocytes appear as critical elements of the cortico-limbic networks, whose function is required for correct cognitive function, which is a remarkable finding. The temporal and regional control of the manipulations of the identified astrocytic pathways revealed that astrocytes integrate neuronal circuits in cortico-limbic regions to compute cognition.
Despite the lack of national and international events during the pandemic, we were invited to deliver talks online that allowed the presentation of novel results generated in this project. After this exceptional period, we returned to presential events, and we were able to present our data to national and international audiences. These presentations potentiated the outreach of the data obtained in the scope of this project and fostered novel collaborations. We received very positive feedback from the consultants and peers. We also chaired symposia and workshops in relevant international meetings (e.g., the FENS Forum in Paris and the ISN-APSN Meeting in the USA). Also important were the outreach activities that we carried out in the community, namely by disclosing the secrets of the human brain at schools in our region to children and adolescents.
We published research and review papers in the research grant's scope, resulting from in-house and collaborative efforts. The students involved in the project team carried out the works of their MSc and/or PhD theses with success. All these outputs are detailed below.

We acknowledge the Bial Foundation both for the relevant financial support and for the continuous management aid, that led to the success of this project.

Project outputs

Expected and achieved output indicators

Output indicators	Expected	Achieved
Advanced training: PhD theses	2	1 defended 4 ongoing
Advanced training: Master theses	2	2 defended 1 ongoing
Journal article	2	11
Organization of seminars and conferences	3	2 Symposia 1 Workshop
Conference presentation	10	17 Oral communications 15 Poster presentations
Awards		4
Actions of scientific dissemination	6	Outreach activities to society

PhD Theses

Joana Filipa da Silva Rodrigues
Defense in 04/2023
"The role of Ral GTPase in glial development and function"

= Ongoing
João Filipe Oliveira Viana
"The role of the astrocytic metabotropic glutamate receptor 5 in the pathophysiology of depression"

Sara Barsanti
"Gliotransmission in depression: astrocytes as promising targets to more effective therapeutics"

João Luís Fernandes Machado
"Decoding the neuron-astrocyte dialogue that supports cognitive processing"

Daniela Sofia da Costa Abreu
"Neuron-astrocyte signaling in depression: learning from astrocytes how to effectively treat depression"

MSc theses

João Luís Fernandes Machado
Defense in 12/2021
"The role of the astrocytic mGluR5 in behavior, dependent on the ventral hippocampus and the prefrontal cortex"

Francisca Soledade Santos
Defense in 12/2021

"The role of astrocytic FOXO1 in the mouse brain and its modulation of behavior"

= Ongoing
Alexandra Catarina Machado Veiga
"The role of astrocytic Foxo1 in the dorsal hippocampus"

José Duarte Gomes Dias
"The role of astrocytic mGluR5 in depressive-like behavior"

Peer-reviewed publications:

- Viana JF, Machado JL, Abreu DS, Veiga A, Barsanti S, Tavares G, Martins M, Sardinha VM, Guerra-Gomes S, Domingos C, Pauletti A, Wahis J, Liu C, Calì C, Henneberger C, Holt MG, **Oliveira JF.** 2023. Astrocyte structural heterogeneity in the mouse hippocampus. Glia. 71:1667–1682.
- Cunha-Garcia D, Monteiro-Fernandes D, Correia JS, Neves-Carvalho A, Vilaça-Ferreira AC, Guerra-Gomes S, Viana JF, **Oliveira JF**, Teixeira-Castro A, Maciel P, Duarte-Silva S. 2023. Genetic Ablation of Inositol 1,4,5-Trisphosphate Receptor Type 2 (IP3R2) Fails to Modify Disease Progression in a Mouse Model of Spinocerebellar Ataxia Type 3. Int J Mol Sci. 24:10606.
- Martins-Macedo J, Mateus-Pinheiro A, Alves C, Veloso F, Gomes ED, Ribeiro I, Correia JS, Silveira-Rosa T, Alves ND, Rodrigues AJ, Bessa JM, Sousa N, **Oliveira JF**, Patrício P, Pinto L. 2023. StressMatic: A Novel Automated System to Induce Depressive- and Anxiety-like Phenotype in Rats. Cells. 12:381.
- Oliveira JF*, Araque A*. 2022 Astrocyte regulation of neural circuit activity and network states. **Glia**. 70:1455–1466.
- Machado-Santos AR, Loureiro-Campos E, Patrício P, Araújo B, Alves ND, Mateus-Pinheiro A, Correia JS, Morais M, Bessa JM, Sousa N, Rodrigues AJ, **Oliveira JF***, Pinto L*. 2022. Beyond New Neurons in the Adult Hippocampus: Imipramine Acts as a Pro-Astrogliogenic Factor and Rescues Cognitive Impairments Induced by Stress Exposure. **Cells**. 11:390.
- Escartin C, Galea E, ... **Oliveira JF**, ... Sofroniew MV, Verkhratsky A. (2021) Reactive astrocyte nomenclature, definitions, and future directions. **Nature Neuroscience** 1–14.
- Canedo T, Portugal CC, Socodato R, Almeida TO, Terceiro AF, Bravo J, Silva AI, Magalhães JD, Guerra-Gomes S, **Oliveira JF**, Sousa N, Magalhães A, Relvas JB, Summavielle T. 2021. Astrocyte-derived TNF and glutamate critically modulate microglia activation by methamphetamine. **Neuropsychopharmacol**. 46:2358–2370.
- Loureiro-Campos E, Mateus-Pinheiro A, Patrício P, Soares-Cunha C, Silva J, Sardinha VM, Mendes-Pinheiro B, Silveira-Rosa T, Domingues AV, Rodrigues AJ, **Oliveira J**, Sousa N, Alves ND, Pinto L. 2021. Constitutive deficiency of the neurogenic hippocampal modulator AP2γ promotes anxiety-like behavior and cumulative memory deficits in mice from juvenile to adult periods. **eLife**. 10:e70685.
- Falcón-Moya R, Pérez-Rodríguez M, Prius-Mengual J, Andrade-Talavera Y, Arroyo-García LE, Pérez-Artés R, Mateos-Aparicio P, Guerra-Gomes S, **Oliveira JF**, Flores G, Rodríguez-Moreno A. 2020. Astrocyte-mediated switch in spike-timing-dependent plasticity during hippocampal development. **Nature Communications** 11:4388.
- Batiuk MY, Martirosyan A, Wahis J, Vin F de, Marneffe C, Kusserow C, Koeppen J, Viana JF, **Oliveira JF**, Voet T, Ponting CP, Belgard TG, Holt MG. 2020. Identification of region-specific astrocyte subtypes at single cell resolution. **Nature Communications** 11:1–15.

- Guerra-Gomes S, Cunha-Garcia D, Nascimento DSM, Duarte-Silva S, Loureiro-Campos E, Sardinha VM, Viana JF, Sousa N, Maciel P, Pinto L, **Oliveira JF**. IP3R2 null mice display a normal acquisition of somatic and neurological development milestones. **European Journal of Neuroscience**.

Publication in bioRxiv including recent data of this project:

- Viana JF, Guerra-Gomes S, Abreu DS, Machado JL, Barsanti S, Gonçalves M, Martín-Monteagudo C, Sardinha VM, Nascimento DSM, Tavares G, Irmler M, Beckers J, Korostynski M, Sousa N, Navarrete M, Teixeira-Castro A, Pinto L, **Oliveira JF**. 2023. Astrocytic Foxo1 regulates hippocampal spinogenesis and synaptic plasticity to enhance fear memory.
https://www.biorxiv.org/content/10.1101/2023.05.01.538923v1

Organization of scientific events/symposia

- ISN Workshop – Satellite Event of the ISN-ESN Meeting in Porto 2023
"Astrocytic mechanisms involved in the modulation of brain circuits and behavior"
5-6 Aug 2023; ICVS – School of Medicine, Braga, Portugal

- Symposium on "Astrocytes control brain circuits underlying cognitive function"
International Society for Neurochemistry-APSN Meeting, Honolulu, Aug 2022

- Symposium on "Astrocytes control brain circuits underlying behavior"
FENS Forum, Paris, Jul 2022

Oral communications in international meetings

- ISN Workshop, Braga, Portugal, Aug 2023
"The involvement of astrocyte calcium-dependent signaling in fear memory"
João Filipe Oliveira

- FENS Regional Meeting 2023, Algarve, Portugal, 3-5 May 2023
João Luís Machado "Astrocyte structural heterogeneity in the mouse hippocampus"

- FENS Regional Meeting 2023, Algarve, Portugal, 3-5 May 2023
Daniela Sofia Abreu "The involvement of astrocyte calcium-dependent signaling in fear memory"

- International Astrocyte School 2023, 26th March- 1st April 2023
"Astrocyte structural heterogeneity in the mouse hippocampus".
João Filipe Viana

- International Society for Neurochemistry-APSN Meeting, Honolulu, Aug 2022
"The involvement of astrocyte calcium-dependent signaling in behavior"
João Filipe Oliveira

- FENS Forum, Paris, Jul 2022
"The involvement of astrocyte calcium-dependent signaling in behavior"
João Filipe Oliveira

- Satellite Event of the FENS Forum 2022, Paris, Jul 2022

"Electrophysiological properties of basal cortico-limbic activity involved in cognition and emotion"
João Filipe Oliveira

- João Oliveira | The involvement of astrocytes in cognitive function | NICO NeuroWebinar University of Turin | Nov 2020 | Virtual

Oral communications in national meetings

- MSc Talks Faculty of Medicine – University of Lisboa Nov 2022
"The involvement of astrocytes in cortico-limbic function and behavior"

- VI Symposium of the Portuguese Glial Network, Porto, Oct 2022
"The involvement of astrocyte calcium-dependent signaling in fear memory"
João Filipe Oliveira

- Pint of Science, Braga, May 2022
"Emocional-MENTE Seguindo as estrelas do cérebro para contrariar os efeitos nocivos do stress"
João Filipe Oliveira

- Bial Symposium, Porto, Apr 2022
"The role of astrocytes in complex cognitive processing"
João Filipe Oliveira

- Jornadas da Ciência, Braga, Apr 2022
"Descodificar o diálogo entre neurónios e astrócitos para entender o funcionamento do cérebro"
João Filipe Oliveira

- Faculty of Medicine, University of Lisbon, MSc-PhD Program, Nov 2021
"The involvement of astrocytes in cortico-limbic function and behavior, 3 stories:
- The final story
- What comes next?
- What really happened"
João Filipe Oliveira

- IMM Neurosciences Seminar, University of Lisbon, June 2021
"The involvement of astrocytes in cortico-limbic function and cognition"
João Filipe Oliveira

- CEDOC Seminars, CEDOC Nova Medical School, Virtual Dec 2020
"The involvement of astrocytes in cognitive function"
João Filipe Oliveira

- IX Ciclo de Conferências Científicas para Professores de 3º Ciclo e Secundário, Virtual, Jun 2020
"Descodificar o diálogo entre neurónios e astrócitos para entender melhor o funcionamento do cérebro"
João Filipe Oliveira

Poster communications in international meetings

XVI European Meeting on Glial Cells in Health and Disease, Berlin, Germany, June 2023

"The involvement of astrocyte calcium-dependent signaling in behavior"
Daniela Sofia Abreu, João Filipe Viana, Sónia Guerra-Gomes, Francisca Soledade, João Luís Machado, Sara Barsanti, Diana Sofia Marques Nascimento, Luísa Pinto, Andreia Teixeira-Castro, João Filipe Oliveira

"Astrocyte structural heterogeneity in the mouse hippocampus"
João Luís Machado, João Filipe Viana, Daniela Sofia Abreu, Alexandra Veiga, Gabriela Tavares, Manuella Martins, Vanessa Morais Sardinha, Sónia Guerra-Gomes, Cátia Domingos, Alberto Pauletti, Jérôme Wahis, Chen Liu, Corrado Calì, Christian Henneberger, Matthew G. Holt, João Filipe Oliveira

"Structural analysis of astrocytes in different experimental conditions"
Sara Barsanti, João Luís Machado, João Filipe Viana, Alexandra Veiga, Daniela Sofia Abreu, Jerôme Wahis, Chen Liu, Matthew Holt, João Filipe Oliveira

"Astrocytic Foxo1 regulates hippocampal spinogenesis and synaptic plasticity and enhances fear memory"~
João Filipe Viana, Daniela Sofia Abreu, Sónia Guerra-Gomes, Cristina Martín-Monteagudo, João Luís Machado, Sara Barsanti, Diana Sofia Marques Nascimento, Marta Navarrete, Andreia Teixeira-Castro, João Filipe Oliveira

FENS Regional Meeting 2023 (FRM2023), Algarve, Portugal 2023

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13th FENS Forum of Neuroscience, July 2022

"The involvement of astrocyte calcium-dependent signaling in behavior"
Daniela Sofia Abreu, João Filipe Viana, Sónia Guerra-Gomes, Francisca Soledade, João Luís Machado, Diana Sofia Marques Nascimento, Luísa Pinto, Andreia Teixeira-Castro, João Filipe Oliveira

"Morphological analysis of astrocytes in different preparations"
João Luís Machado, João Filipe Viana, Alexandra Veiga, Eduardo Teixeira, Daniela Sofia Abreu, Jerôme Wahis, Chen Liu, Matthew Holt, João Filipe Oliveira

"Heterogeneity of the astrocyte morphology within the mouse hippocampus"
João Filipe Viana, João Luís Machado, Daniela Sofia Abreu, Gabriela Tavares, Manuella Martins, Vanessa Morais Sardinha, Sónia Guerra-Gomes, Cátia Domingos, Alberto Pauletti, Jérôme Wahis, Chen Liu, Christian Henneberger, Matthew Holt, João Filipe Oliveira

Poster communications in national meetings

VII Symposium – PT Glial Network, Algarve, Portugal, May 2023

"The involvement of astrocyte calcium-dependent signaling in behavior"
Daniela Sofia Abreu, João Filipe Viana, Sónia Guerra-Gomes, Francisca Soledade, João Luís Machado, Sara Barsanti, Diana Sofia Marques Nascimento, Luísa Pinto, Andreia Teixeira-Castro, João Filipe Oliveira

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VI Symposium – PT Glial Network, Porto, Portugal, October 2022

"The involvement of astrocyte calcium-dependent signaling in behavior"
Daniela Sofia Abreu, João Filipe Viana, Sónia Guerra-Gomes, Francisca Soledade, João Luís Machado, Diana Sofia Marques Nascimento, Luísa Pinto, Andreia Teixeira-Castro, João Filipe Oliveira

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Awards

Best Glial Image Award, VII Symposium – PT Glial Network 2023 – João Viana
FENS Regional Meeting 2023 IBRO grant – João Viana
SPN Travel Award 2021 – João Viana
SPN Travel Award 2021 – João Machado