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Escola de Medicina

ICVS/3B's
Associate Laboratory
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The role of astrocytes in complex cognitive processing

Fellowship 207/14

João Filipe Oliveira, PhD

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Summary

The present document reports the works and actions performed, and it sums up the findings and outputs of the research project **"The role of astrocytes in complex cognitive processing" - Grant 207/14** carried in the Life and Health Sciences Institute of the School of Medicine (ICVS), University of Minho.

This report describes the extensive work performed to address the proposed goals. At this stage, we do believe that these goals were fully accomplished. As initially proposed, we have used complementary state-of-the-art techniques such as *in vivo* electrophysiology, innovative behavior, anatomical and molecular analyses, to study mouse models of astrocytic dysfunction and seek a role of astrocytes in the computation of cognitive function, dependent on the cortico-limbic networks, namely on the prefrontal cortex and interconnected brain regions such as the hippocampus.

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 resulted ultimately in an increased consistence of the data obtained, confirmed by the high-quality publications obtained so far (2 additional publications are being written and they will be submitted soon), and by the very positive feedback that we have received from the consultants and peers when we presented data at national and international congresses.

Astrocytes appear as critical elements of the cortico-limbic networks, whose function is required for correct cognitive function, which is a quite remarkable finding.

We acknowledge the Bial Foundation both for the relevant financial support, and for the continuous management aid.

Aims

In recent years, the understanding of synaptic modulation by neuron-astrocyte interactions has evolved considerably, contributing to build up the concept of the "tripartite synapse". This concept is based on the dynamic dialogue between astrocytes and neurons that complements and modulates the communication between pre- and post-synaptic structures. However, it remains elusive how this interaction between neurons and astrocytes translates into network computation of behavior. Astrocytes were described to release gliotransmitters (such as glutamate, GABA, ATP or D-Serine) by means of several mechanisms, being exocytosis the more extensively studied. This process is mediated by the vesicular machinery and SNARE complex formation between vesicles and the target membrane, ultimately resulting in the release of the vesicular content. Transmitter release is essential for astrocyte signaling and a disruption of this phenomenon is expected to impact on the function of neuronal networks, with consequences for the computation of higher brain functions. Besides, astrocytes integrate neuronal signals by complex calcium (Ca^{2+}) elevations that control intracellular mechanisms that in turn drive the neuron-astrocyte dialogue, modulating the activity of cells and networks. It is now recognized that Ca^{2+} elevations in astrocytes appear spatially distributed in global (soma and main processes) and/or focal regions (microdomains). Although it is observed that global astrocytic Ca^{2+} signaling contributes to synaptic communication, its role in circuit computation and cognitive performance is still poorly understood. A detailed behavioral, structural and molecular characterization should provide us with putative mechanisms underlying the roles of astrocytic Ca^{2+}.

In this project we aimed at studying how astrocyte intracellular and extracellular signaling, and its modulation could impact cognitive function. To address this main aim, we studied two mouse models of astrocytic dysfunction to disclose: (1) if astrocyte signaling and its rescue could modulate cognitive function and its underlying mechanisms; (2) if astrocyte calcium signaling controls intracellular pathways that provide molecular control of the structure and function of cortico-limbic networks responsible for cognitive function. Works carried out in the first part resulted in 3 publications (Sardinha et al. 2017; Tavares et al. 2017; Kafetzopoulos et al. 2018), one special topic edited in *Frontiers in Cellular Neuroscience* (Oliveira et al. 2016), one PhD thesis and one MSc thesis. The second part resulted in one publication (Guerra-Gomes, Viana, et al. 2018) (two additional publications are currently being written), one review (Guerra-Gomes, Sousa, et al. 2018)

and one PhD thesis, and one MSc thesis just delivered. Altogether work resulting from this grant was presented in both national and international meetings.

Methods

To address astrocytic signaling in the first part of this project, we studied the transgenic dnSNARE mouse model that displays a conditional blockade of transmitter release by exocytosis, selectively in astrocytes. This was achieved by allowing the conditional expression of the dominant negative domain of vesicular SNARE protein synaptobrevin II (dnSNARE), which interferes with the SNARE complex formation, impairing vesicular release. Four weeks after the induction of transgene transcription, the levels of transgenic protein reached its maximum. The dnSNARE transgenes are expressed exclusively by astrocytes and display inter-subject variability. The quantification of dnSNARE transgene expression levels allowed to discriminate high "expressor" subjects to be analyzed throughout.

Mice were first evaluated by performing *in vivo* electrophysiological recordings of local field potentials from neuronal populations of cognitive related brain regions: dorsal hippocampus and prefrontal cortex. This functional network evaluation was followed by a thorough assessment of cognitive ability of these mice, by performing a battery of behavioral tests. These tests addressed different cognitive tasks mainly dependent from the performance of the hippocampus-prefrontal network. This functional assessment was complemented by a morphological characterization of neurons and astrocytes to address structural correlates of network function.

In the second part, we took advantage of the inositol 1,4,5-trisphosphate receptor type 2 knockout (IP_3R2 KO) mouse model, which lacks global Ca^{2+} signaling in astrocytes. We confirmed if the receptor deletion caused developmental inconsistencies, and next we carried out detailed behavioral, structural and molecular characterization of this mouse model at different ages.

Results and Discussion

Our findings in the first part of the work demonstrated a specific neural desynchronization in the theta rhythm between the dorsal hippocampus and prefrontal cortex in the dnSNARE mice, without

any alteration of levels of neuronal activity. Moreover, the blockade of gliotransmitter release in astrocytes triggers a critical cognitive impairment in tasks classically attributed to neuronal circuits of the hippocampus-prefrontal cortex network. More specifically, dnSNARE mice faced an increased difficulty when performing in reference memory tasks of Morris water maze (MWM) and hole-board test (HB), and revealed a clear deficit in tasks involving spatial recognition and long-term memory, such as the novel object recognition (NOR) and two-trial place recognition (2TPR) tests. Further analysis of electrophysiological recordings showed a direct correlation between the loss of theta coherence in dorsal hippocampus-prefrontal link and poor consolidation of reference memory. The structure evaluation of the dorsal hippocampus and prefrontal cortex revealed that the neuronal dendritic trees appear to be intact in dnSNARE mice. However, astrocytes undergo drastic process atrophy, specifically in GFAP+ cells that also express dnSNARE transgenes.

Interestingly, the intraperitoneal supplementation with the NMDAR co-agonist D-serine — that is known to be released by exocytosis in astrocytes and to be significantly decreased in the brains of dnSNARE mice — completely restored theta synchronization and rescued the learning and memory deficits in transgenic mice.

In the second part, we demonstrated that IP_3R2 KO mice retain a normal developmental maturation, as compared with WT littermates. The detailed behavioral characterization of this mouse model showed that IP_3R2 KO mice display enhanced cognitive performance in hippocampal-dependent tasks. This finding was in fact surprising, and therefore we explored it further. We found *Foxo1* as the most active transcription factor controlling the increased expression of astrocyte-specific genes related with fine cytoskeleton modulation and spinogenesis, which could underlie the cognitive enhancement observed. Moreover, specific overexpression of FOXO1 in hippocampal astrocytes of C57BL/6J mice was enough to recapitulate the enhanced fear memory observed in IP_3R2 KO mice. Finally, we explored the role of astrocytic Ca^{2+} signaling in cortico-limbic performance in aged mice that display cognitive decline, namely in one task highly dependent on the prefrontal cortex. We observed a preserved cognitive performance in aged IP_3R2 KO mice, an altered neuron/astrocyte ratio and a dendritic refinement of medial prefrontal cortex neurons.

Conclusions and Recommendations

The results obtained in this project provide the first evidence of a mechanism by which astrocytic signaling is required for entrainment of distant cortico-limbic circuits, being mandatory for cognitive

performance. Moreover, our findings suggest that D-serine may be the gliotransmitter maintaining the synchronization of the theta rhythm between these circuits required for learning and memory consolidation. Further studies should be performed to unravel the astrocytic contribution to different cognitive tasks, in different brain regions. Moreover, we demonstrated for the first time that interfering with the major source of global Ca^{2+} signaling in astrocytes does not influence postnatal development. Therefore, the IP_3R2 KO mouse model is suitable to study astrocyte involvement in the behavioral response, in all developmental stages and adulthood. Our results highlight an important role of IP_3R2-dependent Ca^{2+} signaling in astrocytes in the regulation of cognitive behavior in the adult and aged brain. In the adult age, we found that IP_3R2 KO mice display an enhanced cognitive performance at hippocampal-dependent tasks. Our results provided the first evidence of a transcription factor, *Foxo1*, which is highly enriched in astrocytes and whose activity seems to be controlled by global Ca^{2+} signaling in astrocytes. *Foxo1* is regulating the expression of relevant astrocytic-related genes in the hippocampus - *Sdc2* and *Ezr* - that have an important role in PAP morphology and spinogenesis, which in turn should control synaptic plasticity phenomena to enhance cognitive performance. Finally, in healthy aging, we found that cognitive function dependent on key of the cortico-limbic circuits, the prefrontal cortex, was preserved in mice lacking IP_3R2-dependent Ca^{2+} signaling in astrocytes. These results open a new window in the search for the basis of cognitive function.

Altogether, these findings provided information about unprecedented roles of astrocytes in the modulation of brain circuits responsible for cognitive computation, that pave the way a for a deeper understanding of the complexity of cellular networks that produce brain outputs.