Rio de Janeiro, 04 January 2019.

Prof. Alessandro Prinetti
Chair
Committee for Aid and Education in Neurochemistry
International Society for Neurochemistry

Dear Dr. Prinetti,

Please find enclosed my report regarding the ISN CAEN Award 1B, granted to me on 15 February 2017. I am very grateful to the Committee for the aid granted. This grant was essential for me to complete the proposed project and to support related projects in my research group. I consider this award offered me a very positive outcome and encourage the Committee to maintain this form of support to the Neuroscience community. Please do not hesitate to contact me should any questions occur.

Sincerely,

Mychael V. Lourenco
Assistant Professor of Neuroscience
Institute of Medical Biochemistry Leopoldo de Meis
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Brazil

Main Report

Awardee: Dr. Mychael Lourenco, Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro

Background

Alzheimer's disease (AD) is a severe disorder of memory/cognition affecting more than 35 million people worldwide [1]. To date, no effective preventive approach or treatment has been developed, and pharmacological interventions have had limited benefits [2]. Hallmarks of AD neuropathology include synapse loss, formation of intracellular neurofibrillary tangles composed of hyperphosphorylated tau, and accumulation of extracellular amyloid-β (Aβ) in senile plaques [3, 4]. Although it was originally thought that amyloid plaques were the toxic aggregates responsible for neurodegeneration and cognitive dysfunction, mounting evidence has identified small, soluble Aβ aggregates, known as Aβ oligomers (AβOs), as central neurotoxins in AD [5]. AβOs bind to synapses [6, 7] and trigger neurotoxic cascades resulting in synapse failure/loss reminiscent of AD [8-10]. AβOs further disrupt synaptic plasticity, as evidenced by impaired long-term potentiation (LTP) in the hippocampus [5, 8, 11, 12], providing a likely basis for memory impairment in AD [13, 14, 15].

Physical exercise has emerged as a non-pharmacological approach with demonstrated benefits for both brain and body, including in metabolic dysfunction caused by diabetes and/or AD. Significant evidence has indicated that brain-derived neurotrophic factor (BDNF) and other neurotrophins are released in the brain upon exercise. We have been interested in understanding how exercise could be neuroprotective in AD models and it has come to our attention the recent description of irisin, an exercise-triggered factor (generated from a precursor protein termed FNDC5) that is expressed both in the muscle and in the brain and that reduces peripheral insulin resistance in obese mice [16,17]. For instance, irisin appears to have neuromodulatory effects in central neurons, including BDNF upregulation [18].

Irisin is an exercise-induced myokine recently identified as a regulator of peripheral energy metabolism. Irisin is produced from cleavage of a precursor protein, fibronectin type III domain-containing protein 5 (FNDC5), in muscle and is released in the circulation [16, 17]. Importantly, FNDC5/irisin is expressed in the brain, notably in the hippocampus, where it

regulates the expression of brain-derived neurotrophic factor (BDNF) [18]. Brain FNDC5/irisin expression is controlled by PGC-1α [18], a transcriptional co-activator that is downregulated in AD brains.

Notably, both BDNF and PGC-1α have been reported to exert neuroprotective actions in experimental models of AD [19, 20]. Collectively, these observations raise the possibility that hippocampal FNDC5/irisin may constitute a novel neuromodulator with pro-cognitive actions.

Our hypothesis was that downregulation of FNDC5/irisin impairs synapse and memory function in AD. Thus, replenishing brain FNDC5/irisin might protect against synapse and cognitive impairment in AD. In this grant proposal, we aimed to:

1) Determine whether reduced brain FNDC5/irisin expression contributes to memory deficits.

2) Establish whether exercise-derived FNDC5/irisin mediates neuroprotection in mouse models of AD.

Summary of results and conclusions

To first address whether reduced brain levels of FNDC5/irisin would result in impaired cognition, we used lentiviral vectors harboring shRNAs targeting *FNDC5/irisin*. These lentiviral vectors were injected into the lateral ventricle of male 2-3 month-old WT C57BL/6 mice, and were allowed to express for four weeks. After this period, mice were subjected to memory tests, including novel object recognition, contextual fear conditioning and two-day radial arm water maze. Appropriate controls were performed.

Our results indicated that downregulation of brain FNDC5 was indeed effective and triggered selective novel object recognition memory impairment, but not fear or spatial memory (Fig. 3 in Lourenco et al, Nat Med, 2019; [21]). Importantly, knockdown of FNDC5/irisin did not result in any evident weight or locomotion alteration (Extended Fig. 5 in [21]). Taken together, these results indicate that lower brain levels of FNDC5/irisin could result in memory deficits and potentially account for AD-linked cognitive failure.

We next aimed to understand whether FNDC5/irisin could mediate the beneficial actions of exercise in AD models. We found that irisin levels were indeed higher in brains of mice undergoing exercise. Consistently, AD mice that exercised had better performance in novel object recognition memory tasks than sedentary ones (Fig. 6 and Extended Fig. 10 in [21]). Most interestingly, however, was the fact that intraperitoneal injections of a selective anti-

FNDC5/irisin antibody ablated any beneficial action of exercise on memory in AD mice, when compared to mice receiving control irrelevant immunoglobulins (Fig. 6 in [21]). Thus, our results suggest that peripheral FNDC5/irisin levels may be relevant for neuroprotection induced by regular physical exercise. Future work is required to elucidate the molecular intricacies of exercise actions in the brain, and to assess the clinical relevance of stimulating 'exercise factors' in AD.

I consider that the most important outcome of this award was the completion of an ambitious project to comprehensively understand the neuroprotective potential of exercise-linked FNDC5/irisin in mouse models of AD. Results, with significant contributions of the funding provided by ISN CAEN, have been included in the manuscript "Exercise-linked FNDC5/irisin rescues synaptic and memory defects in Alzheimer's models" [21], now published online in Nature Medicine. This is an important outcome not only because it may reveal yet unexplored therapeutic roads in AD, but also because it uncovers novel molecular mechanisms of exercise in the brain and offer glimpses on the actions of the still barely studied irisin. After obtaining these intriguing but compelling results, we look forward to better understanding the brain actions of exercise. Therefore, this project notably funded by ISN also opened new scientific roads for our research group. Additionally, two review articles have been published by our group [22, 23], and both properly acknowledged the ISN funding granted to our lab.

Financial Report

We were awarded a total of US$ 5,000 from the ISN CAEN 1B funds that were instrumental to develop the proposed activities. Please find below a summary of our expenses under the total granted budget:

Description	**Value (USD)**
ISN/CAEN 1B	5,000.00
Total - Award	**5,000.00**
Basic chemicals and reagents (Sigma Aldrich, Life Technologies)	1,000.00
Reagents for Western blotting (antibodies, gel reagents)	1,500.00

Reagents for qPCR (primers, fluorescent DNA labeling kits)	1,500.00
Reagents for cell culture	1,000.00
Total – Expenses	**5,000.00**

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