2023 IMPACT CIRCLE

Project Title: **Reprograming the proteostasis network to sustain brain function during aging and extend healthspan.**

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Unmet Need/Primary Question: Decades of research have defined the hallmarks of aging, biological processes that determine when and how organisms age (1,2). Brain aging is the main risk factor to develop mild cognitive impairment and neurodegenerative diseases, associated with the abnormal deposition of protein aggregates in the brain. Thus, the pathways that sustain the proteome homeostasis (termed "proteostasis") are substantially altered in the elderly (3). Proteostasis requires the complex and dynamic coordination of folding, quality control and degradation mechanisms to reduce the load of abnormal protein aggregation (4). One of the main nodes of the proteostasis network altered during aging involves the function of the endoplasmic reticulum, a central site for protein production in the cell. When misfolded proteins accumulate inside the ER, a dynamic signaling network known as the unfolded protein response (UPR) is activated, which aims to restore proteostasis and sustain cell function (5, 6). Adaptation to ER stress required the reprograming of gene expression to stablish adaptive responses that improve the activity of multiple processes involved in proteostasis control. In fact, the UPR is governed by the concert action of three main transcription factors, ATF6, XBP1 and ATF4, regulating distinct subsets of target genes. Our laboratory has been pioneering in defining the role of the UPR in brain diseases, normal brain function and more recently brain aging. In collaboration with Pablo Sardi in Genzyme-Sanofi, we recently developed a gene therapy to artificially boost the proteostatic capacity of the brain by delivering an active form of the transcription factor XBP1, which improved synaptic plasticity and memory of aged animals (7). However, ATF6 has remained completely unstudied in aging and brain function. Upon ER stress, ATF6 is cleaved and activated as a potent transcription factor that translocates to the nucleus to engage gene expression programs involved in folding, quality control and protein degradation pathways (**Figure 1**, annex) (8, 9). The role of ATF6 in human diseases has been poorly explored until recently, where targeting this pathway is beneficial in different context (10), including retinal degeneration (11), autoimmunity (12), heart disease (13, 14) and stroke (15). Our collaborator Luke Wiseman (Scripps Institute) discovered compound 147, a selective molecule that activates ATF6 to artificially boost the UPR. Interestingly, the intravenous administration of compound 147 protected the heart and brain from ischemia/reperfusion (I/R) damage (14), indicating this compound 147 can efficiently reach the brain presenting a neuroprotective activity. We have also developed a different approach to artificially deliver the processed active from of ATF6 into the brain using a gene therapy approach (17), showing outstanding effects that are superior to XBP1 in different diseases (unpublished). Thus, ATF6

represent a completely unexplored new pathway that could be intervened to improve brain health span. We have obtained preliminary evidence indicating that the administration of adeno-associated viruses (AAV) to express active ATF6 into the hippocampus of mice recovers the defects in memory and synaptic plasticity naturally occurring in aged animals.

Novel Hypothesis: The pathways that determine the decay of brain function as we age are not well understood. Thus, it is necessary to evaluate new concepts and identify novel targets. Because the decay in the buffering capacity of the proteostasis network is a central pillar of the aging process, and ATF6 is a master regulator of the UPR, we propose that the artificial activation of the ATF6 pathway may delay normal brain aging. Here we propose to uncover the possible contribution of the ATF6 signaling branch to brain aging and develop a proof-of concept study aiming to globally improve neuronal proteostasis by engaging UPR-dependent gene expression programs to extend brain healthspan.

Project Proposal: We plan to determine the significance of the ATF6 pathway as a central component antagonizing brain aging (annex Figure). Our supporting data indicates that the delivery of active ATF6 into the hippocampus of mice using AAVs improves cognition and synaptic plasticity of aged animals. Our experimental strategy will include the establishment of a collaborative network with Buck researchers and external collaborators to test a pharmacological strategy and a gene transfer approach to artificially engage the ATF6 pathway in the brain of aging mice, followed by a functional analysis of cognition, proteomic changes and histopathological alterations including the content of senescent cells. We aim to deliver AAVs into the ventricle of aged mice (18 months) to express ATF6 in neurons or administrate intravenously the compound 147 and assess cognitive and motor tests followed by electrophysiological (LTP) and detailed morphological characterization of dendritic spines, senescence, inflammation and neuronal health. Finally, we aim to determine the global impact of ATF6 expression on the proteomic changes observed in the hippocampus during aging as we recently reported on a large brain aging study (7).

Description of Potential Impact: The brain is a complex organ that controls thought, memory, emotion, touch, motor skills, vision, breathing, temperature, hunger and every process that regulates our body. As we age, the function of our brain decays, having enormous consequences in our quality of life and our families. The progressive loss of the proteostasis capacity contributes to neuronal dysfunction and may represent one of the main risk factors to accumulate abnormal protein aggregates and develop neurodegenerative diseases (reviewed in 3). Understanding the pathways that mediate brain aging are fundamental to intervene the process to sustain health. We have undertaking a non-conventional approach to address this fundamental question where instead of targeting one gene (the classical approach of pharmaceuticals and research labs), we plan to engage a full network of processes that as a whole will improve neuronal proteostasis and cell function (holistic view). We believe that the concept behind our possible findings is "rejuvenation": we aim to artificially engage an adaptive capacity that young cells naturally have and that is loss as we age. We predict that ATF6-deppedent transcriptional programs will enforce folding, quality control, protein degradation pathways, and other unknown processes to be uncovered, that altogether will improve neuronal function and produce healthy proteins (i.e. synaptic proteins). Biomedicine is suffering transformative changes because of the concept of geroscience: our health system (that is currently a "sick care system") will turn into a "health care system", were medicine will seek to develop strategies that avoid the appearance of disease by targeting the biological pillars of aging. Since gene

therapy with AAVs is a reality in the US due the recent approval of several technologies based on AAVs, if this proposal is successful (and all follow-up initiatives), this knowledge may translate into the development of cutting-age approaches to sustain brain function as we age.

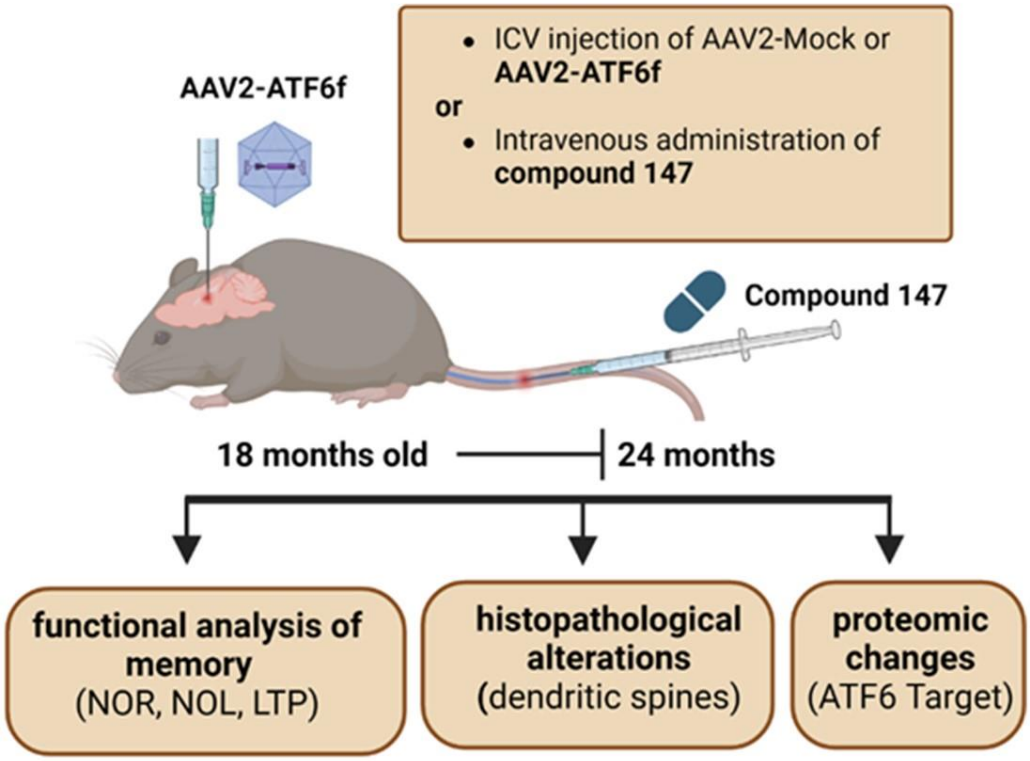

Figure. Idea and workflow: We plan to define the possible role of the UPR transcription factor ATF6 in brain aging and how it determines the functional decay on its function. We plan to develop a 1-year project (proof-of-concept) to test strategies to artificially engage the UPR using pharmacological and gene therapy strategy and restore the buffering capacity of the proteostasis network to restore neuronal function. Animals of 18 months of age that already show cognitive impairment, synaptic alterations and accumulation of senescent cells (see our characterization in (7)) will be manipulated with two aproaches to artificially engage the ATF6 pathway and improve proteostasis: a gene therapy (stereotaxis injection of AAV2-ATF6f into the hippocampus) and a pharmacological strategy (IP delivery 3 times per week of compound 147) to either deliver active ATF6 or induce the activation of the endogenous stress sensor. 22-24 month later, cognitive assessment will be performed (new object location (NOL) and new object recognition (NOR)), in addition to electrophysiological measurements in hippocampal slices (long term potentiation (LTP)) and morphological characterization (dendritic spines), and protein expression profiling (proteomics, collaboration with B. Schilling).