SUMMARY:

I. Title and summary of the proposal research.

Neurovascular pathways in aging and neurodegenerative diseases: integrating cardiovascular health and brain function

Aging is a main risk factor for neurodegenerative diseases (ND), such as Alzheimer's disease (AD) and frontotemporal dementia (FTD) [1]. The burden of age-associated brain pathology and cognitive decline is especially high in Chile, the South American country projected to have the highest aging population in the coming decades. Life expectancy in Chile rises by 4.2 years per decade, leading to a rise in the proportion of individuals older than 60 years from 15.7% to 32.8% by 2050 [3]. With this demographic shift, the incidence of ND is expected to double in the next 20 years, becoming one of the leading causes of death among the elderly in Chile [4].

The burden of age-associated cognitive decline and brain pathology is influenced not only by genetics but also by potentially modifiable environmental risk factors, such as social determinants of health (SDH) and lifestyle choices that are detrimental to physical and mental health [5]. In particular, cardiovascular health (CVH) metrics are associated with cognitive impairment and the incidence of ND in aging individuals [6]. Approximately half of clinically probable AD cases present with microvascular infarcts, and the extent of cognitive impairment is exacerbated in cases with concurrent vascular pathology [7]. Notably, CVH could be improved through psychosocial and behavioral interventions, however, prevention trials require specific biomarkers to target the effect of neurovascular integrity on brain function, which are lacking [8].

Currently, the characterization of brain function in neuroimaging studies of ND and dementia is overwhelmingly overrepresented, often failing to account for the energetic demands supplied by brain vasculature [9]. In this project, we will develop data- and model-driven approaches to characterize neurovascular pathways in aging and ND. We will combine structural and functional magnetic resonance imaging (MRI) neuroimaging, assessments of CVH and SDH, and computational models for mechanistic inference. Our main goal is to investigate the integrity and dynamics of neurovascular coupling as specific biomarkers for pathological aging and ND, linked to CVH and predictive of cognitive decline and disease status. This will be achieved by addressing the following three aims:

1. **Characterize vascular structure and dynamics**. We will analyze vascular structure and dynamics in healthy aging and ND patients, correlating cerebral perfusion and the hemodynamic response function (HRF) with white-matter hyperintensities (WMHI) measured with fluid attenuated inversion recovery (FLAIR), a surrogate marker of cerebral small vessel disease, previously linked to increased likelihood of cognitive impairment and ND [10].

2. **Develop next generation biophysical models**. We will create advanced biophysical models capable of disentangling vascular and neural contributions to neuroimaging biomarkers for ND, providing a clearer understanding of the underlying mechanisms.

3. **Investigate neurovascular integrity and environmental risk factors**. We will explore the connections between neurovascular integrity and other environmental risk factors, enhancing our comprehension of how these factors interplay in the progression of ND.

We hypothesize that cerebral blood flow volume, latency and amplitude will reflect vascular integrity, as captured by WMHI lesions. Moreover, we hypothesize that the inversion of a biophysical model of whole-brain activity will disentangle neural and vascular contributions to MRI-based biomarkers,

establishing direct connections between cerebral blood flow dynamics and mechanistically-interpretable variables, such as the intensity of the local vasodilatory signal, and alterations in the biomechanical properties of blood vessels. Finally, we expect to find correlations between mechanistically-interpretable parameters of vascular integrity and CVH and SDH assessments, given the impact of social and economic disparity on CVH [11].

Our proposal is posed to profoundly impact clinical research while also producing concrete translational applications. Despite large international collaborative consortiums aimed at consolidating neuroimaging-based biomarkers for health and pathological aging trajectories, these efforts struggle to inform the specific contributions of vascular and neuronal factors to the developed biomarkers . Data sources such as functional MRI (fMRI) and WMHI are analyzed independently, even though the vascular contribution to the hemodynamic response measured by fMRI is well established [13]. Moreover, this contribution is frequently modeled as noise to be removed from the data instead of being parsimoniously incorporated to the process of biomarker discovery [12]. Neuroimaging-based biomarkers are routinely adopted in traditional clinical trials investigating potential treatments for ND [14, 15]. However, trials assessing the outcome of preventive interventions targeted to mitigate CVH risk factors cannot succeed without an adequate and specific baseline characterization of neurovascular integrity [8], currently unavailable with the existing neuroimaging data sources and analysis methodologies.

Finally, our study goes beyond the development of data-driven biomarkers, aiming to provide a novel mechanistic characterization for the study of how CVD relates to the variance in brain atrophy, cognitive deficits, pathological protein accumulation, and disease trajectories observed across patients.

II. Research question and problem of the research proposal.

The advent of non-invasive neuroimaging has revolutionized our understanding of ND and dementia, as well as our diagnostic and prognostic capabilities. In spite of this success, there have been few advances concerning the mechanisms underlying neuroimaging-based biomarkers. In particular, fMRI measures oxygenated blood flow as a proxy for neural activity, yet aging individuals and ND patients often present vascular damage capable of exerting significant influence on the recorded signals. While other techniques such as arterial spin labeling (ASL) provide more directly interpretable measures of blood flow, they are not as widespread as blood oxygenation level dependent (BOLD) fMRI. A profound knowledge gap exists concerning the link between CVH, neurovascular integrity, and their influence on MRI- and fMRI-based biomarkers. As a result of this gap, with the current analytical methodologies even the most sophisticated MRI technologies are unable to selectively inform the vascular and neuronal dimensions contributing to the measured alterations. This information is crucial to assess the efficacy of interventions aimed at mitigating CVH risk factors contributing to ND and dementia, and to understand how CVH mediates risk factors related to social and economic disparities. Thus, without advancing our integrated understanding of CVH and brain function, basic and translational research faces significant challenges, including a) interpretability problems concerning available biomarkers, b) insufficiently specific biomarkers to conduct prevention trials, c) an overall inadequate understanding of environmental risk factors.

Our research will address the following research questions aimed at overcoming these challenges in the field:

1. How does healthy and pathological aging impact neurovascular dynamics, specifically in terms of the amplitude and latency of the cerebral blood flow supply that occurs in response to neural activation?
2. Is there a relationship between the dynamics of cerebral blood flow supply (measured using fMRI and ASL) and surrogate markers of vascular damage (quantified in terms of WMHI)?

3. What are the separate contributions of neural and vascular sources to fMRI-based biomarkers of ND and dementia?
4. What are the mechanisms behind these contributions, expressed in terms of mechanistically-interpretable variables (neural excitation/inhibition, vasodilatory signal, biomechanical properties of vascular tissue)?
5. How does CVH mediate the effect of social and economic disparities on the risk of developing ND and dementia?

III. Characteristics of high novelty and disruption.

Our project is posed to draw novel connections between neurovascular integrity and brain function in aging individuals and ND patients. While large international consortia compile imaging data from cohorts of ND and dementia patients, and from aging individuals with significant risk factors, our proposal is the first to directly integrate BOLD fMRI data (vascular dynamics), ASL (cerebral perfusion), and FLAIR-detected WMHI (vascular structure) to yield robust quantitative and mechanistic metrics of brain vascular integrity.

Furthermore, our proposal pioneers the leveraging of biophysical simulations to separately infer neural and vascular mechanisms underlying fMRI-based biomarkers for ND and dementia. The lack of specificity regarding neural and vascular sources is a striking deficiency of nearly all existing fMRI-based biomarkers, as fMRI measures a surrogate marker of neural activity fundamentally mediated by vascular response. Our critical analysis of these contributions is long overdue in the field, and further highlights the novelty and timeliness of our proposal.

Even though our project requires new data to develop and validate our novel methodology, once established it will be straightforwardly applicable to large pre-existing cohorts of patients and healthy controls examined with neuroimaging. This broad applicability maximizes the disruptive potential of our methods. An integral part of this proposal is the widespread application of the developed methodology to data from the Multi-Partner Consortium to Expand Dementia Research in Latin America (ReDLat), a large collaborative consortium including participants from Chile and the region. These collaborations enhance the disruptive aspect of our proposal by allowing us to connect our mechanistic interpretation of the data with comprehensive assessments of CVH and SDH. Finally, the results of this proposal will serve as the basis of future trials designed to assess the outcome of preventive interventions targeting CVH risks for ND and dementia, representing a concrete and disruptive translational application.

IV. Uncertainty involved in the research proposal.

Our proposal is composed of multiple aims with varying degrees of uncertainty. This layered approach guarantees that the most uncertain or speculative analyses are grounded on robust results, contributing to the overall risk mitigation of the project.

Extensive previous results demonstrate the variability of the hemodynamic response measured with fMRI and its interpretation in terms of the structure and dynamics of neurovascular coupling. On the other hand, WMHI measured with FLAIR are validated as makers of small vessel integrity. While the detection of cerebral microinfarcts is an alternative method it requires high-field MRI sequences that are not widely available. Thus, hypothesizing a link between these two neuroimaging domains is well supported by available evidence, even though uncertainty remains as this is a novel endeavor.

The model-based inference of vascular and neural mechanisms carries more relative uncertainty in our project. Our previous research shows that biophysical models of whole-brain activity (namely, the dynamic mean field model) can be fitted to fMRI data from aging individuals and ND patients to extract mechanistically-interpretable parameters. However, to date, this has never been attempted by also including neurovascular coupling as part of the model optimization. The worst-case scenario would require adopting more detailed models of neural activity (e.g. neural mass models, models including adaptation) and vascular coupling, which are likely to provide an improved goodness of fit at the expense of higher computational complexity. This added complexity could potentially slow down the analysis process and require more computational resources, but it would also enhance the accuracy and robustness of our findings.

V. Transformative potential and high reward regarding its results or the development of the research process.

The transformative potential of our project is very high, as it promises to correct the long-standing and systematic conflation of vascular and neural effects in neuroimaging assessments of pathological aging, ND and dementia. By separately informing these two contributions and their mechanistic basis, our project will open the way to study neurovascular pathways in large pre-existing datasets and to design protocols for acquiring new data, bearing in mind our methodological advances. An especially transformative dimension of our proposal is its translational application to assess the outcome of prevention trials. This is highly relevant in the context of ND and dementia, given the lack of effective treatment options. Our approach will support interventions targeted at mitigating risk factors at the behavioral level, including measures to minimize the detrimental effects of CVH.

While our research is expected to produce significant rewards at the global level, it will be especially impactful within the context of Chilean public health. Chile faces a rapid increase in the prevalence of ND due to its aging population, which will expand considerably in the coming decades. This increase is also driven by preventable risk factors that are overrepresented in the Chilean population. Nationwide surveys conducted in recent years indicate that more than half of Chile's adult population present CVH risks, including hypertension, overweight/obesity, hyperlipidemia, and nicotine dependency. Importantly, past research supports associations between CVH and cognitive impairment in the Chilean population. This suggests the feasibility of addressing these risk factors, heightening the transformative impact of our proposal. By advancing our understanding of neurovascular integrity and its contributions to ND and dementia, our project aims to provide a robust foundation for future prevention and intervention strategies. This will not only enhance our diagnostic and prognostic capabilities but also pave the way for effective public health measures tailored to the unique challenges faced by the Chilean population.

VI. Methodologies.

Participant cohorts. We will recruit 300 adult participants (ranging between 20 and 80 years, with at least 100 participants in the range 50-80 years). In this sample, we will obtain task and resting state BOLD fMRI, T1 and T2 images, FLAIR to quantify WMHI lesions, DTI, and ASL to quantify cerebral perfusion. Tasks performed during BOLD fMRI acquisition will consist of an hypercapnic challenge (breath holding task) designed to map spatial variability in the HRF due to variations in the underlying vasculature [16]. The task-based HRF shape estimation will be compared with a resting state estimation based on the pre-existing dataset from the Multi-Partner Consortium to Expand Dementia Research in Latin America (ReDLat) [17], including neuroimaging data (BOLD fMRI, T1, T2, FLAIR) subdivided in patients recruited in USA and across 10 centers in Latin America (Latam), and also includes healthy controls with demographics matching patients (Latam, N=327; USA,

N=197). Patient groups include AD (Latam, N=288, USA, N=119) and FTD (Latam, N=205, USA, N=143).

Assessment of CVH and SDH. For CVH, we will measure self-reported physical activity levels and diet, smoking status, body mass index, cholesterol level, blood sugar level, and blood pressure. Socioeconomic factors will be assessed with the ESOMAR scale, and social determinants of health (SDH) with a validated questionnaire from ReDLat [5]

Neuroimaging data analysis. Neuroimaging data preprocessing will be based on the ReDLat protocol (fMRIPrep) to ensure harmonization between datasets. This will result in whole-brain inter-areal functional connectivity (FC) and structural connectivity (SC), and anatomical maps for WMHI, brain perfusion, and gray matter atrophy w-scores. A DTI-based procedure will be applied to determine the cortical burden of WHMI and compared with brain perfusion measured with ASL. The estimation of the HRF shape will be pursued using task-based methodology (general linear models applied to the hypercapnic challenge task) and applying a deconvolution algorithm to resting state data (rsHRF toolbox) [18]. After assessment of consistency between methodologies, the resulting HRF parameters will be correlated with local cerebral perfusion, cortical WMHI burden, and with measures of CVH and SDH.

Biophysical model. An overview of the modeling procedure is shown in Figure 1 (adapted from [19]). The implemented DMF network model will simulate spontaneous brain activity across the entire brain with individual brain areas represented as nodes interconnected by white matter pathways (SC), fitted to optimize individual whole-brain (FC) of each group of participants. Based on our previous research [19], parameter optimization will encompass the scaling of the global coupling, parameters related to neuronal E/I balance (feedback current inhibition), and related to the neurovascular coupling interpreted as the effect of excitatory activity on the vasodilatory signal, the effect of vascular stiffness on blood inflow/outflow, and the characteristic blood transit time in the vessels. Following previous studies, we will allow for regional variability in these parameters by introducing anatomical priors related to E/I balance (gray matter atrophy) and vascular integrity (WMHI cortical burden, cerebral perfusion) [19].

VII. Bibliography.

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VIII. Supplementary Figures.

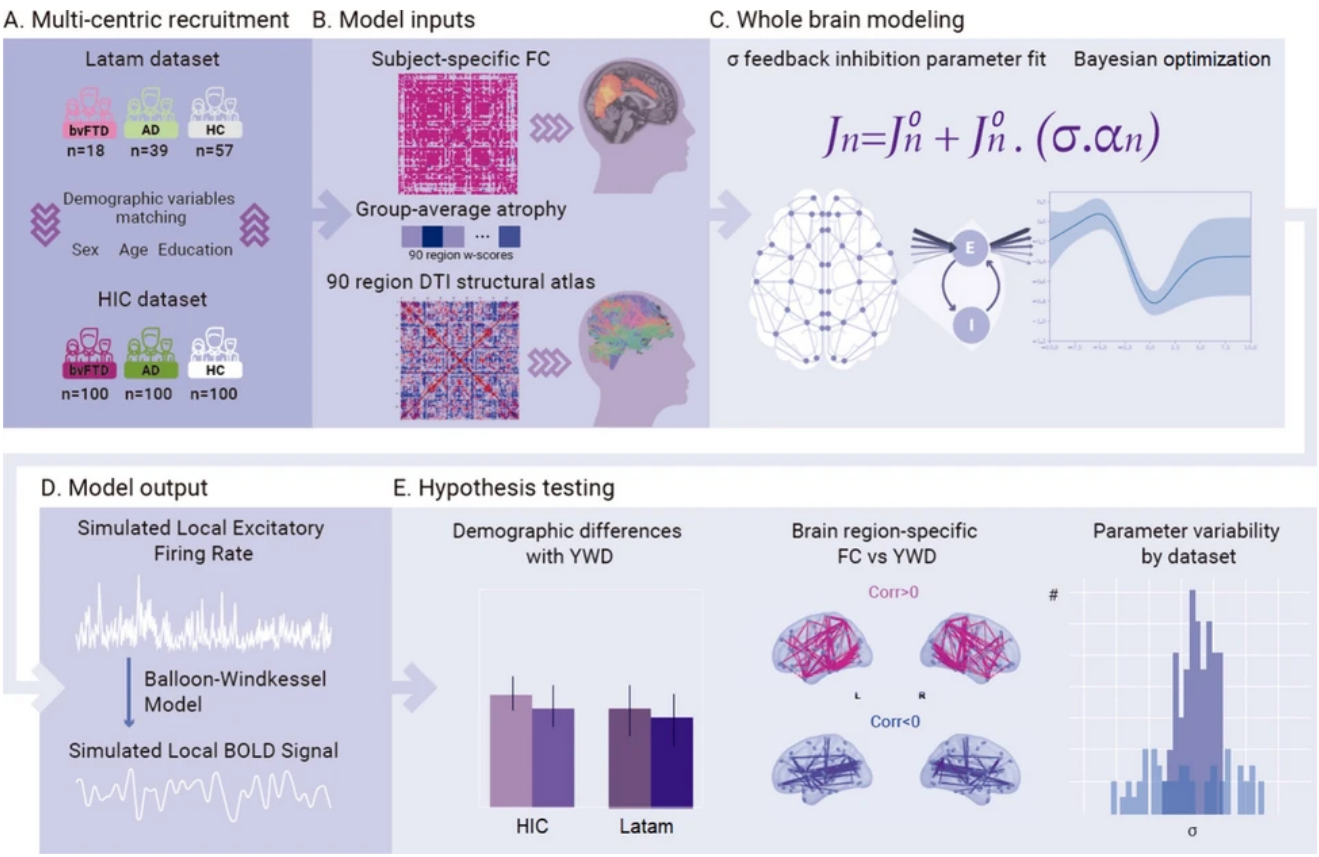

Figure 1. Overview of the dynamic mean field (DMF) modeling procedure. A) The model is applied to different groups with resting state fMRI data, defined in terms of disease status, as well as the socio-economic backgrounds and geographic origins (Latin America vs. USA). **B)** The optimization target is the subject-specific functional connectivity matrix (FC), and the local dynamics of the model are connected via the structural connectome (SC) inferred from DTI recordings. **C)** To allow regional heterogeneity, the model parameters are modulated using spatial maps with relevant priors, including gray matter atrophy, cerebral perfusion (ASL) and cortical WMHI burden (FLAIR). **D)** The model simulates the local firing rates, which are converted to the measured hemodynamic response using the Balloon-Windkessel model for neurovascular coupling. Instead of adopting fixed parameters for this model, we propose to include it in the optimization procedure, allowing the inference of mechanistically-interpretable parameters related to the vasodilatory signal and vessel tissue biomechanics. **E)** After fitting the model, the resulting parameters are used to test hypotheses, such as inter-site differences (Latin America vs. high income countries), and correlations with CVH and SDH. Figure adapted from [19].