PROPOSAL RESEARCH:

1. THEORETICAL BACKGROUND AND PROPOSAL OVERVIEW

1.1. Neurodegenerative diseases and cardiovascular health

Neurodegenerative diseases (ND) present a significant public health challenge for Latin American countries, especially those experiencing rapidly increasing life expectancy[1,2], such as Chile. Chile is projected to have the highest aging population in the region in the coming decades[3,4]. These conditions, including Alzheimer's disease (AD)[5] and frontotemporal dementia (FTD)[6], are characterized by the progressive and irreversible deterioration of brain tissue, leading to both general and domain-specific cognitive impairments[7]. Given the current lack of effective treatment, early detection and risk mitigation strategies[8] are crucial. Cardiovascular health (CVH) emerges as a key predictor of healthy aging[9] and a natural target for interventions addressing modifiable risk factors[8]. However, our understanding of neurovascular pathways in aging and ND remains insufficient for the development of reliable and specific biomarkers, particularly those based on non-invasive functional and structural neuroimaging techniques commonly used in clinical and research settings[10]. Additionally, the mechanisms linking cardiovascular diseases (CVD) and ND are not yet fully understood[11]. Addressing these challenges requires the development of mechanistic models capable of disentangling the vascular and neural contributions to neuroimaging-based biomarkers. Such models would be widely applicable to existing data, thereby enhancing our understanding of CVH and CVD as risk factors for ND[12].

1.2. Brain vascular integrity in neurodegenerative diseases

Convergent evidence indicates links between compromised brain vasculature during aging, ND and dementia[13,14]. Studies using transcranial Doppler show an inverse correlation between cerebral blood flow (CBF) velocity and the likelihood of developing ND[15], while reductions in CBF predict disease progression in AD patients[16]. A large-cohort longitudinal study indicates that CBF and other factors related to brain vasculature present changes preceding the detectability of classical biomarkers based on amyloid and tau proteins[17]. Cerebrovascular reactivity is also related to disease status in ND and dementia, indicating impaired vasodilation believed to reflect downstream amyloid-mediated vasculopathy[18,19]. Vascular contributions influence the blood-oxygen-level-dependent (BOLD) signal in functional magnetic resonance imaging (fMRI), reflecting the complex interplay of CBF and the cerebral metabolic rate of oxygen consumption ($CMRO_2$)[20]. The connection between these two factors can be disrupted by vascular structural compromise—i.e., altered cerebrovascular structure, reduced blood vessel elasticity, and atherosclerosis—and by reduced vascular reactivity to chemical modulators, which can potentially alter the BOLD response in aging and ND without concomitant changes to neural activity[13,21]. This points towards the need to disentangle vascular and neural components in the development of fMRI-based biomarkers of pathological aging trajectories[22,23]. Compromised vascular integrity also plays a role in healthy aging, where diminished blood flow speed and cerebral perfusion has been reported, together with a decline of cerebrovascular reactivity[24-26]. An example of age-related vascular effects on the BOLD signal can be found in the disruption of the normal shape of the hemodynamic response function (HRF), i.e., the shape of the BOLD signal arising to an impulse activation[27-30].

The causal connection between vascular structure and dynamics compromised and ND remains obscure[31]. It has been proposed that advanced aging together with vascular risk factors may result in reduced brain perfusion, challenging the metabolic needs of vulnerable neurons and triggering a progressive circulatory insufficiency, ultimately causing neurodegeration, formation of senile plaques, neurofibrillary tangles and amyloid angiopathy[13,14]. According to this model, the early mitigation of vascular risk factors could contribute to reduce and/or delay the onset of ND in aging populations[10].

1.3. State of the art non-invasive methods to assess vascular integrity

The dynamics of peripheral vascular function can be investigated using multiple neuroimaging methods, such as blood oxygenation-level dependent (BOLD) MRI, arterial spin labeling (ASL); phase contrast; high-resolution MRI and dynamic MR oximetry to quantify oxygen saturation[32]. Among these options, fMRI BOLD imaging has the advantage of being the standard MRI-based measure of neural activity, of widespread availability in the majority of large-cohort neuroimaging studies[33]. In contrast to ASL, however, the BOLD signal does not directly reflect CBF[23]. Resting-state fMRI has been intensively used to investigate healthy aging, ND and dementia[34]. Brief neural events initiate a sequence of metabolic and vascular events leading to the HRF as measured with BOLD fMRI, the shape of this response reflecting cerebrovascular reactivity and neuro-vascular coupling integrity[35], while being sensitive to alterations in healthy and pathological aging[27-

[29]. A standard non-invasive methodology to investigate the HRF is the hypercapnic challenge, consisting of a sequence of breath holding tasks performed during BOLD signal acquisition with the simultaneous recording of respiration via a belt attached to the participants[36]. Breath holding elevates the blood concentration of CO2 leading a vasodilatory response which allows the estimation of cerebrovascular reactivity via changes in the BOLD signal[37]. An alternative is a data-driven framework for the inference of HRF shape parameters based on resting state fMRI using BOLD signal deconvolution[38,39]. In contrast to task-based HRF measures, this can be applied to large databases of pre-existing resting-state data gathered under standardized conditions.

Standard neuroimaging-based assessment of vascular structure relies on white-matter hyperintensities (WMHI) measured using the magnetic resonance imaging fluid attenuated inversion recovery (FLAIR) sequence[40]. WMHI lesions are a surrogate marker of cerebral small vessel disease, correlate with age and increased likelihood of cognitive impairment and dementia, and are related to CVH (**Fig. 1**)[41-44].

1.4. Rationale and proposal description

This proposal consists of a novel multi-staged and multimodal approach to integrate vascular assessments to the neuroimaging toolbox available for the study of healthy and pathological aging trajectories. Even though neuroimaging-based markers of altered vascular dynamics and integrity exist[13,41,43], they have not been assessed simultaneously in populations of aging individuals and patients diagnosed with ND. Therefore, it is currently unknown how metrics of neurovascular coupling dynamics (BOLD HRF parameters) simultaneously relate to CBF (measured with ASL), and cerebrovascular reactivity (measured with hypercapnic challenge) in healthy aging and dementia, and how they relate to WMHI lesions as structural markers of vascular compromise. Also, the mechanistic contribution of these alterations to brain activity and connectivity measured with fMRI remains to be elucidated[13,14]. Thus, without advancing this integrated understanding of vascular integrity, CVH and brain function, basic and translational research faces significant challenges, including a) interpretability problems concerning available neuroimaging data[22], b) insufficiently specific biomarkers to conduct prevention trials[10], c) an overall inadequate understanding of environmental risk factors, given the known link between socioeconomic status (SES), social determinants of health (SDH) and CVH[45].

2. HYPOTHESIS

Based on previous reports of task-based HRF estimates in aging, we hypothesize that older healthy controls will present increased HRF latency and decreased amplitude relative to younger participants[28], and that this effect will be heightened in cognitively impaired patients[29]. We also hypothesize an inverse relationship between global and regional WMHI lesion volume and the HRF amplitude and latency, pointing towards an effect of compromised vascular integrity on the local oxygenated blood flow supply[46]. Fitting a biophysical model to the empirical data, we expect to find that the cortical distribution of WMHI burden will modulate model parameters related to cerebral blood flow supply in response to the local vasodilatory signal, reflecting compromised vascular integrity[40]. Conversely, we hypothesize that HRF changes in healthy aging without significant WMHI burden will be better captured by neural parameters related to excitation-inhibition balance, indexed by the local grey matter atrophy values (**Fig. 2**)[47]. Finally, we expect correlations between model-based measures of vascular integrity and markers of cardiovascular health, given the impact of SES, SDH on CVH[45].

3. GENERAL AND SPECIFIC OBJECTIVES

The main goal of this proposal is the development of quantitative and mechanistic biomarkers capable of disentangling neural and vascular effects of aging, ND and dementia, and investigate their association with CVH and other modifiable risk factors.

This goal will be achieved by developing the following three specific aims of the project:

- **Aim 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

- **Aim 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.
- **Aim 3**: Investigate neurovascular integrity and environmental risk factors. We will explore the connections between neurovascular integrity and other environmental factors, enhancing our comprehension of their interplay in aging and ND progression.

4. METHODOLOGY

4.1. Experimental plan overview

This project will include an initial phase of data acquisition and analysis for methodological validation, where the HRF shape parameters will be inferred from task data (hypercapnic challenge)[36] and compared with the results of data-driven estimation based on resting state signals[39]. HRF shape parameters will be correlated with regional CBF (measured with ASL) and with the cortical burden of WMHI lesions (projected to the cortical surface using probabilistic tractography obtained from diffusion tensor imaging [DTI])[48]. After examining their convergence, these metrics will be correlated with age in the healthy control group and extrapolated to the ReDLat cohort (N>1000) in the second phase of the project[49]. A model-based approach will be implemented to optimize the reproduction of empirical fMRI functional connectivity combining on realistic biophysical assumptions and multiple empirical data sources at the single subject level[47,50,51]. By optimizing the model to the data, we aim to disentangle mechanisms associated with neural contributions (interpretable as changes in neural excitation/inhibition parametrized by regional variations in grey matter atrophy) and vascular damage (interpretable vasodilatory signal strength, biomechanical properties of vascular tissue, parametrized by regional variations in HRF shape parameters and CBF). Fitting the model to individual subjects will result in mechanistic biomarkers that will be correlated with measures of CVH and SDH in a large cohort of controls and patients from Latin America (Latam) and high-income countries (HIC)[52].

4.2. Recruitment plan

We will recruit 300 adult participants (between 20 and 80 years, with at least 100 participants in the range 50-80 years). Subjects will be recruited through the Memory and Neuropsychiatric Clinic (CMYN and GERO FONDAP 15150012, University of Chile-Hospital del Salvador, directed by Co-I Dr. Slachevsky), and the BrainLat institute (directed by Co-I Ibañez, with recruitment of FONDECyT 1210195, and ReDLat project, Subjects will receive clinical, neurological, and neuropsychological evaluations. The screening process will consist of visits to the clinical centers, a neurological exam, cognitive tests, and medical history assessment. Participants will have no history of drug abuse, neurologic, or psychiatric disorders, and will show functional independence (Clinical Dementia Rating Scale-CDR = 0)[53]. Subjects will be excluded due to inadequate fluency in Spanish; inability to give informed consent or to complete neuropsychological tasks; criteria for psychiatric or neurologic disorders; recent history of substance abuse; visual and auditory deficits impeding cognitive testing; presence of ferromagnetic implants. From all participants, we will obtain informed consent, which will be submitted to the local Ethics Committee for approval.

4.3. Pre-existing data

The study will also include a cohort of pre-existing data from the Multi-Partner Consortium to Expand Dementia Research in Latin America (ReDLat), a multi-partner-funded network for dementia research set to acquire cognitive, neuropsychological, imaging, genetic and epigenetic data from patients and controls across 10 centers spanning 6 Latin American countries, with harmonized neuroimaging acquisition parameters and preprocessing, and variability in terms of geographic, socioeconomic and other environmental factors[49]. The ReDLat cohort can be 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). Consensus groups diagnosed participants with AD based on the National Institute of NINCDS-ADRDA clinical criteria[54,55]. This dataset will be used in our project to develop the methodology in ND and dementia patients, as a large-scale validation of our methodology in an independent sample of aging individuals, and to investigate the relationship between neurovascular integrity, CVH, cognition, and SDH in a heterogeneous multi-site sample including participants with diverse SES[52]. Criteria for FTD inclusion are focused on the most extended presentation of FTD (behavioral variant FTD; bvFTD)[56], and include fulfilling revised criteria for probable bvFTD as determined by a consensus group, with prominent changes in social behavior and personality (verified by caregivers), and frontal atrophy/hypoperfusion/hypometabolism[57,58]. Patient diagnoses are also

supported by an extensive battery of neurologic and neuropsychiatric assessments, in accordance with the ReDLat protocol (see "Participant assessment") below. Participants with a history of neurologic disorders, primary language deficits, psychiatric disorders, or substance abuse are excluded from the study. All data to be included in this study was obtained with the approval of the relevant Institutional Review Boards from each institution. Participants provided informed consent following the Helsinki Declaration, National Institutes of Health guidelines, and local regulations.

4.4. Participant assessment

The assessment off all participants included in our study will be based on the ReDLat harmonized protocol covering episodic memory, executive function, processing speed, language function, and neuropsychiatric symptoms (Neuropsychiatric Inventory Questionnaire) and the Mini–Mental State Examination (MMSE)[59]. Socioeconomic factors will be assessed with the ESOMAR scale, and social determinants of health (SDH) with a validated questionnaire from ReDLat[52]. Cardiovascular health will be assessed using self-reported physical activity levels and diet, smoking status, body mass index, cholesterol level, blood sugar level, and blood pressure[60].

4.5. Neuroimaging data acquisition

For the pre-existing data, volumetric T1, T2, T2*, FLAIR and BOLD resting state data was acquired from all participants at independent ReDLat participating centers in accordance with the ReDLat neuroimaging acquisition protocol and the Organization for Human Brain Mapping (OHBM) recommendations. During scanning sessions, participants were instructed to refrain from focusing on specific thoughts without falling asleep, keep their eyes closed, and avoid movements that could cause artifacts. Data collection included 3D structural volumetric and ~10-minute-long resting state fMRI sequences from all participants. For data acquired during this project, neuroimaging recordings will be obtained in a 3T scanner with scanning parameters based on ReDLat's neuroimaging protocol to ensure harmonization between datasets. T2* and FLAIR sequences will be included to assess vascular effects. fMRI will be acquired during rest with eye closed and during a breath holding task to investigate anatomical variability in the HRF (see "Hypercapnic challenge task" below). DTI will be acquired for personalized connectome estimation and to project the WMHI lesion burden to the cortical surface[48]. ASL data will be acquired with eyes closed, instructing subjects to avoid falling asleep.

4.6. Hypercapnic challenge task

The hypercapnic challenge task is implemented to measure cerebrovascular reactivity, used for the assessment of compromised vasculature[30,36]. Subjects will be monitored using a by a respiratory belt (Biopac Systems, Santa Barbara, CA) connected to the stimulus presentation computer as a parallel device. After calibration, subjects will perform 20 repetitions of a block with two phases: normal breathing and breath hold, with the timing of each breathing cued by different auditory tones. The physiological respiratory signal will be used to inform when they inhaled the target volume. Another auditory cue will indicate the timing of the release, 11 s after the initial tone. Previous research demonstrates that the neural activation due to the auditory tone is negligible in comparison to the changes induced by the vasodilatory response[30].

4.7. Structural and functional data pre-processing

The pre-processing of acquired data and the pre-existing ReDLat cohort will be harmonized. Structural data (T1) will be processed using DARTEL-SPM12 to estimate grey matter structural features[61]. Tissues will be segmented to estimate total intracranial volume (TIV)[62], and MNI standard space will be used for image normalization. Data will be smoothed using an isotropic 12 mm Gaussian kernel, surface based-morphometry will be processed using FreeSurfer on native space, volume, area and cortical thickness from a 400 region parcellation based on regional functional homogeneity (Schaefer parcellation)[63] will be obtained and normalized by TIV. FSL will be used to perform the following preprocessing steps to functional task and fMRI data: removal of the first five volumes, slice timing correction, head-motion correction, linear trend removal, Gaussian spatial filtering with 3 mm kernel, identification and removal of noisy components related to head motion using ICA as implemented in FSL MELODIC[64], and normalization to MNI152 space (FSL FLIRT). Data will be high-pass filtered (Butterworth filter) with 0.04 Hz cutoff to reduce low-frequency drift[65]. Given the objective of accounting for variability related to vascular response, regression of mean WM and CSF signals will not be implemented[66], however, residual head motion timeseries plus their first three derivatives will be regressed out of the data to account for remaining motion artifacts[67,68]. In resting state data, volumes with

relative displacements > 1 mm will be removed and interpolated, retaining data with less than 10% volumes removed; in task data, trials with > 1 mm relative displacements will be omitted from further analysis[67].

4.8. DTI data pre-processing

For the DTI analysis, regions in the structural connectivity network were defined based on the same parcellation used to extract functional time series (Schaefer parcellation)[63]. DTI data will be preprocessed using FSL diffusion toolbox (Fdt; standard parameters)[69]. The probtrackx Fdt tool will be used to estimate crossing fibers. The connectivity from a seed voxel i to another voxel j will be defined by the normalized count of fibers passing through both voxels, with >5000 streamlines per voxel. Both grey and white matter voxels will be seeded, and this procedure will be repeated for each region in the Schaefer parcellation, resulting in a probability matrix indicating the normalized streamline count between regions. A threshold will be applied (0.1% top connections) to this matrix to yield the SC matrix.

4.9. ASL data pre-processing

ASL data processing and analysis will be performed using FSL 6.0.5 (FMRIB, Oxford, United Kingdom) including the Bayesian Inference for Arterial Spin Labelling MRI (BASIL) toolbox[70], MCFLIRT for head motion correction and BET for non-brain tissue removal[64]. After spatial smoothing, CBF maps will be quantified from perfusion-weighted images (averaged pairwise subtracted control label images) by applying the standard general kinetic model according to widely followed recommendations and using voxel-wise calibration with the M0 image, and correction for partial volume effect with spatial regularization[71]. Finally, CBF maps will be mapped to MNI152 standard space.

4.10. Hemodynamic response shape estimation

The HRF shape will be parametrized using the onset delay or latency, full-wifth half maximum (FWHM), and time-to-peak, estimated from BOLD data measured during hypercapnic challenge using an auto-regressive model applied to the average response in each region of the Schaefer parcellation[30,36]. The rest based HRF shape estimation will be performed with a Python implementation of a deconvolution algorithm included in the rsHRF toolbox[39]. This algorithm assumes that BOLD fluctuations are generated by a hidden point-process and models the relationship between both signals with a linear time invariant (LTI) system via convolution with the HRF[38]. The solution of the LTI system can be obtained applying least-squares to an equivalent optimization problem, yielding the three parameters that characterize the estimated HRF (onset delay or latency, FWHM, time-to-peak). These parameters will be available for each participant and for each ROI in the Schaefer parcellation. This method will be validated using previous HRF parameter maps[38] and comparing with the hypercapnic challenge task results[36].

4.11. Regional and total WMHI burden

The detection of WMHI will be based on the segmentation of white matter lesions in raw FLAIR images, performed with the lesion prediction algorithm (LPA) as implemented in the Lesion Segmentation Toolbox (version 2.0.15), based on the calculation of a lesion probability score for each voxel[72]. The resulting lesion probability maps will be smoothed using a Gaussian kernel with FWHM at 1 mm for voxels with a lesion probability >0.1. Voxels with no direct neighbors will be deleted from the lesion maps. Lesion size maps will then be acquired from the probability maps considering voxels with a probability >0.5 and lesion sizes with a threshold >0.015. The total WMHI volume in cubic centimeters will be defined as the voxel size multiplied by the total number of voxels labeled as lesions in the cerebrum. The resulting WMHI volume will be normalized by the total intracranial volume in each subject[60]. Using the probabilistic tractography obtained from the DTI data combined with a registration of individual white matter volumetric data to a standard space, we will determine the connectivity of WMHI lesions to cortical regions of interest (ROI) in the Schaefer parcellation[48]. For ReDLat participants we will use a high-quality reference DTI dataset[73]. We will count all streamlines with endpoints in each ROI and use this quantity to normalize the number of streamlines that pass through at least one voxel within a segmented WMHI lesion, yielding a metric that estimates the cortical burden of white matter lesions. The results of this analysis will be validated against results in the previous report that introduced this methodology[48].

4.12. Vascular integrity and hemodynamic response

To address the hypothesis of HRF sensitivity to vascular damage in SVD, we will correlate the cortical WMHI burden of each ROI with the HRF shape parameters. For each group of patients in each dataset we will correlate the individual cortical WMHI burden values with each of the three shape parameters, yielding a

coefficient of determination (R2) and its associated p-value for each ROI in the parcellation. Alternatively, we will estimate the anatomical overlap between WMHI burden and HRF shape parameters by computing their correlation across all ROIs in the parcellation, and then submitting the individual R2 values to group-level statistical analysis. This analysis will be repeated using the ASL data to assess the link between the HRF shape parameters and the CBF.

4.13. Regional atrophy w-scores

Grey matter atrophy w-scores will be obtained from T1 images, processed using the voxel-based morphometry method implemented in the Computational Anatomy toolbox (CAT12) within Matlab r2022a[61]. Preprocessing steps will include bias-field correction, noise reduction, skull stripping, segmentation, and normalization to the Montreal Neurological Institute (MNI) space at a 1.5 mm isotropic resolution. Regional atrophy values will be obtained using the Schaefer parcellation[63].

4.14. Dynamic mean field model of whole-brain activity

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 (structural connectome) (**Fig. 4**). Within each ROI, the behavior of ensembles of interconnected excitatory and inhibitory neurons is captured by a set dynamical equations[51]. In the model, excitatory synaptic currents $I^{(E)}$ are mediated by NMDA receptors, while inhibitory currents $I^{(I)}$ are mediated by $GABA_A$ receptors, and excitatory (E) and inhibitory (I) neuronal pools are reciprocally connected, whereas inter-areal connections only occur at the excitatory-to-excitatory level and are weighted by the white matter fiber density, with a global scaling parameter G. The simulated currents $I^{(E)}$ and $I^{(I)}$ are converted into firing rates via neuronal response transformations shaped as sigmoid functions. The parameters of the DMF are determined to replicate resting state conditions, such that each isolated node exhibits the characteristic noisy spontaneous activity with a low firing rate ($r^{(E)} \sim 3Hz$) observed in electrophysiology experiments[51], which requires E-I balance achieved by an inhibitory feedback current (FIC)[74]. Our past research has demonstrated that healthy aging and dementia impacts on E-I balance via altered FIC, depending on local grey matter atrophy values[73].

4.15. Neurovascular coupling model

To convert the simulated mean field firing rages into a BOLD signal, we will implement the hemodynamic model proposed by Stephan et al. where the excitatory firing rate of the n-th ROI, $r_n^{(E)}$, leads to a corresponding increase in a local vasodilatory signal, s_n, undergoing auto-regulatory feedback[75]. The local blood inflow f_n^{in} changes proportional to s_n and positively influences the total supplied blood volume and deoxyhemoglobin content according to a characteristic response time, τ, representing the average time blood takes to traverse the venous compartment. The blood outflow, f_n^{out}, is a function of volume with a parameter that represents the vessel stiffness. Neuropathology studies reveal that WMHI are associated with reduced vessel density and capacity, increased cerebrovascular stiffness, causing causes venous insufficiency and vasogenic edema[40]. The mathematical formulation of the hemodynamic model allows the introduction of separate parameters determining the effect of excitatory activity on the vasodilatory signal (η), the relationship between blood volume and the difference between inflow and outflow via the vascular stiffness (α) and the characteristic transit time (τ).

4.16. Model fitting and comparison

For each participant, we will first adjust the global coupling G to maximize the similarity between empirical and simulated FC matrices, determined by the Structural Similarity Index (SSMI)[76]. In parallel, the fitting procedure will iteratively adjust the regional FIC to achieve the desired spontaneous firing rate. Afterwards, we will use anatomical priors to optimize relevant model parameters introducing spatial heterogeneities[73,77]. Given a baseline model parameter π_n^0 and an anatomical prior with value μ_n, we introduce the modulated parameter as $\pi_n = \pi_n^0(1 + \sigma\mu_n)$. Thus, after optimizing σ to maximize the goodness of fit with the empirical data, the resulting value can be interpreted as the influence of the prior μ_n on parameter π_n at the n-th node: if $\sigma \approx 0$, the model parameter is independent of the anatomical prior, while a positive/negative dependence results in $\sigma > 0$ and $\sigma < 0$, respectively. We will modulate the FIC modulated by grey matter atrophy, maximizing the match between empirical and simulated FC via the SSIM. We will also modulate the hemodynamic coupling parameters η, α and τ informed by the map of cortical WMHI burden[48], to maximize the similarity between empirical and simulated HRF shape parameters. In both cases, we will obtain parameters σ using randomized maps to construct a null model[77]. For each group, we will compare the contribution of neural E-I disbalance to whole-brain FC by contrasting the goodness of fit values obtained

modulating FIC with grey matter atrophy, the values obtained modulating hemodynamic coupling parameters, and their combination.

4.17. Link to measures of cardiovascular health

This includes self-reported physical activity levels and diet, smoking status, body mass index and waist-to-hip ratio, cholesterol, low-density lipoprotein and triglycerides level, blood sugar and glycated hemoglobin level, and blood pressure[60]. We will employ linear models to investigate the correlation between data-driven metrics (local and global WMHI burden, CBF, HRF shape parameters) and model-based metrics (modulation of vascular and E-I balance parameters η, α τ, and FIC, using age, gender, and cognitive capacity as covariates of no interest.

4.18. Link to social determinants of health

SDH are available based on a multidimensional index integrating upbringing, nutrition, home environment, adverse life experiences, violence, discrimination, basic needs, education, occupation, employment, finances, healthcare, and social neighborhood[52]. This assessment protocol is harmonized and cross-culturally validated. A similar analysis to that presented above (section 4.17) will be followed to study correlations with SDH data.

4.19. Statistical analyses and power calculation

The effect of patient group on regional WMHI and HRF shape parameters will be assessed using ANCOVA with age, gender, and the result of principal component analysis (PCA) applied to cognitive/neuropsychological data as covariates. The relationship between these variables will be assessed using partial linear correlations, controlled using the same set of covariates. When performing one statistical test per each ROI, the effect of multiple comparisons on statistical significance will be controlled using Benjamini-Hochberg False Discovery Rate (FDR) with α=0.5. For a sample of > 100 ND patients per sub-type, we estimate 80% power at α = .05 to detect an effect with Cohen's d = 0.5 and 99.9% to detect an effect of d=1 in across group comparisons; and 80% power to detect correlations with R=0.25, and 99.9% power to detect correlations with R=0.4. The significance of the model goodness of fit (SSIM) will be determined by running a large number (N≈1000) of independent simulations using randomized version of the anatomic prior maps and constructing an empirical permutation p-value. For each participant, the model parameters σ will be compared using ANCOVA with age, gender, and MMSE scores as covariates of no interest.

5. SUMMARY OF NOVELTY, DISRUPTION & UNCERTAINTY

The proposal is posed to draw novel and impactful connections between neurovascular integrity and brain function in aging individuals and ND patients assessed using neuroimaging methodologies. In contrast to the efforts of ongoing large international consortia, the strength of our proposal resides in the modeling of vascular contributions instead of regressing them from data as covariates of no interest[66]. We will provide a novel integration between 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, we also pioneer the use of biophysical simulations to disentangle neural and vascular contributions to neuroimaging data. Current biomarkers lack specificity in this regard, given that fMRI measures a surrogate marker of neural activity fundamentally mediated by vascular response[22]. We propose a critical analysis of these contributions which is long overdue in the field, further highlighting the novelty and timeliness of our proposal. Once established, our methodological framework will be straightforwardly applicable to large pre-existing cohorts of patients and healthy controls examined with neuroimaging, which underscores the disruptive potential of our proposal. As part of the conducted research, we will analyze 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[49]. This collaboration adds to the disruptive aspect of our proposal by allowing us to establish connections between mechanistically interpretable parameters of vascular and neural impairment with comprehensive assessments of CVH and SDH across a heterogeneous population[52]. Finally, we expect that the end deliverables 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[10].

Our proposal is composed of multiple aims with varying degrees of uncertainty, constituting a layered approach guaranteeing that the most uncertain part of the analysis is grounded on robust results,

contributing to the overall risk mitigation of the project. Even though our previous research establishes the feasibility of using biophysical models to study whole-brain activity in aging individuals and ND patients[47], 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 and vascular coupling[78,79], 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.

6. TRANSFORMATIVE POTENTIAL, REWARD & SCOPE

The transformative potential, reward and scope are summarized in **Table 1.** This proposal carries a very high transformative potential, as it directly addresses the long-standing issue of systematic conflation of vascular and neural effects in neuroimaging assessments of pathological aging, ND, and dementia. Instead of removing the estimated vascular effects from the data, we propose to capitalize this information in the development of biomarkers factoring neurovascular integrity, which are considered fundamental for the future formulation of prevention trials addressing CVH-related risk factors, an especially transformative translational dimension of our proposal. Even though this proposal will produce e significant rewards at the global level (by integrating the developed methodologies to large pre-existing datasets), it will be especially impactful within the context of Chilean public health. Not only Chile is facing a rapid increase in the prevalence of ND due to its aging population, but also displays a high prevalence of preventable risk factors, including those related to CVH. 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[80]. Importantly, past research supports associations between CVH and cognitive impairment in the Chilean population[81]. These previous results underscore the possibility of mitigating the social and economic impact of the proliferation of ND and dementia, adopting measures aimed to prevent risk factors.

Table 1. Summary of transformative potential, reward, and scope of this proposal.

	Field of research/area of application	**Short term (3-4 years)**	**Medium term (5-10 years)**	**Long term (>10 years)**
Transformative potential	Fundamental research	Novel understanding of mechanisms underlying vascular and neural contributions to fMRI-based biomarkers.	Large neuroimaging protocols incorporate measurements to address vascular contributions and to develop multimodal non-invasive MRI-driven vascular integrity biomarkers	Causal mechanisms linking cerebrovascular impairment, ND and dementia, leading to more powerful biomarkers for early diagnosis.
	Translational applications	Specificity of current biomarkers is improved by disentangling neural and vascular sources	Specific biomarkers allow to test the efficacy of prevention trials addressing CVH and CVD	Specific biomarkers are leveraged for personalized medicine and interventions to mitigate the risk of ND and/or slow their progression
Reward and scope	Society	Improved awareness of how SES and SDH contribute to ND prevalence, mediated by CVH	Improved awareness of how SES and SDH contribute to ND prevalence, mediated by CVH	Diminished social burden of ND and dementia
	Economy	More affordable methods to address vascular integrity in vivo	More affordable methods to address vascular integrity in vivo	Diminished economic burden of ND and dementia
	Public health	Improved diagnosis and prognosis. Improved and more specific epidemiological data	Support of novel trials for CVH risk factor mitigation	Diminished incidence of ND and dementia

7. WORK TEAM

We are an interdisciplinary team with complementary backgrounds covering the main areas of expertise needed for this project. The expertise and role of each researcher is summarized in **Table 2.**

Table 2. Work team members, position and role in the project, and relevant expertise.

Name	Position	Role in the project	Relevant expertise
Enzo Tagliazucchi	Director	Project coordination. Neuroimaging data analysis, statistical analyses.	Neuroimaging and machine learning.
Josephine Cruzat	Co-director	Project coordination. Implementation and analysis of biophysical modeling.	Biophysical modeling applied to fMRI data.
Andrea Slachevsky	Co-investigator	Participant recruitment and assessment, neuroimaging and CVH assessments.	Clinical and translational research in aging and ND.
Agustín Ibañez	Co-investigator	Director of ReDLat. Interaction between the project and the ReDLat consortium.	Coordination of large-cohort research consortia.
Vicente Medel	Co-investigator	Link between neuroimaging markers, CVH, and other environmental variables.	Neuromaging analysis of factors influencing aging.
Claudia Durán-Aniotz	Co-investigator	Formulation and validation of biomarkers for vascular and neural contributions.	Biomarker discovery in aging, ND, and dementia.
Patricio Orio	Co-investigator	Implementation of neurobiological models of whole-brain activity.	Realistic modeling of neuroimaging data.
N.N.	Support staff	Project manager.	Collaborative research projects coordination.
N.N.	Support staff	Lab manager.	Laboratory operations, staff management and compliance with safety standards.
N.N.	Technical staff	Conduct neuropsychological assessments	Neuropsychologist.
N.N.	Technical staff	Ensuring high-quality neuroimaging data.	MRI Engineer
N.N. (x4)	Thesis student	Data analysis, biophysical modeling, statistical analyses, etc.	Advanced students of related disciplines.

8. WORK PLAN

Table 3. Gantt chart with the timeline of the project

	Year 1		Year 2		Year 3		Year 4	
	Sem 1	Sem 2	Sem 1	Sem 2	Sem 1	Sem 2	Sem 1	Sem 2
Subject recruitment and acquisition (**Aims 1-3**)	■	■	■	■	■	■	■	■
Structural and functional data preprocessing	■	■	■	■	■	■	■	■
Task-based HRF shape estimation (**Aim 1**)	■	■	■					
Rest-based HRF shape (**Aim 1**)	■	■	■					
Vascular integrity metrics integration (**Aim 1**)		■	■	■				
Journal publication & conference presentation			■	■				
Processing of data for model fitting (**Aim 2**)			■	■				
Biophysical model fitting and analysis (**Aim 2**)				■	■	■		
Journal publication & conference presentation					■	■		
Extrapolation to the ReDLat dataset (**Aims 1-3**)				■	■	■	■	■
Integration with CVH, SES, SDH (**Aim 3**)					■	■	■	■
Journal publication & conference presentation						■	■	■
Assessment of future funding opportunities						■	■	■

9. CRITICAL MILESTONES & MITIGATION PLAN

Table 4. Summary of critical milestones and the associated mitigation plan.

Critical Milestones	Risk Factors	Achievement Indicator	Compliance Period	Mitigation Activities
Piloting hypercapnic challenge task	Poor data quality	Clear HRF shape evident in the BOLD signals	M 1-18	Decrease scanning time duration, add breaks to keep participants engaged
Piloting MRI, FLAIR, fMRI, DTI; ASL	Poor data quality	Achieving modality-dependent quality standards	M 1-6	Additional measures to limit head motion (shorter scans and longer breaks, vacuum cushions)
HRF estimation	Lack of agreement between task and rest-based estimation	Regional agreement between task and rest-based estimation	M 12-18	Optimization of deconvolution model parameters. Meeting with Dr. D. Marinazzo, main developer of the algorithm and toolbox
Biophysical model fitting	Poor fit to the empirical data	Goodness of fit comparable with previous studies/publications	M 18-36	Implementation of more sophisticated models (neural mass models)
Extrapolation of findings to the ReDLat dataset	Lack of consistent results. Poor goodness of fit of model	Results consistent with previous milestones, good model generalization	M 24-48	Enhancing harmonization between datasets by subsampling the ReDLat dataset

10. CO-INVESTIGATORS CURRICULAR BACKGROUND

Andrea Slachevsky, MD, PhD, is a full Professor of the Faculty of Medicine, Universidad de Chile, directs the Neuropsychiatry and Memory Disorders Clinic (CMYN), Neurology Department, Hospital del Salvador and Faculty of Medicine, Universidad de Chile, and is principal investigator of the FONDAP Center, GERO (Center for Geroscience, Brain Health and Metabolism). She is a cognitive neurologist with extensive experience in clinical care and research in aging and dementia. Her research includes the development of neuropsychological tests and biomarkers for the diagnosis of dementia, cognitive ageing, and evaluation of the burden and cost of dementia disorders. She has been principal investigator for several research projects on cognitive disorders in brain diseases and has published more than 80 articles, with an *h*-index of 35, as well as several books and book chapters.

Agustín Ibañez, PhD, is the Director of the Latin American Brain Health Institute (BrainLat) at Universidad Adolfo Ibáñez. With over 350 publications and numerous citations, Dr. Ibañez has an extensive research background in brain health, particularly focusing on the impact of social determinants on neurodegenerative diseases such as frontotemporal dementia (FTD) and Alzheimer's disease (AD). His work integrates genetic, imaging, and behavioral data through advanced computational frameworks. Dr. Ibañez has secured funding from prestigious international bodies, including NIH, Alzheimer's Association, and Horizon 2020. He has led the neuroimaging core of the largest longitudinal study on aging and dementia in South America and has established significant international collaborations. He co-founded the Latin American and Caribbean Consortium on Dementia (LAC-CD) and the Multi-partner Consortium to Expand Dementia Research in Latin America (ReDLat). He has a relevant track record (+350 publications, citations >18,600; h-index >77; i10-index >*315*, with over 150 publications in the last five years, including works in top-ten journals (e.g., *Nature Medicine, World Psychiatry*, *Lancet Neurology, Nature Reviews Neurology*, *Nature Human Behaviour*, *Nature Aging, Nature Communications, JAMA Neurology*, *Brain*, *Alzheimer' & Dementia, Neuron, Neurology,* among others). In this project, Dr. Ibañez will apply his expertise to investigate neurovascular pathways in aging and ND, leveraging his extensive network and resources to support the research's goals. His role will include mentoring, ensuring adherence to research objectives, and providing scientific direction based on the latest advancements in dementia research.

Vicente Medel, PhD, is professor and principal investigator at BrainLat institute, Universidad Adolfo Ibañez. With degrees a philosophy from Universidad de Chile, and master's and PhD degrees in Neuroscience from Pontificia Universidad Católica de Chile, Dr. Medel has extensive research experience analyzing neuroimaging data, including EEG, MRI, and magnetic resonance spectroscopy, and using computational models in health and ND diseases. In this project, Dr. Medel will play a crucial role in the project, conducting detailed analyses of neuroimaging data and cardiovascular health metrics, and collaborating on the development of computational models to disentangle neuronal and vascular contributions to biomarkers of ND. He will oversee results interpretation, contribute to the writing and publication of scientific articles, and foster collaborations with other researchers and laboratories.

Claudia Durán-Aniotz, PhD, is an Associate Professor at Universidad Adolfo Ibañez and co-Director of the Brain Health Institute (BrainLat) in Chile. She is deeply committed to dementia and brain health research, focusing on biomedical research on Alzheimer's disease, will play a crucial role in the project. Collaborating with the Center for Geroscience, Mental Health, and Metabolism (GERO) under the FONDAP Program, she focuses on identifying peripheral inflammatory biomarkers as dementia risk factors in Chile. As Director of the Corporación Profesional Alzheimer y otras Demencias (COPRAD), she promotes public policies like the National Alzheimer's Plan and works to improve patients' quality of life. Dr. Durán-Aniotz will bring her expertise in biomarker identification and translational research, enhancing the project's ability to develop effective intervention strategies and understand dementia risk factors in the Chilean population.

Patricio Orio, PhD, researcher at the Centro Interdisciplinario de Neurociencia de Valparaíso, full professor at the Faculty of Sciences, Universidad de Valparaíso, and Director of the Biophysics and Computational Biology PhD Program at the University of Valparaíso, will play a crucial role in the project. An expert in network topology, Dr. Orio's research focuses on how neural dynamics and network topology shape the behavior of neural networks, particularly their unique features like scale-free and small-world properties, and their ability to exhibit multistability and chaotic behavior. His expertise will be invaluable in understanding how these network characteristics contribute to the stability and dynamics of neural networks in the context of neurodegenerative diseases and the coupling with the cardiovascular structure.

11. FIGURES

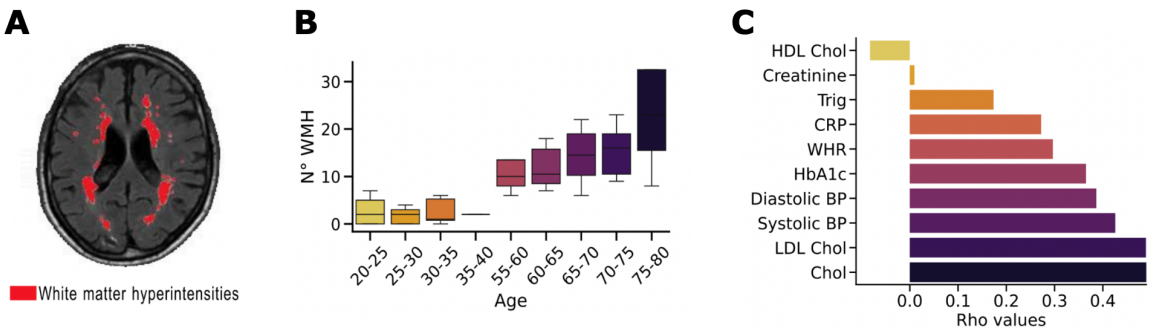

Figure 1. Preliminary results by our group linking WMHI, age and metrics related to CVH[60]. **A)** An example of WMHI lesions shown in red. **B)** Number of WMHI lesions as a function of age in a sample of healthy aging individuals. **C)** Correlation coefficient for markers of CVH and the total number of WMHI lesions. HDL: high-density lipoprotein; Chol: Cholesterol; Trig: Triglycerides; CRP: C-Reactive protein; WHR: Waist-to-hip ratio; HbA1c: Glycated hemoglobin; BP: blood pressure; LDL: low-density lipoprotein.

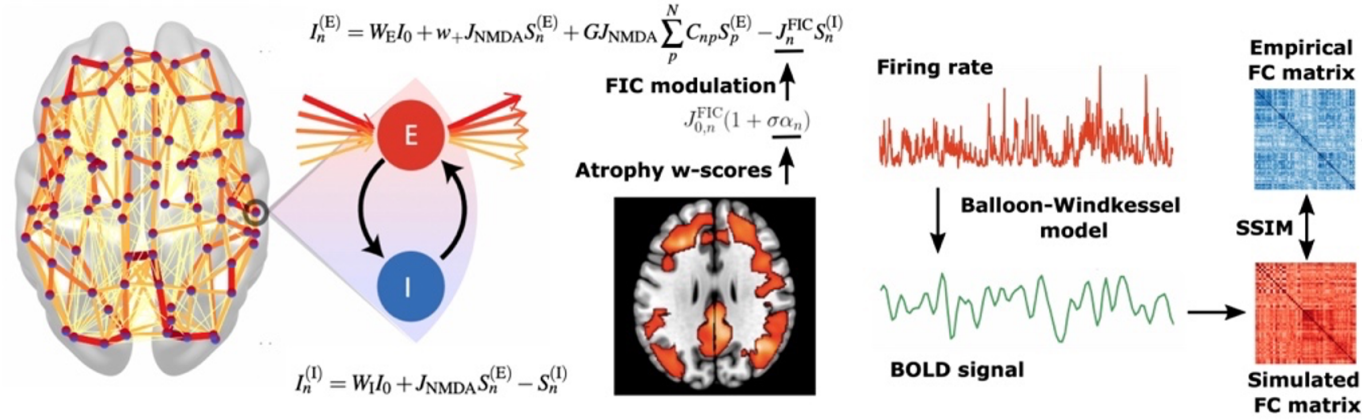

Figure 2. Overview of the DMF model[82]. Each region in the parcellation comprises a set of excitatory (E) and inhibitory (I) populations, with long-range excitatory coupling and local $E-I$ interactions. In this example, the feedback inhibitory current (FIC) is modulated by grey matter density. The simulated firing rate time series are converted to BOLD signals via the Balloon-Windkessel model of neurovascular coupling, and then used to compute a simulated FC matrix, compared with the empirical FC matrix with the SSIM index.

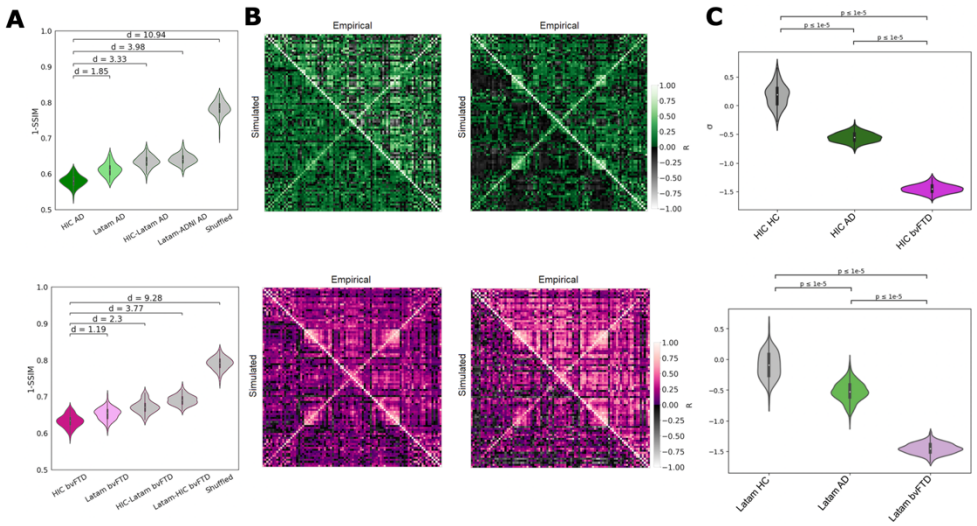

Figure 3. Previous results by our group showing that excitation-inhibition balance is altered in AD and FTD depending on regional grey matter atrophy values[47]. **A)** DMF model goodness of fit per patient group and location. B) Above/below diagonal show empirical and simulated fMRI FC matrices for AD (up), FTD (bottom), Latam (Left) and USA (Right) ReDLat data. C) Parameter σ indexing excitation-inhibition balance per patient group and location. Latam: Latin America, HIC: High income countries, AD: Alzheimer's disease, bv: behavioral variant frontotemporal dementia

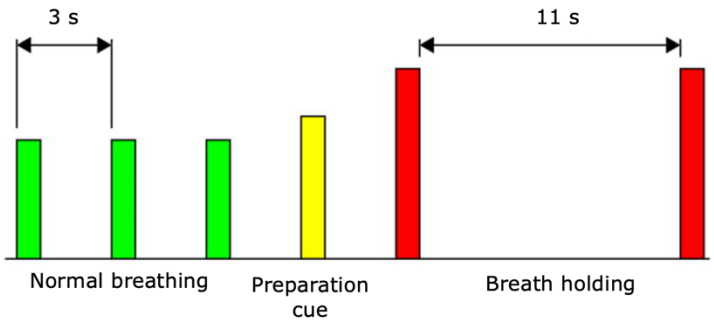

Figure 4. Schematic illustration of the breath holding task (hypercapnic challenge) that will be performed by participants during acquisition of BOLD fMRI for the purpose of estimating regional HRF shape parameters[30]. An initial block with auditory cues for normal breathing (separated by 3 s each) is followed by a preparation cue, and cues to breathe until reaching a target volume (determined with a respiratory belt) and holding breathing for 11 s, until the presentation of another auditory cue for exhalation.