1 - General information

Project code: PNRR-MAD-2022-12376667

Project topic: C2) Malattie croniche non trasmissibili, ad alto impatto sui sistemi sanitari e socio-assistenziali: eziopatogenesi e meccanismi di malattia

PI / Coordinator: Vecchio Fabrizio

Applicant Institution: Casa di cura San Raffaele Pisana

Call section: Malattie Croniche non Trasmissibili (MCnT) ad alto impatto sui sistemi sanitari e socio-assistenziali

Proposal title: Brain connectivity and complexity parameters to monitor disease progression in dementia patients and anti-inflammatory nanotherapeutics in a preclinical model of Alzheimer's disease.

Duration in months: 24

MDC primary: Neurologia

MDC secondary: Neurologia

Project Classification IRG: Brain Disorders and Clinical Neuroscience

Project Classification SS: Clinical Neuroscience and Neurodegeneration - CNN

Project Keyword 1: Alzheimer¿s disease and other dementias.

Project Request: **Animals:** [X] **Humans:** [X] **Clinical trial:** [ ]

Project total financing request to the MOH: € 1.000.000

Free keywords: EEG; functional connectivity; neuroinflammation; nanoparticles

Declarations

In case of a Synergy grant application 'Principal Investigator'(PI) means 'corresponding Principal Investigator on behalf of all Principal Investigators', and 'Host Institution' means 'corresponding Host Institution'.

1) The Principal Investigator declares to have the written consent of all participants on their participation and on the content of this proposal, as well as of any researcher mentioned in the proposal as participating in the project (either as other PI, team member or collaborator).	[X]
2) The Principal Investigator declares that the information contained in this proposal is correct and complete.	[X]
3) The Principal Investigator declares that all parts of this proposal comply with ethical principles (including the highest standards of research integrity — as set out, for instance, in the European Code of Conduct for Research Integrity — and including, in particular, avoiding fabrication, falsification, plagiarism or other research misconduct).	[X]
4) The Principal Investigator is only responsible for the correctness of the information relating to his/her own organisation. Each applicant remains responsible for the correctness of the information related to him and declared above.	[X]

Personal data protection

The assessment of your grant application will involve the collection and processing of personal data (such as your name, address and CV), which will be performed pursuant to Regulation (EC) No 45/2001 on the protection of individuals with regard to the processing of personal data by the Community institutions and bodies and on the free movement of such data. Unless indicated otherwise, your replies to the questions in this form and any personal data requested are required to assess your grant application in accordance with the specifications of the call for proposals and will be processed solely for that purpose. Details concerning the purposes and means of the processing of your personal data as well as information on how to exercise your rights are available in the privacy statement. Applicants may lodge a complaint about the processing of their personal data with the European Data Protection Supervisor at any time.

Abstract

Alzheimer's Disease (AD) is multifactorial disorder where the non-linear interaction between genetic, biological and environmental factors accounts for interindividual clinical variability. The lack of an effective cure and the preclinical data on animal models, made it clear that, once neurodegeneration is too advanced, treatments have limited effects. Early treatments require new reliable tests to identify the initial manifestations of the disease. Since early diagnosis might increase the effectiveness of therapeutic interventions, our study will focus on the identification of early biomarkers of AD. The impairment of functional cortical connectivity can be detected in early and prodromal AD through neurophysiological assessment and EEG measurements. In this study we will investigate complex cortical connectivity networks, which are already deteriorated in early AD, as a reliable marker of cognitive progression. Our goal is to identify neurophysiological indices and life-style factors to characterize early AD and mild cognitive impairment (MCI). MCI is characterized by measurable cognitive deficit, but not overt dementia. Half of the MCI patients progresses to or, more accurately, is already in a prodromal form of AD. The expected results of this project will potentially offer a new test to discriminate with accuracy the cases of MCI due to AD. These patients would be the ideal candidate to undergo early interventions.
Concurrently, the study of AD onset and progression in a mouse model, will allow to evaluate the causal effect of inflammation on the disease phenotype and to develop a new class of biomimetic nanoparticles (the leukosomes) able to target and overcome the blood brain barrier to resolve neuroinflammation. In AD, the chronic activation of M1 microglia triggers the release of pro-inflammatory cytokines and chemokines, which can lead to further neurodegeneration. Inhibition of microglia over-activation and neuroinflammation could represent a promising strategy for the treatment of AD. Unfortunately, targeting brain inflammation through a systemic pharmacological approach does not hold ground clinically, because of poor delivery across the blood brain barrier. We will develop next-generation targeted therapies based on the specific action of biomimetic anti-inflammatory nanoparticles at the site of brain damage. We hypothesize that the selective neutralization or downregulation of the pathogenic and pro-inflammatory signaling, will significantly improve the therapeutic outcome and inhibit or slow down the progression of AD.
The project will be divided in the following three specific aims:
- Specific Aim 1: To characterize AD and MCI subjects performing clinical, psycho-cognitive, metabolic, physical functioning and different neurophysiological investigations, to identify standardized multidimensional biomarkers of AD. These data will be compared between patients and a group of age- sex- and education-matched healthy subjects.
- Specific Aim 2: To characterize changes in brain connectivity and complexity in a sporadic AD-like mouse model and to correlate the neurophysiological indices to AD hallmarks and neuroinflammation.
- Specific Aim 3: To evaluate the effect of anti-inflammatory biomimetic nanoparticles to target neuroinflammation in AD mouse model and to ameliorate AD phenotype.

In order to best review your application, do you agree that the above non-confidential proposal title and abstract can be used, without disclosing your identity, when contacting potential reviewers? Yes

2 - Participants & contacts

Operative Units

Institution that perform as UO	CF Institution	Department / Division / Laboratory	Role in the project	Southern Italy	SSN
1 - Casa di cura San Raffaele Pisana	10636891003	Department of Neuroscience and Neurorehabilitation, Brain Connectivity Laboratory	Project coordination and EEG data analyses and statistics of all data		X
2 - Azienda Ospedaliero-Universitaria di Sassari	02268260904	Unit of Endocrinology, Nutrition and Metabolism Disorders AOU Sassari	Patients' recruitment and data collection	X	X
3 - IRCCS Fondazione Policlinico Universitario A. Gemelli	13109681000	UOC neurologia	Animal model study coordination, recordings and analyses		X

Principal Research Collaborators

Key Personnel Name	Operative Unit	Role in the project
1 - MIRAGLIA FRANCESCA	Casa di cura San Raffaele Pisana	She will be the Co-Pi of the project and will analyzed electrophysiological data
2 - Deriu Franca	Azienda Ospedaliero-Universitaria di Sassari	She will coordinate OU2 and contribute to neurophysiological, metabolic and nutritional characterization of AD, MCI and control subjects
3 - Podda Maria Vittoria	IRCCS Fondazione Policlinico Universitario A. Gemelli	She will coordinate OU3 and perform LFP recordings to identify neurophysiological indices
4 - TASCIOTTI ENNIO	Casa di cura San Raffaele Pisana	He will oversee optimization of nanoparticles composition and the effect of anti-inflammatory molecules
5 - IODICE FRANCESCO	Casa di cura San Raffaele Pisana	He will analyze and integrate clinical data during intermediate and final reports
6 Under 40 - LI PUMA DOMENICA DONATELLA	IRCCS Fondazione Policlinico Universitario A. Gemelli	She will set up the in vivo model of recurrent HSV-1 infection and perform molecular analyses
7 Under 40 - Paciello Fabiola	IRCCS Fondazione Policlinico Universitario A. Gemelli	She will perform behavioral experiments to evaluate cognitive dysfunctions and morphological analyses

Key Personnel Name	Co-PI	Resp. CE	Resp. Animal	Birth Date	Gender
1 - MIRAGLIA FRANCESCA	X	X		09/03/1985	F
2 - Deriu Franca				02/02/1965	F
3 - Podda Maria Vittoria			X	30/05/1970	F
4 - TASCIOTTI ENNIO				20/08/1977	M
5 - IODICE FRANCESCO				07/08/1985	M
6 Under 40 - LI PUMA DOMENICA DONATELLA				22/01/1986	F
7 Under 40 - Paciello Fabiola				14/06/1987	F

Additional research collaborators under 40 to hire

Key Personnel Name	Operative Unit	Birth Date	Gender	Role in the project	Degree	Actual Pos. and Inst.
0 - Ventura Lucia	Azienda Ospedaliero-Universitaria di Sassari	15/01/1991	F	She will perform the functional and neurophysiological tests on subjects.	MSc in Rehabilitation Sciences	PhD Student, University of Sassari
1 - Loi Nicola	Azienda Ospedaliero-Universitaria di Sassari	06/11/1990	M	He will perform the neurophysiological tests on subjects.	MSc in Neuropsychobiology	PhD, University of Sassari
2 - Cano Antonella	Azienda Ospedaliero-Universitaria di Sassari	13/06/1983	F	She will evaluate the nutritional status of the recruited subjects	MSc in pharmacy	Non-medical resident, University of Sassari

2.1 Administrative data of participating

Operative Unit Number 1:

Address: Via di Val Cannuta 247, Rome, 00166 Italy
PEC: sr.roma.fiscale@legalmail.it

Operative Unit Number 2:

Address: Viale San Pietro 43/b, Sassari, 07100 Italy
PEC: franca.deriu@ss.omceo.it

Operative Unit Number 3:

Address: Largo Francesco Vito 1, Rome, 00168, Italy
PEC: grantoffice.gemelli@pec.it

Operative Unit Number 4:

Address: -
PEC: -

Operative Unit Number 5 (self financing):

Address: -
PEC: -

2.2 Principal Investigator (PI) Profile

Last Name: Vecchio
First Name: Fabrizio
Last name at birth:
Gender: M

Title: Principal investigator
Country of residence: ITALY
Nationality: Italiana
Country of Birth: ITALY
Date of birth: 06/07/1975
Place of Birth: Roma

Official H index (Scopus or Web of Science): 39.0

Scopus Author Id:8068004300 **ORCID ID:**0000-0002-6004-9522 **RESEARCH ID:**K-7527-2016

Contact address

Current organisation name: Casa di cura San Raffaele Pisana
Current Department / Faculty / Institute / Laboratory name: Department of Neuroscience and Neurorehabilitation, Brain Connectivity Laboratory

Street: Via di Val Cannuta 247
Postcode / Cedex: 00166
Town: Roma
Phone:+393478031201
Phone 2: 0652253767

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Department of Human Physiology and Pharmacology High Resolution EEG section, University of Rome "La Sapienza"	PhD	PhD in Neurophysiology	2002	2005
University of Rome "La Sapienza"	Master's Degree / Laurea Magistrale	Master Degree in Biomedical Electronic Engineering	1996	2002

Personal Statement:

Fabrizio Vecchio will coordinate the whole project and the UO1. He will be responsible for EEG data analyses in both humans and animal models and for the centralized statistical analyses of all data generated in the study.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
eCampus University	Department of Theoretical and Applied Sciences	Novedrate (Como)	Associate Professor of Physiology	2021	2022
IRCCS San Raffaele Pisana	Brain Connectivity Laboratory	Rome	Researcher	2013	2022
Associazione Fatebenefratelli per la Ricerca (AFaR)	Department of Neuroscience	Rome	Researcher	2003	2013
University of Foggia	Department of Medical Sciences	Foggia	Contract project	2010	2013
IRCCS Centro S. Giovanni di Dio Fatebenefratelli	Department of Neuroscience	Brescia	Contract project	2008	2009

Other awards and honors

-From2014 Ass Editor: Journal of Alzheimer's Disease
-International Scientific Advisory Board of ECCN2015
-2018-2019 Co-Director of SIG (Special Interest Group) "Functional Brain Connectivity as Revealed by EEG/MEG" IFCN (Int Federation of Clin Neurophys)
-From2020 Co-Director of SIG "Advanced EEG-MEG Techniques in Clinical Neurophysiology" IFCN
-From2020 partner responsible of "EEG/High Density EEG" group for a national network of IRCCS
-Editorial Boards: Translational Neuroscience, Symmetry

Other CV informations

He is devoted to the study of brain connectivity in the past 20 years.

Selected peer-reviewed publications of the PI valid for minimum expertise level

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
Early dementia diagnosis, MCI-to-dementia risk prediction, and the role of machine learning methods for feature extraction from integrated biomarkers, in particular for EEG signal analysis	Article	1-8	April 7	2022	10.1002/alz.12645	35388959	0	L
Performance prediction in a visuo-motor task: the contribution of EEG analysis	Article	297-308	16	2022	10.1007/s11571-021-09713-x	35401869	0	F
Analysis of complexity in the EEG activity of Parkinson¿s disease patients by means of approximate entropy	Article	NOT_FOUND	March 28	2022	10.1007/s11357-022-00552-0	35344121	0	L
Neuronavigated Magnetic Stimulation combined with cognitive training for Alzheimer¿s patients: an EEG graph study	Article	159-172	44	2022	10.1007/s11357-021-00508-w	34970718	0	F
Human Brain Networks in Physiological and Pathological Aging: Reproducibility of Electroencephalogram Graph Theoretical Analysis in Cortical Connectivity	Article	41-51	12	2022	10.1089/brain.2020.0824	33797981	0	F
Brain network modulation in transradial amputee with finger perception restored through biomimetic intraneural stimulation	Article	5369-5372	42	2021	10.1007/s10072-021-05525-3	34406536	0	F

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
Graph theory on brain cortical sources in parkinson¿s disease: The analysis of `small world¿ organization from eeg	Article	7266	21	2021	10.3390/s21217266	34770573	1	F
Entropy modulation of electroencephalographic signals in physiological aging	Article	111472	196	2021	10.1016/j.mad.2021.111472	33766746	0	L
tDCS effects on brain network properties during physiological aging	Article	785-792	473	2021	10.1007/s00424-020-02428-8	32623523	2	F
Contribution of Graph Theory Applied to EEG Data Analysis for Alzheimer's Disease Versus Vascular Dementia Diagnosis	Article	871-879	82	2021	10.3233/JAD-210394	34092648	3	F
Approximate entropy of brain network in the study of hemispheric differences	Article	1220	22	2020	10.3390/e22111220	33286988	5	L
Neurophysiological hallmarks of neurodegenerative cognitive decline: The study of brain connectivity as a biomarker of early dementia	Review	34	10	2020	10.3390/jpm10020034	32365890	6	L
Human brain networks: a graph theoretical analysis of cortical connectivity normative database from EEG data in healthy elderly subjects	Article	575-584	42	2020	10.1007/s11357-020-00176-2	32170641	9	F
Classification of Alzheimer's Disease with Respect to Physiological Aging with Innovative EEG Biomarkers in a Machine Learning Implementation	Article	1253-1261	75	2020	10.3233/JAD-200171	32417784	9	F
Cortical connectivity from EEG data in acute stroke: A study via graph theory as a potential biomarker for functional recovery	Article	133-138	146	2019	10.1016/j.ijpsycho.2019.09.012	31648028	13	F
Acute cerebellar stroke and middle cerebral artery stroke exert distinctive modifications on functional cortical connectivity: A comparative study via EEG graph theory	Article	997-1007	130	2019	10.1016/j.clinph.2019.03.017	31005052	7	F
Tracking Neuronal Connectivity from Electric Brain Signals to Predict Performance	Review	86-93	25	2019	10.1177/1073858418776891	29781389	7	F
Sustainable method for Alzheimer dementia prediction in mild cognitive impairment: Electroencephalographic connectivity and graph theory combined with apolipoprotein E	Article	302-314	84	2018	10.1002/ana.25289	30014515	21	F
Transcranial direct current stimulation generates a transient increase of small-world in brain connectivity: an EEG graph theoretical analysis	Article	1117-1127	236	2018	10.1007/s00221-018-5200-z	29441471	17	F
Learning processes and brain connectivity in a cognitive-motor task in neurodegeneration: Evidence from EEG network analysis	Article	471-481	66	2018	10.3233/JAD-180342	30282357	3	F

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

Selected peer-reviewed publications of the PI for the evaluation CV

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**
Early diagnosis of Alzheimer's disease: the role of biomarkers including advanced EEG signal analysis. Report from the IFCN-sponsored panel of experts	Review	1287-1310	131	2020	10.1016/j.clinph.2020.03.003	32302946	40
¿Small World¿ architecture in brain connectivity and hippocampal volume in Alzheimer¿s disease: a study via graph theory from EEG data	Article	473-485	11	2017	10.1007/s11682-016-9528-3	26960946	42
Connectome: Graph theory application in functional brain network architecture	Review	206-213	2	2017	10.1016/j.cnp.2017.09.003	30214997	66
EEG characteristics in "eyes-open" versus "eyes-closed" conditions: Small-world network architecture in healthy aging and age-related brain degeneration	Article	1261-1268	127	2016	10.1016/j.clinph.2015.07.040	26603651	54
Cortical connectivity and memory performance in cognitive decline: A study via graph theory from EEG data	Article	143-150	316	2016	10.1016/j.neuroscience.2015.12.036	26724581	57
Alpha, beta and gamma electrocorticographic rhythms in somatosensory, motor, premotor and prefrontal cortical areas differ in movement execution and observation in humans	Article	641-654	127	2016	10.1016/j.clinph.2015.04.068	26038115	73
Human brain networks in cognitive decline: A graph theoretical analysis of cortical connectivity from EEG data	Article	113-127	41	2014	10.3233/JAD-132087	24577480	70
Resting state cortical electroencephalographic rhythms are related to gray matter volume in subjects with mild cognitive impairment and Alzheimer's disease	Article	1427-1446	34	2013	10.1002/hbm.22005	22331654	69
Resting state cortical EEG rhythms in Alzheimer's disease: Toward EEG markers for clinical applications: A review	Review	223-236	62	2013	10.1016/B978-0-7020-5307-8.00015-6	24053043	92
Human brain cortical correlates of short-latency afferent inhibition: A combined EEG-TMS study	Article	314-323	108	2012	10.1152/jn.00796.2011	22457460	40

** Autocertificated

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Italian Ministry of Health	Associazione Fatebenefratelli per la Ricerca Biomedica	2008	Does rehabilitation with a 10-Hz sensory stimulation improve brain rhythms and cognitive-motor performance in neurological patients?	Collaborator	150.000,00	https://www.salute.gov.it/portale/p5_11.jsp
Italian Ministry of Health	IRCCS Oasi Troina	2008	Prediction of cognitive decline in mild cognitive impairment (MCI) subjects carrying genetic risk factors based on quantitative EEG and transcranial magnetic stimulation markers	Coordinator	450.000,00	https://www.salute.gov.it/portale/p5_11.jsp

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Italian Ministry of Health	Centro San Giovanni di Dio Fatebenefratelli	2011	Neurophysiological markers of neuroplasticity in normal and pathological aging	Collaborator	33.000,00	https://www.salute.gov.it/portale/temi/documenti/ricercaSanitaria/C_17_pagineAree_3878_listaFile_itemName_23_file.pdf
Italian Ministry of Health	IRCCS San Raffaele Roma	2013	NEUROMASTER: NEUROnavigated MAgnetic STimulation in patients with mild-moderate Alzheimer disease combined with Effective cognitive Rehabilitation	Coordinator	336.910,00	https://www.salute.gov.it/imgs/C_17_pagineAree_4357_listaFile_itemName_1_file.pdf
H2020 European Commision	Neuroconnect	2021	Intelligent digital tools for screening of brain connectivity and dementia risk estimation in people affected by mild cognitive impairment	Collaborator	513.750,00	https://ec.europa.eu/info/research-and-innovation/funding/funding-opportunities/funding-programmes-and-open-calls/horizon-2020_en
Giovan Battista Baroni Foundation	IRCCS San Raffaele Roma	2021	Stimolazione tDCS per la riabilitazione nei soggetti colpiti da ictus con deficit dell'arto superiore	Coordinator	50.000,00	https://www.fondazionebaroni.it/assets/6_vincitori_bandi_fondazione-baroni_2020__21.04.2021.pdf

2.3 CO-PI Profile

Last Name: MIRAGLIA
First Name: FRANCESCA

Last name at birth:
Gender: F

Title: She will be the Co-Pi of the project and will analyzed electrophysiological data

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: POTENZA

Nationality: ITALIANA
Date of birth: 09/03/1985

Official H index (Scopus or Web of Science): 16.0

Scopus Author Id:56202717100 **ORCID ID:**0000-0002-4642-3952 **RESEARCH ID:**K-6415-2016

Contact address

Current organisation name: Casa di cura San Raffaele Pisana
Current Department / Faculty / Institute / Laboratory name: Department of Neuroscience and Neurorehabilitation, Brain Connectivity Laboratory

Street: VIA DI VAL CANNUTA 247
Postcode / Cedex: 00166 **Town:** ROMA
Phone:+393281512811 **Phone 2:** 3281512811

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Catholic University of The Sacred Heart, Rome, Italy	PhD	Neuroscience	2013	2016
La Sapienza University of Rome, Italy	Master's Degree / Laurea Magistrale	Biomedical Engineering	2008	2011
La Sapienza University of Rome, Italy	Bachelor Degree / Laurea Triennale	Clinical Engineering	2005	2008

Personal Statement:

Dr. Francesca Miraglia has been working in the EEG data analysis since 2012. She maturated expertise in studying innovative algorithm of brain connectivity in physiological and pathological aging. She has a solid technical background in the graph theory analysis. She will be the Co-Pi of the project and will be engaged in electrophysiological data analysis. The goal of her activity will be to analyze electrophysiological data from EEG of elderly, MCI subjects and AD patients and of mice. These analyses will be computed to detect networks brain features suitable for the characterization of brain synaptic plasticity mechanisms and disease characterization. She will compute functional connectivity and network analyses with advanced algorithms based on graph theory and on brain complexity.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
IRCCS San Raffaele Roma	Brain Connectivity Laboratory	via di Val Cannuta 247, 00166 Rome	Researcher	2012	2022
Catholic University of the Sacred Heart of Rome	Department of Neurology	Largo A. Gemelli, 8, 00168 Rome	Post Doc	2016	2019
Catholic University of the Sacred Heart of Rome	Department of Neurology	Largo A. Gemelli, 8, 00168 Rome	PhD student	2013	2016

Other awards and honors

Co-founder of academic innovative spin off Neuroconnect
Adjunct Professor in Fundamentals of Biomedical Engineering,Roma Tre University 2019-2020
Guest Editor of Sensors,Special Issue "Biomedical Signal Acquisition and Processing Using Sensors" and of Symmetry,Special Issue "Cognitive Neuroscience and Symmetry"
Reviewer of International peer review journals since 2015 and Grant Application Dunhill Medical Trust
Examiner of MSc thesis The University of Sydney Master of Philosophy Engineering & IT

Other CV informations

She is devoted to the study of brain connectivity in the past 10 years.

Selected peer-reviewed publications of the Co-PI valid for minimum expertise level

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
Brain Connectivity and Graph Theory Analysis in Alzheimer¿s and Parkinson¿s Disease: The Contribution of Electrophysiological Techniques	Review	402	12	2022	10.3390/brainsci12030402	35326358	0	F
Brain Networks Modulation in Young and Old Subjects during Transcranial Direct Current Stimulation Applied on Prefrontal and Parietal Cortex	Article	2150056	32	2022	10.1142/S0129065721500568	34651550	1	F
Brain sources' activity in resting state before a visuo-motor task	Article	NOT_FOUND	18	2021	10.1088/1741-2552/abe7ba	33601343	0	F
Assessing the dependence of the number of EEG channels in the brain networks¿ modulations	Article	33-36	167	2021	10.1016/j.brainresbull.2020.11.014	33242521	3	F
The brain network organization during sleep onset after deprivation	Article	36-44	132	2021	10.1016/j.clinph.2020.10.016	33254098	1	F
Graph theory on brain cortical sources in parkinson¿s disease: The analysis of `small world¿ organization from eeg	Article	7266	21	2021	10.3390/s21217266	34770573	1	C
Small World Index in Default Mode Network Predicts Progression from Mild Cognitive Impairment to Dementia	Article	2050004	30	2020	10.1142/S0129065720500045	31957512	9	F
Brain electroencephalographic segregation as a biomarker of learning	Article	168-174	106	2018	10.1016/j.neunet.2018.07.005	30075353	9	F
Searching for signs of aging and dementia in EEG through network analysis	Review	292-300	317	2017	10.1016/j.bbr.2016.09.057	27693849	29	F

Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
EEG characteristics in "eyes-open" versus "eyes-closed" conditions: Small-world network architecture in healthy aging and age-related brain degeneration	Article	1261-1268	127	2016	10.1016/j.clinph.2015.07.040	26603651	54	F
Small-worldness characteristics and its gender relation in specific hemispheric networks	Article	1-11	310	2015	10.1016/j.neuroscience.2015.09.028	26384963	16	F

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Italian Ministry of Health and Agenzia Italiana del Farmaco	IRCCS Fondazione Policlinico Gemelli	2019	INTERCEPTOR PROJECT	Collaborator	340.000,00	https://www.interceptorproject.com/
IRCCS SAN RAFFAELE ROMA	IRCCS SAN RAFFAELE ROMA	2016	NEURORIABILITAZIONE PERSONALIZZATA: approccio tecnologico innovativo per la valutazione strumentale dell'impatto delle procedure riabilitative in pazienti con esiti di ictus	Coordinator	150.000,00	https://www.sanraffaele.it/
Italian Ministry of Health	IRCCS SAN RAFFAELE ROMA	2016	GR-2016-02361802 Prediction of conversion from mild cognitive impairment to Alzheimer's disease based on TMS-EEG biomarkers	Collaborator	117.750,00	https://www.salute.gov.it/imgs/C_17_bandi_135_listaFile_itemName_12_file.pdf

2.3 Research Collaborators n. 2

Last Name: Deriu
First Name: Franca

Last name at birth: Deriu
Gender: F

Title: She will coordinate OU2 and contribute to neurophysiological, metabolic and nutritional characterization of AD, MCI and control subjects

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Ossi

Nationality: Italiana

Date of birth: 02/02/1965

Official H index (Scopus or Web of Science): 19.0

Scopus Author Id:6603706285 **ORCID ID:**0000-0001-8235-6091 **RESEARCH ID:**D-3049-2018

Contact address

Current organisation name: Azienda Ospedaliero-Universitaria di Sassari

Current Department / Faculty / Institute / Laboratory name: Unit of Endocrinology, Nutrition and Metabolism Disorders AOU Sassari

Street: Viale San Pietro 43/b

Postcode / Cedex: 07100 **Town:** Sassari

Phone:+393486266306 **Phone 2:**

Education / training

Educational institution and location	Degree	Field of study	From year	To year
University of Sassari, Italy	Specialization / Specializzazione	Neurologist	1989	1996
University of Turin, Turin, Italy	PhD	Neurophysiology	1989	1994
University of Sassari, Sassari, Italy	Master's Degree / Laurea Magistrale	Medicine and Surgery	1983	1989

Personal Statement:

The overall goal of this project is to identify novel biomarkers of Alzheimer's Disease (AD) to advance knowledge on processes contributing to AD development and to evolution of mild cognitive impairment (MCI) in AD. Prof. Franca Deriu will contribute to neurophysiological, metabolic and nutritional characterization of AD, MCI and control subjects. She has a broad background in basic neurophysiology, clinical neurophysiology and neurology. Research throughout her scientific career focused on the physiological mechanisms underlying complex cerebral functions, at the brainstem and cortical levels, such as somatosensory integration, nervous circuit excitability and plasticity, investigated through non-invasive neurophysiological methods, both in healthy and neurological populations.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Aou Sassari	Unit of Endocrinology, Nutrition and Metabolism Disorders	Sassari (Italy)	Consultant Neurologist	2021	2022
University of Sassari	Department of Biomedical Sciences	Sassari (Italy)	Full professor in Physiology	2021	2022
University of Sassari	Department of Biomedical Sciences	Sassari (Italy)	Associate Professor in Physiology	2005	2020
University College London (UCL) Institute of Neurology	Sobell Department of Motor Neuroscience and Movement Disorders	London (UK)	Assistant Researcher in Neurophysiology	2003	2004
University of Sassari	Department of Biomedical Sciences	Sassari (Italy)	Senior Researcher in Human Physiology	2001	2005
University of Sassari	Department of Biomedical Sciences	Sassari (Italy)	Fellow Researcher in Human Physiology	1995	2001
University of Turin	Department of Neuroscience	Turin (Italy)	Assistant Professor of Physiological Psychology	1994	1995

Other awards and honors

2007 The most productive researcher at University of Sassari Award

2015 Winner of the 2014 combined ESMAC/SIAMOC meeting¿s best clinical and best methodological paper award

2017 Best Presentation Award for the presentation titled ¿Anatomo-Physiological basis of Vagal and Trigeminal Nerve Stimulation (funded by GLEM, Lyon) at the 8th World meeting of Auriculotherapy, Singapore

2018 Grant for Multiple Sclerosis Innovation Award (funded by Merck) Berlin

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Fondazione di Sardegna	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2022-2024	Study of the physiologic ageing and of related pathologies with a multidisciplinary approach.	Coordinator	115.000,00	https://www.fondazionedisardegna.it/
Fondazione Italiana Sclerosi Multipla (FISM)	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2021-2023	Measuring the impact of common exercise programs on subjective and objective fatigue during daily living activities in people with multiple sclerosis	Coordinator	131.460,00	https://aism.it/gli-studi-sostenuti-con-il-bando-fism-2020
Fondazione di Sardegna	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2021-2023	Investigation of neural adaptations within face and hand motor networks following musical instruments practice	Coordinator	15.000,00	https://www.fondazionedisardegna.it/
Grant for Multiple Sclerosis Innovation (GMSI)	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2018-2019	The effects of eccentric strength training on limb spasticity and muscle weakness in people with multiple sclerosis.	Coordinator	30.000,00	https://www.grantformultiplesclerosisinnovation.org/en_NA/gmsi_2018/winners.html

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Fondazione Italiana Sclerosi Multipla (FISM)	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2017-2019	Efficacy of contralateral training in the management of muscle weakness and fatigue in people with Multiple Sclerosis	Coordinator	128.500,00	https://aism.it/gli_studi_sostenuti_con_il_bando_fism_2016
Italian Foundation for Multiple Sclerosis (FISM)	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2014-2015	Neurophysiological, dynamometric, and clinical assessment of the cross-training effect in patients with multiple sclerosis: a pilot study.	Coordinator	30.000,00	https://aism.it/sites/default/files/Compendio_della_ricerca_AISM_FISM_2014.pdf
Fondazione Banco di Sardegna	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2012-2013	Dance as a rehabilitative method to improve gait and postural instability in patiemts with Parkinson¿s Disease	Coordinator	18.000,00	https://www.fondazionedisardegna.it/
Regione Autonoma della Sardegna (RAS)	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2010-2012	Trigeminal stimulation as non-pharmacological therapy alternative and/or coadjutant vagal nerve stimulation in the treatment of drug-resistant epilepsy.	Coordinator	54.540,00	https://www.regione.sardegna.it/
Italian Foundation for Multiple Sclerosis (FISM)	Department of Biomedical Sciences, University of Sassari, Sassari, Italy. Scientific	2009-2011	Neurophysiological and neuroradiological study of brainstem circuits in patients with multiple sclerosis	Coordinator	60.000,00	https://www.aism.it/italian_multiple_sclerosis_society_aism
Italian Ministry of Education, University and Research (MIUR), Italy	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2008-2010	Study of the excitability of the trigeminal motor system in healthy subjects and in patients affected by oromandibular dystonia and evaluation of its response to stimulation of trigeminal and extra-trigeminal afferents	Coordinator	70.300,00	https://prin.cineca.it/php5/home/prin.php?info=&username=deriuf&SESSION=81f4c8f522b1d5d87ce389527ff69da4202205111741&anno=2007&info=

2.4 Research Collaborators n. 3

Last Name: Podda
First Name: Maria Vittoria

Last name at birth:
Gender: F

Title: She will coordinate OU3 and perform LFP recordings to identify neurophysiological indices

Nationality: Italiana

Date of birth: 30/05/1970

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Alghero

Official H index (Scopus or Web of Science): 16.0

Scopus Author Id:7004555504 **ORCID ID:**0000-0002-2779-8417 **RESEARCH ID:**K-8947-2016

Contact address

Current organisation name: IRCCS Fondazione Policlinico Universitario A. Gemelli

Current Department / Faculty / Institute / Laboratory name: UOC neurologia

Street: L.go Francesco Vito, 1

Postcode / Cedex: 00168

Town: Roma

Phone:+393205309673

Phone 2: 0630154966

Education / training

Educational institution and location	Degree	Field of study	From year	To year
University of Sassari, Italy	Specialization / Specializzazione	Nutrition Sciences	2000	2005
University of Pavia, Italy University of Edinburgh, UK	PhD	Neuroscience	1997	2000
University of Sassari, Italy	Master's Degree / Laurea Magistrale	Biology	1992	1994

Personal Statement:

Maria Vittoria Podda has solid background and expertise in plasticity, behavioral and tDCS studies in animal models through a wide array of techniques to explore molecules, neural circuits and behavior. She will coordinate OU3 research activities on animal model aimed at characterizing the role of the neuroinflammation in AD and at identifying novel neurophysiological (connectivity) biomarkers associated with AD phenotype and efficacy of treatment based on nanoparticles.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Università Cattolica Sacro Cuore/ IRCCS Fondazione Policlinico Universitario A. Gemelli	Dept Neuroscience (Physiology)/UOC Neurologia	Rome, Italy	Associate Professor	2016	2022
Università Cattolica Sacro Cuore	Institute of Human Physiology	Rome, Italy	Assistant Professor	2005	2016
Università Cattolica Sacro Cuore	Institute of Human Physiology	Rome, Italy	Postdoctoral fellow	2002	2005
University of Sassari, School of Medicine	Department of Biomedical Sciences	Sassari, Italy	Postdoctoral fellow	2001	2002
University of Illinois, Chicago	Department of Physiology and Biophysics	Chicago, IL, US	Research assistant	1993	1993

Other awards and honors

1999-2000 Regione Sardegna Awards Fondo Giovani Ricercatori
2014 Member of International Scientific Committee for research grant applications to Austrian Science Fund.
2016-2018 Università Cattolica intramural grants Linea D1
2017 best scientific poster Award, XVII National congress of the Italian Society for Neuroscience
2019 Best publication award Università Cattolica del Sacro Cuore

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Thomas Jefferson University	Università Cattolica del Sacro Cuore	2022	¿Comparative analysis of functional recovery following intranasal vs intracarotid administration of exosomes derived from human bone marrow mesenchymal stem cells (hMSCs) in two animal models of ischemic stroke¿	Collaborator	96.000,00	https://www.jefferson.edu/
Fondazione Baroni	Università Cattolica del Sacro Cuore	2021	Riabilitazione robotica e neuro-stimolazione non-invasiva su modello murino di ischemia cerebrale: nuovi approcci terapeutici per favorire il recupero funzionale post-ictus	Coordinator	50.000,00	https://www.fondazionebaroni.it/assets/6_vincitori_bandi_fondazione-baroni_2020__21.04.2021.pdf
Fondazione Roma	Università Cattolica del Sacro Cuore	2016	Post-stroke brain connectivity and functional outcome following traditional and enhanced neurorehabilitation by non-invasive brain stimulation. NCDS-2013-00000349.	Coordinator	192.500,00	https://www.fondazioneroma.it/

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Office Naval Research Global	Università Cattolica del Sacro Cuore	2015	Modulation of brain functions by transcranial direct current stimulation (tDCS): molecular events and functional outcome. N62909-15-1-2002	Collaborator	408.000,00	https://www.onr.navy.mil/en/Science-Technology/ONR-Global
Ministero del Lavoro, della Salute e delle politiche sociali	Università Cattolica del Sacro Cuore	2009	In vitro and ex vivo studies of electromagnetic fields: effects on stem cells and risk assessment of health care workers	Coordinator	164.000,00	https://www.lavoro.gov.it/Pagine/default.aspx
MIUR	Università Cattolica del Sacro Cuore	2008	Ruolo dei canali del calcio voltaggio-dipendenti nell¿ipereccitabilità neuronale in un modello sperimentale della sindrome dell¿X Fragile	Coordinator	21.428,00	https://www.miur.gov.it/

2.5 Research Collaborators n. 4

Last Name: TASCIOTTI
First Name: ENNIO

Last name at birth:
Gender: M

Title: He will oversee optimization of nanoparticles composition and the effect of anti-inflammatory molecules

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Sezze

Nationality: italiana
Date of birth: 20/08/1977

Official H index (Scopus or Web of Science): 37.0

Scopus Author Id:23971656200 **ORCID ID:**0000-0003-1187-3205 **RESEARCH ID:**J-8309-2015

Contact address

Current organisation name: Casa di cura San Raffaele Pisana
Current Department / Faculty / Institute / Laboratory name: Department of Neuroscience and Neurorehabilitation, Brain Connectivity Laboratory

Street: via di Val Cannuta 247
Postcode / Cedex: 00166 **Town:** Roma
Phone:+393488873879 **Phone 2:**

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Scuola Normale Superiore of Pisa	PhD	Molecular Medicine	2000	2004
University of Pisa, Italy	Master's Degree / Laurea Magistrale	Biology	1998	2000

Personal Statement:

Dr. Tasciotti is an expert in the use of micro-, nano- and bio-materials for drug delivery. By combining nanotechnology with cell biology, he created innovative platforms that work within the rules dictated by the pathophysiology of different diseases. In particular, he focused on developing injectable nanoparticles to overcome biological barriers, target inflammation, deliver drugs to diseased tissues and tune immune response. Dr. Tasciotti pioneered the creation of bioinspired nanoparticles able to prevent macrophage clearance, target inflamed vessels, cross the endothelial layer, and increase therapeutic accumulation in the cytoplasm of target cells. In this project he will oversee the synthesis, characterization and pharmacological use of anti-inflammatory leukosomes (Aim 3).

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
IRCCS San Raffaele	Human Longevity program	Roma, It	Director	2021	2022
San Raffaele University	Department of Nutrition Sciences	Roma, It	Full Professor	2021	2022
Houston Methodist Research Institute	Department of Orthopedics and Sports Medicine	Houston, TX, USA	Full Professor	2018	2020
Houston Methodist Hospital	Department of Orthopedics and Sports Medicine	Houston, TX, USA	Founder and Director Center for Musculoskeletal Regeneration	2016	2020
Houston Methodist Hospital	Department of Orthopedics and Sports Medicine	Houston, TX, USA	Associate Professor	2016	2018
Houston Methodist Research Institute	Department of Nanomedicine	Houston, TX, USA	Chairman Department of Nanomedicine	2010	2015
Houston Methodist Research Institute	Department of Nanomedicine	Houston, TX, USA	Associate Professor	2010	2015
University of Texas, Health Science Center	Department of Biomedical Engineering	Houston, TX, USA	Assistant Professor	2008	2010
AREA Science Park	Center for Biomolecular Medicine	Trieste, IT	Program Manager Molecular Imaging Lab	2005	2006

Other awards and honors

2011 Best Italian Cancer Research Scientists Award, ISSNAF, Washington DC
2012 TMHRI President¿s Award for Transformational Excellence
2012 Moran Foundation Award in Translational Research
2013 Italy-America Chamber of Commerce ¿PrimiDieci Under Forty Award¿
2015 TMHRI President¿s Award for Transformational Excellence
2016 Career Award. NanoScience Symposium, Houston
2017 and 2018 Houston Men of Distinction Award
2020 Winner of the American Chemical Society Nano Championship

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Cancer Prevention and Research Institute of Texas (CPRIT)	Houston Methodist Research Institute, Houston, TX	2016-2020	Targeting the metastatic sarcoma niche using leukocyte biomimetic nanoparticles	Collaborator	1.135.000,00	https://www.cprit.texas.gov/grants-funded/grants/rp180394
Kleberg Foundation	Houston Methodist Research Institute, Houston, TX	2017-2020	A New Biomimetic Approach in the Treatment of Cardiovascular Inflammation	Coordinator	820.000,00	https://www.klebergfoundation.org/grant-guidelines/medical-research/

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
National Cancer Institute	Houston Methodist Research Institute, Houston, TX	2017-2020	Biomimetic nanovesicles to overcome multiple physiological barriers for primary and metastatic triple negative breast cancer therapy	Coordinator	370.000,00	https://grantome.com/grant/NIH/R56-CA213859-01A1
Cancer Prevention and Research Institute of Texas (CPRIT)	Houston Methodist Research Institute, Houston, TX	2016-2020	: Targeting the Inflammatory Cancer Stem Cell Microenvironment of Triple Negative Breast Cancer with Leukocyte-mimetic Nanovesicles	Collaborator	850.000,00	https://www.cprit.texas.gov/grants-funded/grants/rp170

2.6 Research Collaborators n. 5

Last Name: IODICE
First Name: FRANCESCO

Last name at birth: FRANCESCO
Gender: M

Title: He will analyze and integrate clinical data during intermediate and final reports

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: SANTA MARIA CAPUA VETERE

Nationality: Italiana
Date of birth: 07/08/1985

Official H index (Scopus or Web of Science): 11.0

Scopus Author Id:56008627200 **ORCID ID:**0000-0003-4817-8089 **RESEARCH ID:**Z-5875-2019

Contact address

Current organisation name: Casa di cura San Raffaele Pisana
Current Department / Faculty / Institute / Laboratory name: Department of Neuroscience and Neurorehabilitation, Brain Connectivity Laboratory

Street: Via Lardaria 9B
Postcode / Cedex: 00168 **Town:** ROMA
Phone:+393924605481 **Phone 2:**

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Catholic University of the Sacred Heart, Rome	PhD	Medicine - Neuroscience	2017	2020
Catholic University of the Sacred Heart, Rome	Specialization / Specializzazione	Medicine - Neuroscience	2010	2016
Catholic University of the Sacred Heart, Rome	Single-cycle master's degree / Laurea magistrale a ciclo unico	Medicine	2004	2010

Personal Statement:

Dr. Francesco Iodice is a neurologist engaged since 2010 in the Neurophysiology Unit where he gained expertise in the study of the central and peripheral nervous system with neurophysiological methods. He has joined several clinical trials related to diagnostic and therapeutic aspects of neurodegenerative diseases. In this project, he will analyze and integrate clinical data during intermediate and final reports.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Società Italiana di Neurologia	Sezione Giovani	Italy	Past-President	2020	2021
Società Italiana di Neurologia	Sezione Giovani	Italy	President	2018	2020

Other awards and honors

No Award and Honors

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Italian Ministry of Health and Agenzia Italiana del Farmaco	IRCCS Fondazione Policlinico Gemelli	2019	INTERCEPTOR PROJECT	Collaborator	340.000,00	https://www.interceptorproject.com/

2.7 Research Collaborators n. 6 - Under 40

Last Name: LI PUMA
First Name: DOMENICA DONATELLA

Last name at birth:
Gender: F

Title: She will set up the in vivo model of recurrent HSV-1 infection and perform molecular analyses

Nationality: italiana

Date of birth: 22/01/1986

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: PETRALIA SOTTANA

Official H index (Scopus or Web of Science): 17.0

Scopus Author Id:55370288500 **ORCID ID:**0000-0001-6729-6967 **RESEARCH ID:**J-7524-2018

Contact address

Current organisation name: IRCCS Fondazione Policlinico Universitario A. Gemelli

Current Department / Faculty / Institute / Laboratory name: UOC neurologia

Street: Largo Francesco Vito,1

Postcode / Cedex: 00168 **Town:** ROMA

Phone:00393339152957 **Phone 2:** 06-3015-4966

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Università Cattolica del Sacro Cuore, Rome, Italy	PhD	Neuroscience	2011	2014
Università Cattolica del Sacro Cuore, Rome, Italy	Master's Degree / Laurea Magistrale	Medical Biotechnology	2008	2010
Università Cattolica del Sacro Cuore, Rome, Italy	Bachelor Degree / Laurea Triennale	Health Biotechnology	2005	2008

Personal Statement:

Dr. Domenica Li Puma has been working in Alzheimer's disease field since 2011 studying the molecular and cellular mechanisms underlying synaptic plasticity, learning and memory in experimental models of neurodegenerative diseases (e.g. Alzheimer's). Most of her work has been focused on role of beta-amyloid and tau in the pathophysiology of Alzheimer¿s disease. She also investigated the relative contribution of Aß deposits, tau hyperphosphorylation and neuroinflammation to synaptic dysfunction and cognitive decline induced by repeated Herpes simplex virus type 1 replications in the brain. In the project she will perform molecular and morphological experiments to investigate leukosomes treatment on the AD hallmarkers by taking advantage of the sporadic model of AD.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Università Cattolica del Sacro Cuore, Rome, Italy	Department of Neuroscience	Largo F. Vito, 1 -00168- Rome, Italy	Assistant professor	2017	2022
Università Cattolica del Sacro Cuore, Rome, Italy	Institute of Human Physiology	Largo F. Vito, 1 -00168- Rome, Italy	Post-doctoral fellow	2015	2017
Università Cattolica del Sacro Cuore, Rome, Italy	Institute of Human Physiology	Largo F. Vito, 1 ¿ 00168 - Rome, Italy	PhD	2011	2014

Other awards and honors

2008: Magna cum laude, Health Biotechnology, Università Cattolica
2010: Magna cum laude, Medical Biotechnology, Università Cattolica
2014: Summer Intern in the RIKEN Brain Science Institute
2018: Visiting Researcher in the Taub Institute for Research on Alzheimer¿s disease and the Aging Brain, Columbia University
2019: Oral presentation at Neuroscience 2019
2020: Best publication Award, Università Cattolica
2021: Oral presentation at 25th WCN
2022: Oral presentation at 16th AD/PD Conference

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Italian Ministry of Education, University and Research	Università Cattolica del Sacro Cuore, Rome	2019	2017A9MK4R_004 - Immune-synaptopathies: dissecting the contribution of inflammation to synaptic dysfunctions	Collaborator	148.030,00	-

2.8 Research Collaborators n. 7 - Under 40

Last Name: Paciello
First Name: Fabiola

Last name at birth:
Gender: F

Title: She will perform behavioral experiments to evaluate cognitive dysfunctions and morphological analyses

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Agropoli

Nationality: italiana
Date of birth: 14/06/1987

Official H index (Scopus or Web of Science): 13.0

Scopus Author Id:55614565200 **ORCID ID:**0000-0002-8473-8074 **RESEARCH ID:**K-4967-2018

Contact address

Current organisation name: IRCCS Fondazione Policlinico Universitario A. Gemelli
Current Department / Faculty / Institute / Laboratory name: UOC neurologia
Street: Largo F. Vito, 1
Postcode / Cedex: 00168 **Town:** Roma
Phone:+393389383761 **Phone 2:**

Education / training

Educational institution and location	Degree	Field of study	From year	To year
Università Cattolica del Sacro Cuore, Rome, Italy	PhD	Neuroscience of sensory systems, neurophysiology, structural plasticity	2011	2014
Sapienza University of Rome, Italy	Master's Degree / Laurea Magistrale	Cognitive Neuroscience	2009	2011

Personal Statement:

Fabiola Paciello has been working in the Neuroscience field since 2011. She maturated expertise in studying modulation of neuronal plasticity and neuronal morphology in both physiological and pathological conditions by using electrophysiological, immunohistochemical, and molecular biology techniques. She also has a solid background and expertise in animal model research.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
Università Cattolica del Sacro Cuore	Department of Neuroscience	Rome, Italy	Assistant Professor	2019	2022
Università Cattolica del Sacro Cuore	Institute of Human Physiology	Rome, Italy	Post-doctoral researcher	2018	2019
National Council of Research (CNR)	Institute of Cell Biology and Neurobiology (IBCN)	Monterotondo, Rome, Italy	Post-doctoral researcher	2016	2018
Università Cattolica del Sacro Cuore	Institute of Human Physiology	Rome, Italy	Post-doctoral researcher	2014	2016
Università Cattolica del Sacro Cuore	Institute of Human Physiology	Rome, Italy	PhD student	2011	2014

Other awards and honors

2011-Magna cum laude, Cognitive Neuroscience Master Degree, Sapienza University of Rome, Italy;
2020-Best pubblication award, Università Cattolica del Sacro Cuore, Rome, Italy.

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Alzheimer's Association	Università Cattolica del Sacro Cuore	2021	Engineering proteins as novel candidates for Alzheimer's disease treatment	Collaborator	141.888,00	https://www.alz.org/

2.9 Additional Research Collaborators n. 2 - Under 40 to hire

Last Name: Ventura
First Name: Lucia
Last name at birth:
Gender: F

Title: She will perform the functional and neurophysiological tests on subjects.
Nationality: Italiana
Date of birth: 15/01/1991
Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Sassari

Official H index (Scopus or Web of Science): 2.0
Scopus Author Id:57208689926 **ORCID ID:**0000-0001-7302-743X **RESEARCH ID:**AHE-7782-2022

Contact address

Current organisation name: Azienda Ospedaliero-Universitaria di Sassari
Current Department / Faculty / Institute / Laboratory name: Unit of Endocrinology, Nutrition and Metabolism Disorders AOU Sassari

Street: V.le San Pietro 43/b
Postcode / Cedex: 07100
Town: Sassari
Phone:+393343237482
Phone 2:

Education / training

Educational institution and location	Degree	Field of study	From year	To year
University of Sassari, Sassari, Italy	PhD	Neuroscience	2019	2022
University of Cagliari, Cagliari, Italy	Master's Degree / Laurea Magistrale	Neurorehabilitation and exercise physiology	2014	2018

Personal Statement:

As a clinical research physiotherapist and exercise physiologist, the current goal of my research is the translation of dynamometric and neurophysiological assessments and training methodologies developed in sports and exercise physiology into the assessment and management of neurological disorders. This project aims to monitor markers of neurodegeneration at clinical, neurophysiological and metabolic levels in Mild Cognitive Impairment compared with Alzheimer Diseas and healthy controls. I will perform the functional and neurophysiological tests on subjects.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
University of Sassari	Department of Biomedical Sciences, Physiology Lab	Sassari, Italy	Doctoral researcher	2019	2022
University of Sassari	Department of Biomedical Sciences, Physiology Lab	Sassari, Italy	Post-graduate research fellow	2019	2019

Other awards and honors

No Awards and Honors

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Fondazione Italiana Sclerosi Multipla FISM Grant 2020_R_Single_028	Department of Biomedical Sciences	2021-2023	Measuring the impact of common exercise programs on subjective and objective fatigue during daily living activities in people with multiple sclerosis	Collaborator	131.460,00	https://aism.it/gli-studi-sostenuti-con-il-bando-fism-2020
Fondazione Italiana Sclerosi Multipla (FISM), bando 2020, FISM 2020/R-Single/028	Department of Biomedical Sciences, University of Sassari	2018	The effects of eccentric strength training on limb spasticity and muscle weakness in people with multiple sclerosis: a pilot study.	Collaborator	30.000,00	https://aism.it/gli-studi-sostenuti-con-il-bando-fism-2020

2.10 Additional Research Collaborators n. 3 - Under 40 to hire

Last Name: Loi
First Name: Nicola

Last name at birth: Loi
Gender: M

Title: He will perform the neurophysiological tests on subjects.
Nationality: Italiana
Date of birth: 06/11/1990

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Cagliari

Official H index (Scopus or Web of Science): 0.0

Scopus Author Id:57216319071 **ORCID ID:**0000-0002-1382-0783 **RESEARCH ID:**AHE-7933-2022

Contact address

Current organisation name: Azienda Ospedaliero-Universitaria di Sassari

Current Department / Faculty / Institute / Laboratory name: Unit of Endocrinology, Nutrition and Metabolism Disorders AOU Sassari

Street: Piazza Scirocco 22
Postcode / Cedex: 09030
Town: Elmas
Phone:+393396670579
Phone 2:

Education / training

Educational institution and location	Degree	Field of study	From year	To year
University of Sassari, Italy	PhD	Neuroscience	2018	2022
University of Cagliari, Italy	Master's Degree / Laurea Magistrale	Neuropsychobiology	2014	2017
University of Cagliari, Italy	Bachelor Degree / Laurea Triennale	Toxycology	2011	2014

Personal Statement:

I have a background in neurophysiology and excellent skills in the use of non-invasive neurophysiological techniques in healthy subjects. I have been trained at the University of Sassari and I participated in studies aimed to explore brainstem and cortical functions in neurological conditions using brainstem reflexes, EEG, transcranial magnetic stimulation and event-related potentials.This project aims to monitor markers of neurodegeneration at different levels in Mild Cognitive impairment compared with Alzheimer Disease and healthy controls. I will perform the neurophysiological tests on subjects.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
University of Sassari	Department of Biomedical Sciences, Physiology Lab	Sassari, Italy	Post-doctoral research fellow	2022	2022
University of Sassari	Department of Biomedical Sciences, Physiology Lab	Sassari, Italy	Doctoral researcher	2018	2022

Other awards and honors

No Award and Honors

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Fondazione di Sardegna. D.R. 1265/2021. Prot. 0043658, 09/04/21 [UOR: SI000072 Classif.III/13	Department of Biomedical Sciences; University of Sassari	2020-2023	Investigation of neural adaptations within face and hand motor networks following musical instruments practice.	Collaborator	15.000,00	https://www.fondazionedisardegna.it/

2.11 Additional Research Collaborators n. 4 - Under 40 to hire

Last Name: Cano
First Name: Antonella

Last name at birth:
Gender: F

Title: She will evaluate the nutritional status of the recruited subjects
Nationality: Italiana
Date of birth: 13/06/1983

Country of residence: ITALY
Country of Birth: ITALY
Place of Birth: Alghero

Official H index (Scopus or Web of Science): 5.0

Scopus Author Id:56514743300 **ORCID ID:**0000-0003-1544-9575 **RESEARCH ID:**AHE-7395-2022

Contact address

Current organisation name: Azienda Ospedaliero-Universitaria di Sassari
Current Department / Faculty / Institute / Laboratory name: Unit of Endocrinology, Nutrition and Metabolism Disorders AOU Sassari

Street: Viale San Pietro 43/b
Postcode / Cedex: 07100
Town: Sassari
Phone:+393805990806
Phone 2:

Education / training

Educational institution and location	Degree	Field of study	From year	To year
University of Sassari, Sassari, Italy	Specialization / Specializzazione	Nutritional Science	2019	2022
University of Strathclyde, Glasgow, UK	PhD	Biomedical Sciences	2011	2015
University of Sassari, Sassari, Italy	Master's Degree / Laurea Magistrale	Pharmacy	2002	2009

Personal Statement:

I have a PhD in Biomedical Sciences. My early research was focused on innate immunity and parasitology, acquiring competence in cell culture and molecular biology techniques. Now, I am a resident at the Specialization School of Nutritional Science and I learnt to assess nutritional status in healthy subjects, through the evaluation of body composition, of metabolic rates in static and dynamic conditions and of dietary intake. I will evaluate the nutritional status of the recruited subjects, to provide a full picture of their life-style and to correlate it to the clinical status.

Positions and honors

Positions

Institution	Division / Research group	Location	Position	From year	To year
University of Sassari	Department of Biomedical Sciences, Physiology Laboratory	Sassari, Italy	Non-medical resident (Specialization School in Nutritional Science)	2019	2022
University of Sassari	Department of Biomedical Sciences, Cellular biology Laboratory	Sassari, Italy	Post-doctoral research fellow	2016	2017
Porto Conte Ricerche S.r.l., Tramariglio (Alghero), Italy	Proteomics Laboratory	Alghero, Italy	Post-doctoral research fellow	2015	2016
University of Strathclyde Glasgow, UK	Strathclyde Institute of Pharmacy and BiomedIcal Sciences (SIPBS)	Glasgow, UK	Doctoral researcher	2011	2014
University of Strathclyde Glasgow, UK	Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS)	Glasgow, UK	Post-graduate research fellow	2010	2011

Other awards and honors

Society for General Microbiology Travel Grant, to attend the Free-Living Amoeba Meeting (FLAM) (14-19 July 2013, Vienna, Austria)

British Society for Immunology Travel Award, to attend the Woods Hole Immunoparasitology Meeting (WHIP) (April 2014, Woods Hole, USA).

Grant

Funded by Institution	Researcher inst. where grant is/was performed	Year	Title	Position in Projects	Fund (euro)	Source website grant listed
Fondazione di Sardegna. D.R. rep. n. 605/2022 prot. n. 0019085, 21/01/2022. [UOR: SI000072 Classif.III/13].	Department of Biomedical Sciences, University of Sassari, Sassari, Italy	2022-2024	Study of the physiologic ageing and of related pathologies with a multidisciplinary approach.	Collaborator	115.000,00	https://www.fondazionedisardegna.it/

2.17 Expertise Research Collaborators

Selected peer-reviewed publications of the Research Group / Collaborators

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
Cano Antonella	Sex-Based Differences in Oxygen Cost of Walking and Energy Equivalents in Minimally Disabled Individuals With Multiple Sclerosis and Controls	Article	54-61	24	2022	10.7224/1537-2073.2020-112	35462872	0	O
Cano Antonella	Analysis of sex-based differences in energy substrate utilization during moderate-intensity aerobic exercise	Review	29-70	122	2022	10.1007/s00421-021-04802-5	34550468	3	F
Paciello Fabiola	Auditory sensory deprivation induced by noise exposure exacerbates cognitive decline in a mouse model of alzheimer¿s disease	Article	e70908	10	2021	10.7554/eLife.70908	34699347	0	F
LI PUMA DOMENICA DONATELLA	Ca²⁺-dependent release of ATP from astrocytes affects herpes simplex virus type 1 infection of neurons	Article	201-215	69	2021	10.1002/glia.23895	32818313	4	F
IODICE FRANCESCO	Stroke and digital technology: a wake-up call from COVID-19 pandemic	Review	805-809	42	2021	10.1007/s10072-020-04993-3	33433756	13	F
IODICE FRANCESCO	Direct and indirect neurological, cognitive, and behavioral effects of COVID-19 on the healthy elderly, mild-cognitive-impairment, and Alzheimer¿s disease populations	Review	455-465	42	2021	10.1007/s10072-020-04902-8	33409824	12	F
Loi Nicola	Faces emotional expressions: From perceptive to motor areas in aged and young subjects	Article	1642-1652	126	2021	10.1152/jn.00328.2021	34614362	0	F
Loi Nicola	Emotional Face Expressions Influence the Delay Eye-blink Classical Conditioning	Article	72-79	471	2021	10.1016/j.neuroscience.2021.07.019	34332014	0	F
Loi Nicola	Physiological Differences in Hand and Face Areas of the Primary Motor Cortex in Skilled Wind and String Musicians	Article	141-150	455	2021	10.1016/j.neuroscience.2020.12.023	33359658	0	O

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
Cano Antonella	Energy Expenditure and Oxygen Consumption During Activities of Daily Living in People With Multiple Sclerosis and Healthy Subjects: An Ecological Approach to Estimate Real-Life Fatigue and Fatigability	Article	1482-1489	102	2021	10.1016/j.apmr.2020.12.024	33539804	0	O
Ventura Lucia	Test-Retest Reliability and Known-Groups Validity of Trunk Muscle Tests in People With Multiple Sclerosis: A Cross-Sectional, Case-Control Study	Article	pzab049	101	2021	10.1093/ptj/pzab049	33538837	1	O
Ventura Lucia	Reporting quality of TMS studies in neurological conditions: A critical appraisal of the main gaps, challenges and clinical implications	Review	109293	362	2021	10.1016/j.jneumeth.2021.109293	34293408	0	O
Ventura Lucia	Healthy Women and Men Do Not Show Differences in Tongue Strength and Regular Effort Saliva Swallows as Assessed by Piezo-Resistive Sensors: Results from a Reproducibility Study	Article	NOT_FOUND	November 15	2021	10.1007/s00455-021-10381-6	34779910	0	O
Podda Maria Vittoria	Enhancing Plasticity Mechanisms in the Mouse Motor Cortex by Anodal Transcranial Direct-Current Stimulation: The Contribution of Nitric Oxide Signaling	Article	2972-2985	30	2020	10.1093/cercor/bhz288	31821409	6	C
Loi Nicola	Transcutaneous trigeminal nerve stimulation modulates the hand blink reflex	Article	21116	10	2020	10.1038/s41598-020-78092-w	33273638	0	O
Loi Nicola	The vestibulo-masseteric reflex and the acoustic-masseteric reflex: a reliability and responsiveness study in healthy subjects	Article	1769-1779	238	2020	10.1007/s00221-020-05804-z	32280998	0	F
Cano Antonella	Direct-to-consumer nutrigenetics testing: An overview	Review	566	12	2020	10.3390/nu12020566	32098227	9	O
Ventura Lucia	Effect of eccentric strength training on elbow flexor spasticity and muscle weakness in people with multiple sclerosis: Proof-of-concept single-system case series	Article	1142-1152	100	2020	10.1093/ptj/pzaa055	32266379	1	O

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
Deriu Franca	Role of cutaneous and proprioceptive inputs in sensorimotor integration and plasticity occurring in the facial primary motor cortex	Article	839-851	598	2020	10.1113/JP278877	31876950	6	C
Paciello Fabiola	Targeting dysregulation of redox homeostasis in noise-induced hearing loss: Oxidative stress and ROS signaling	Review	46-59	135	2019	10.1016/j.freeradbiomed.2019.02.022	30802489	59	O
LI PUMA DOMENICA DONATELLA	Recurrent herpes simplex virus-1 infection induces hallmarks of neurodegeneration and cognitive deficits in mice	Article	e1007617	15	2019	10.1371/journal.ppat.1007617	30870531	83	O
MIRAGLIA FRANCESCA	Methods for analysis of brain connectivity: An IFCN-sponsored review	Review	1833-1858	130	2019	10.1016/j.clinph.2019.06.006	31401492	37	O
IODICE FRANCESCO	Cortical connectivity from EEG data in acute stroke: A study via graph theory as a potential biomarker for functional recovery	Article	133-138	146	2019	10.1016/j.ijpsycho.2019.09.012	31648028	13	O
IODICE FRANCESCO	Six-Month Assessment of a Hand Prosthesis with Intraneural Tactile Feedback	Article	137-154	85	2019	10.1002/ana.25384	30474259	59	O
Ventura Lucia	Isokinetic predictors of gait speed increase following high-intensity resistance training of the ankle dorsiflexors in people with multiple sclerosis: A pilot study	Article	102-106	67	2019	10.1016/j.clinbiomech.2019.05.008	31100700	4	O
LI PUMA DOMENICA DONATELLA	LTP and memory impairment caused by extracellular A? and tau oligomers is APP-dependent	Article	e26991	6	2017	10.7554/eLife.26991.001	28696204	61	O
MIRAGLIA FRANCESCA	Small-World Characteristics of Cortical Connectivity Changes in Acute Stroke	Article	81-94	31	2017	10.1177/1545968316662525	27511048	42	O
Deriu Franca	Dance therapy improves motor and cognitive functions in patients with Parkinson's disease	Article	141-144	40	2017	10.3233/NRE-161399	27814308	46	L
Podda Maria Vittoria	Anodal transcranial direct current stimulation boosts synaptic plasticity and memory in mice via epigenetic regulation of Bdnf expression	Article	22180	6	2016	10.1038/srep22180	26908001	109	F
LI PUMA DOMENICA DONATELLA	Extracellular Tau Oligomers Produce An Immediate Impairment of LTP and Memory	Article	19393	6	2016	10.1038/srep19393	26786552	121	O

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
MIRAGLIA FRANCESCA	EEG characteristics in "eyes-open" versus "eyes-closed" conditions: Small-world network architecture in healthy aging and age-related brain degeneration	Article	1261-1268	127	2016	10.1016/j.clinph.2015.07.040	26603651	54	F
Cano Antonella	Acanthamoeba castellanii genotype T4 stimulates the production of interleukin-10 as well as proinflammatory cytokines in THP-1 cells, human peripheral blood mononuclear cells, and human monocyte-derived macrophages	Article	2953-2962	84	2016	10.1128/IAI.00345-16	27481240	11	O
TASCIOTTI ENNIO	The impact of nanoparticle protein corona on cytotoxicity, immunotoxicity and target drug delivery	Review	81-100	11	2016	10.2217/nnm.15.188	26653875	322	L
TASCIOTTI ENNIO	Biomimetic proteolipid vesicles for targeting inflamed tissues	Article	1037-1046	15	2016	10.1038/nmat4644	27213956	194	L
Paciello Fabiola	Molecular targets for anticancer redox chemotherapy and cisplatin-induced ototoxicity: The role of curcumin on pSTAT3 and Nrf-2 signalling	Article	1434-1444	113	2015	10.1038/bjc.2015.359	26469832	69	O
Paciello Fabiola	Rosmarinic acid up-regulates the noise-activated Nrf2/HO-1 pathway and protects against noise-induced injury in rat cochlea	Article	269-281	85	2015	10.1016/j.freeradbiomed.2015.04.021	25936352	53	O
MIRAGLIA FRANCESCA	Cortical Brain Connectivity Evaluated by Graph Theory in Dementia: A Correlation Study between Functional and Structural Data	Article	745-756	45	2015	10.3233/JAD-142484	25613102	35	O
Deriu Franca	Abnormalities of vestibular-evoked myogenic potentials in idiopathic Parkinson¿s disease are associated with clinical evidence of brainstem involvement	Article	995-1001	36	2015	10.1007/s10072-014-2054-4	25567081	33	L
Deriu Franca	Paired neurophysiological and clinical study of the brainstem at different stages of Parkinson's Disease	Article	1871-1878	126	2015	10.1016/j.clinph.2014.12.017	25622530	31	C
TASCIOTTI ENNIO	Biodegradable silicon nanoneedles delivering nucleic acids intracellularly induce localized in vivo neovascularization	Article	532-539	14	2015	10.1038/nmat4249	25822693	257	L

Collaborato	Title	Type	Pag	Vol	Year	DOI	PMID	Cit.**	P.*
LI PUMA DOMENICA DONATELLA	HSV-1 and Alzheimer's disease: More than a hypothesis	Review	97	5	2014	10.3389/fphar.2014.00097	24847267	66	O
MIRAGLIA FRANCESCA	Human brain networks in cognitive decline: A graph theoretical analysis of cortical connectivity from EEG data	Article	113-127	41	2014	10.3233/JAD-132087	24577480	70	O
Deriu Franca	Exploring brainstem function in multiple sclerosis by combining brainstem reflexes, evoked potentials, clinical and MRI investigations	Article	2286-2296	125	2014	10.1016/j.clinph.2014.03.016	24745338	25	L
TASCIOTTI ENNIO	Bromelain surface modification increases the diffusion of silica nanoparticles in the tumor extracellular matrix	Article	9874-9883	8	2014	10.1021/nn502807n	25119793	106	L
Podda Maria Vittoria	Reduced d-serine levels in the nucleus accumbens of cocaine-treated rats hinder the induction of NMDA receptor-dependent synaptic plasticity	Article	1216-1230	136	2013	10.1093/brain/awt036	23518710	40	O
Paciello Fabiola	Noise-induced hearing loss (NIHL) as a target of oxidative stress-mediated damage: Cochlear and cortical responses after an increase in antioxidant defense	Article	4011-4023	33	2013	10.1523/JNEUROSCI.2282-12.2013	23447610	112	O
TASCIOTTI ENNIO	Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions	Article	61-68	8	2013	10.1038/nnano.2012.212	23241654	634	L
Podda Maria Vittoria	A role for neuronal cAMP responsive-element binding (CREB)-1 in brain responses to calorie restriction	Article	621-626	109	2012	10.1073/pnas.1109237109	22190495	97	O
Podda Maria Vittoria	Modulation of LTP at rat hippocampal CA3-CA1 synapses by direct current stimulation	Article	1868-1880	107	2012	10.1152/jn.00319.2011	22236710	124	O
IODICE FRANCESCO	Central cholinergic dysfunction measured "in vivo" correlates with different behavioral disorders in Alzheimer's disease and dementia with Lewy body	Article	533-538	5	2012	10.1016/j.brs.2011.08.009	22019082	44	O

* Position: F=First L=Last C=Correspondent O=Other N=Not applicable

** Autocertificated

3 - Ethics

1. HUMAN EMBRYOS/FOETUSES	
Does your research involve Human Embryonic Stem Cells (hESCs)?	No
Does your research involve the use of human embryos?	No
Does your research involve the use of human foetal tissues / cells?	No
2. HUMANS	
Does your research involve human participants?	Yes
Does your research involve physical interventions on the study participants?	No
3. HUMAN CELLS / TISSUES	
Does your research involve human cells or tissues (other than from Human Embryos/ Foetuses?	No
4. PERSONAL DATA	
Does your research involve personal data collection and/or processing?	Yes
Does your research involve further processing of previously collected personal data (secondary use)?	No
5. ANIMALS	
Does your research involve animals?	Yes
6. ENVIRONMENT & HEALTH and SAFETY	
Does your research involve the use of elements that may cause harm to the environment, to animals or plants?	No
Does your research deal with endangered fauna and/or flora and/or protected areas?	No
Does your research involve the use of elements that may cause harm to humans, including research staff?	No
7. DUAL USE	
Does your research involve dual-use items in the sense of Regulation 428/2009, or other items for which an	No
8. EXCLUSIVE FOCUS ON CIVIL APPLICATIONS	
Could your research raise concerns regarding the exclusive focus on civil applications?	No
9. MISUSE	
Does your research have the potential for misuse of research results?	No
10. OTHER ETHICS ISSUES	
Are there any other ethics issues that should be taken into consideration? Please specify	No

I confirm that I have taken into account all ethics issues described above and that, if any ethics issues apply, I will complete the ethics self-assessment and attach the required documents. [X]

4 - Call-specific questions

Eligibility	
I acknowledge that I am aware of the eligibility requirements for applying as specified in the Call-PNRRXXXX_M6/C2, and certify that, to the best of my knowledge my application is in compliance with all these requirements. I understand that my proposal may be declared ineligible at any point during the evaluation or granting process if it is found not to be compliant with these eligibility criteria.	[X]
I confirm that the proposal that I am about to submit draws substantially don't repeat on an existing or recently finished GRANT funded.	[X]
Data-Related Questions and Data Protection (Consent to any question below is entirely voluntary. A positive or negative answer will not affect the evaluation of your project proposal in any form and will not be communicated to the evaluators of your project.)	
For communication purposes only, the MoH asks for your permission to publish,in whatever form and medium, your name, the proposal title, the proposal acronym, the panel, and host institution, should your proposal be retained for funding.	[X]
Some national and regional public research funding authorities run schemes to fund MoH applicants that score highly in the MoH's evaluation but which can not be funded by the MoH due to its limited budget. In case your proposal could not be selected for funding by the MoH do you consent to allow the MoH to disclose the results of your evaluation (score and ranking range) together with your name, non- confidential proposal title and abstract, proposal acronym, host institution and your contact details to such authorities?	[X]
The MoH is sometimes contacted for lists of MoH funded researchers by institutions that are awarding prizes to excellent researchers. Do you consent to allow the MoH to disclose your name, non-confidential proposal title and abstract, proposal acronym, host institution and your contact details to such institutions?	[X]
The Ministry of Health occasionally could contacts Principal Investigators of funded proposals for various purposes such as communication campaigns, pitching events, presentation of their project's evolution or outcomes to the public, invitations to represent the Ministry of Health in national and international forums, studies etc. Should your proposal be funded, do you consent to the Ministry of Health staff contacting you for such purposes?	[X]
For purposes related to monitoring, study and evaluating implementation of MoH actions, the MoH may need that submitted proposals and their respective evaluation data be processed by external parties. Any processing will be conducted in compliance with the requirements of Regulation 45/2001.	

5 – Description Project

Summary description

The variable course of Alzheimer's disease (AD) and the paucity of adequate cures urges to implement new strategies for its early detection and clinical intervention. Studying the etiopathogenesis and pathophysiological mechanisms of AD is a

necessary prerequisite for the development of new biomarkers and innovative therapies. Contrarily to anatomical structures, cortical connectivity networks are already deteriorated in early AD and could be a reliable marker of early cognitive decline. Our aim is to characterize the neurophysiological parameters in mild AD, in patients with mild cognitive impairment and in matched healthy subjects to identify discriminating criteria. Concurrently, the study of AD onset and progression in a mouse model, will allow evaluating the causal effect of inflammation on disease progression and to develop a new class of biomimetic anti-inflammatory nanoparticles formulated to have the intrinsic ability to induce brain's activated microglia to an M2 phenotype.

Background / State of the art

Alzheimer's Disease (AD) is a multifactorial disorder where the non-linear interaction between genetic, biological and environmental factors accounts for its inherent interindividual clinical variability (PMID:34973457). A better understanding of the disease to develop new therapeutic strategies, and the validation of diagnostic tools to reveal the early signs of AD, are two clinical imperatives that require an urgent solution. From the diagnostic standpoint, while the structural neocortical alterations are evident from the moderate stage of AD, the impairment of functional cortical connectivity can be detected in early and prodromal phases (PMID:24562737), making connectivity an interesting early marker of AD. AD presents not only connectivity disruption but also altered neuroplasticity, which can also be detected at early stages of the disease (PMID:34973457). Last but not least, life-style factors may also influence inflammation status which in turns deteriorate cognitive functions (PMID: 34101789). Notably, the increased levels of inflammatory markers in AD and the identification of AD risk genes associated with innate immunity, strongly suggest neuroinflammation¿s prominent role in AD pathogenesis (PMID:33318676). The development of a novel treatment based on anti-inflammatory nanoparticles (PMID:27213956), could be a relevant therapeutic approach to prevent disease onset or ameliorate AD-like phenotypes.

Description and distribution of activities of each operating unit

Collaboration among the operative units (OUs) involved in this project will be of key relevance for the successful outcome of this proposal. The three OUs will provide complementary, high-level expertise in the main areas of the work plan, namely neurology and clinical management of patients, neurophysiological markers and EEG data recording and analysis (OU1, OU2), animal model management and neurophysiological, behavioral and molecular assessments (OU3), nanoformulation and pharmacology of innovative drug therapies (OU1).
Due to the proximity of their laboratories OU1 and OU3 can easily transfer materials and provide immediate support for the possible technical troubleshooting of experimental conditions. The OUs during the course of previous collaborations have already harmonized the procedures and methods used for data collection, storage and analysis, as well as for the selection of the statistical modelling of the multi-modal markers and the statistical tools to be used to validate the parameters described in the proposal. To further facilitate the project management and the cooperation among the units, both animal model and human clinical, behavioral and neurophysiological (neuroplasticity-EEG in subjects/LFP recordings in mice) data will be stored in a shared storage infrastructure, which will be implemented in the framework of this project. In particular:
OU1 will be responsible for EEG data analyses and for the centralized statistical analyses of all data generated in the study from both animal and human models. It will be also responsible for the nanoformulation and pharmacological assessment of innovative drug therapies.
OU2 will be responsible for patients¿ recruitment, clinical and psycho-cognitive evaluation, assessment of metabolic and nutritional status, physical functioning, neurophysiological measurements of cortical connectivity through EEG, intracortical excitability, plasticity and sensorimotor integration processes, through transcranial magnetic stimulation (TMS) protocols.
OU3 will be responsible for setting up the animal model of AD and will carry out in vivo local field potential (LFP) recordings and behavioral tests as well as molecular and morphological analyses on brain tissues.

5.4 Specific Aims and Experimental Design

Specific aim 1

Aim 1 is focused on the recruitment and clinical and neurophysiological assessments of the human subjects to be enrolled in the study. The criteria established will allow to recruit the following cohorts: mild AD and patients with mild cognitive impairment (MCI). All participants will undergo clinical, psycho-cognitive, metabolic, physical functioning and different neurophysiological investigations. The collected data will be compared between patient groups and a group of age- sex- and education-matched healthy controls (HC). The analysis of the data will identify standardized multidimensional biomarkers to be proposed for the early detection of AD.

Sub-Aim 1.1. Clinical and neuropsychological evaluation. Mild AD e MCI patients will be enrolled according to NINCDS-ADRDA criteria (PMID:10071097). Patients will undergo a complete clinical investigation, through a medical history questionnaire, neurological examination, mini mental state examination (MMSE), and a neuropsychological and complete neuropsychiatric examination. For all participants (AD, MCI and HC), socio-demographic, socio-economic factors and life-style will be investigated using validated questionnaires.

Sub-Aim 1.2. Evaluation of cortical connectivity network. The contribution of functional network analyses will be investigated to characterize the neural correlate of the disconnection syndrome, as a feature of AD. Resting EEG will be recorded, and the EEG signal will be analyzed for: 1) source power spectrum; 2) spectral coherence; 3) cortico-cortical connectivity via graph-theory; 4) complexity analyses. EEG will be analyzed using innovative method to: 1) observe with high spatio-temporal resolution how cognitive decline processes evoke synchronized neuronal activity in connected brain areas; 2) monitor their changes in different level of clinical stages. The data obtained in the other sub-aims will be correlated with the parameters obtained here.

Sub-Aim 1.3. Evaluation of neuroplasticity and sensorimotor integration. Several studies reported that there is a strong relationship between the functionality of the primary motor cortex and the clinical status of AD patients. In particular, it was demonstrated that an alteration of the cholinergic function underlying the sensorimotor integration process would be one of the useful markers to detect neuropathological changes even at the early stage of AD (PMID:34973457). In addition, also cortical plasticity could be a novel biomarker to characterize the clinical progression to dementia in patients with MCI or early stage of AD. Therefore, the cortical excitability, the sensorimotor integration and the cortical plasticity will be investigated in all participants using standardized transcranial magnetic stimulation (TMS) protocols.

Sub-Aim 1.4. Evaluation of metabolic biomarkers.
Since several studies suggested that AD could be the result of a complex and multifactorial condition, nutritional status, dietary habits and physical performance will be evaluated in order to provide a full picture of patients¿ life-style and to correlate it to the clinical status (PMID:34101789). To accomplish this aim, anthropometric measures and body composition will be assessed using body impedance vector analysis, under standardized conditions. The nutritional status will be evaluated through validated mini nutritional assessment score. The adherence to the Mediterranean Diet will be assessed through validated questionnaires; a 3-day dietary diary will be completed by each participant, with caregiver support if needed, to estimate daily energy intake. Physical capabilities will be assessed through a set of validated tests, which provide the following outcomes: handgrip strength, walking speed, walking endurance and fatigability, energy cost of walking, fall risk and walking ability, functional lower extremity strength and transitional movements, dynamic stability, and balance.

Specific aim 2

Aim 2 is focused on the characterization of the changes in brain connectivity and complexity in a sporadic AD-like mouse model, showing progressive signs of neuro-degeneration, neural inflammation and cognitive impairment.
Specifically, C57BL/6 wild type mice: i) will undergo few cycles of Herpes simplex virus-1 (HSV-1) reactivations within the brain (i.e., 2 thermal stress [2×TS]) resulting in increased levels of proinflammatory cytokines (e.g. Interleukin 1ß); ii) will be

subjected to 7 TS [7×TS] resulting in Aß and pTau accumulation increases and irreversible cognitive decline (i.e. AD-like phenotype).
Connectivity indices acquired through LFP recordings and morphological changes in branching complexity and dendritic spines will be correlated with the behavioral and molecular hallmarks of AD and with the evolution of neuroinflammation, to: i) corroborate the validity of the connectivity indices as AD biomarker and ii) identify new molecular or cellular determinants of AD onset and progression, as described in the following Sub-Aims.

Sub-Aim 2.1. To evaluate cognitive functions in 2×TS and 7×TS mice in order to monitor disease onset and progression. We will use behavioral tests evaluating associative (fear conditioning test), spatial and recognition memory (Radial Arm Maze, Novel object recognition test) (PMID:29997473). Motor function (i.e., fore- and hindlimb strength, grid-walking and overall locomotor activity) will be also evaluated, to match functional evaluation in human patients (PMID:31821409).
Sub-Aim 2.2. Characterizing changes in brain connectivity in 2×TS and 7×TS mice and correlating them to changes in cognitive performances and to AD hallmarks. Connectivity indices based on LFP recordings will be calculated as for human EEG data (as reported in Aim1, with less nodes) (PMID:35291824) and correlated to functional (Sub-Aim 2.1) and molecular AD indices (e.g. Aß and pTau).

Sub-Aim 2.3. To evaluate morphological alterations in brain structures. Branching complexity and changes in spine density and perineuronal net in hippocampal and cortical neurons will be evaluated (PMID:34699347) as possible correlates of altered connectivity in 2×TS and 7×TS mice.

Sub-Aim 2.4. Characterize the inflammatory response of AD-like mouse brain. In hippocampal and cortical tissues of 2×TS and 7×TS mice, we will detect changes in the level of pro-inflammatory mediators such as interleukin-1 (IL-1), tumor necrosis factor alpha and anti-inflammatory cytokines, including transforming growth factor ß and interleukin-10 and nitric oxide (by ELISA and Western blotting). We will also evaluate microglia morphology (by Iba1 or TMEM119 immunoreactivity) and the expression of principal microglial markers (e.g. the lysosomal marker CD68, the resting microglia marker F4/80, the M2 state marker CD86) by immunofluorescence analyses. The expression of key genes (i.e. receptors and cytokines) mediating the inflammatory response, will be also evaluated by using RT² Profiler PCR Array profiles.

Specific aim 3

Specific Aim 3 will focus on the evaluation of the effect of anti-inflammatory nanoparticles, the leukosomes, a new class of biomimetic nanotherapeutic for the targeting of inflamed areas of the CNS (PMID:29512198, PMID:29464004). It will be studied the ability to target damaged brain areas, to reduce or resolve neuroinflammation and to ameliorate AD phenotype. All the experiments to determine the pharmacological properties of therapeutic leukosomes will be performed in the mouse model of sporadic AD described in Aim 2. Treatments will be monitored through functional and connectivity indices as described in Sub-Aim2.1 and 2.2. This aim will provide important insights for the use of leukosomes in the treatment of neuroinflammation-related neurodegeneration.

Sub-Aim 3.1. Synthesize biomimetic nanoparticles (leukosomes) using purified leukocyte membrane proteins integrated into synthetic liposomes. A specific formulation of leukosomes will be developed for this project. In order to inherently endow nanoparticles with the ability to stimulate microglia polarization towards an M2 phenotype, freshly isolated and cultured leukocytes will be treated with IL-4 prior to cell membrane proteins extraction. Leukosomes will be manufactured using established microfluidic protocols that guarantee scalability, GMP certification, translational value and approval for clinical use. We expect to characterize nanoparticles¿ size, surface properties, homogeneity, and the proteomic profile of the molecular markers of adhesion to activated endothelial cells and of the membrane receptors that will induce M2 polarization of the microglia.

Sub-Aim 3.2. Determine leukosomes bio-distribution, targeting properties and drug accumulation in the inflamed tissues of the diseased brain areas. We expect that compared to free drugs, leukosomes will allow for a superior accumulation of the therapeutic agent in the inflamed tissues. Leukosomes targeting and biodistribution will be assessed through in vivo fluorescent imaging and ex vivo analysis of explanted tissues and organs.

Sub-Aim 3.3. Load leukosomes with anti-inflammatory payload (dexamethasone) and characterize their pharmacological properties (loading and release kinetics and stability) and their effect in reducing inflammation of the microglia. We will standardize the protocols to encapsulate dexamethasone as a model drug, and to evaluate its release profiles and anti-inflammatory effect on microglia.

Sub-Aim 3.4. Evaluate leukosome treatment (empty or loaded with dexamethasone) to counteract the cellular and molecular hallmarks of AD and neuroinflammation and to prevent the worsening of AD functional impairments. Functional and molecular indices will be assessed in mice subjected to nanoparticle administration and correlated to connectivity indices (as in Sub-Aims 2.1-3), to validate them as biomarkers of treatment efficacy. . Histological evaluation of brain plasticity will be performed to assess if the leukosome treatment also favors a regenerative microenvironment. Behavioral and motor function test will be conducted as described in Aim2.1 to evaluate the impact of the treatment on overall performance of mice.

Experimental design aim 1

100 AD and 100 MCI individuals from the dementia clinical centres of North Sardinia and local sections of patients¿ associations and 100 HC will be recruited for the study. To investigate past/present health status and medications, baseline medical screening will be performed by general clinical and anthropometric examinations. Exclusion criteria will include: major psychosis, genetic, metabolic and other neurological disorders, acute or chronic non-compensated medical illness; any medical or drug-related condition affecting cognitive or mood status; history or current alcohol/illicit drug abuse. All subjects will undergo the evaluations detailed in the following Sub-Tasks

Sub-Task 1.1 Neuropsychological evaluation
The neuropsychological assessment will include Mini-Mental State Examination , Addenbrooke Cognitive Exam and Montreal cognitive assessment. Cognitive domains will be investigated for:
Executive functions: Raven coloured progressive matrices, Frontal Assessment Battery, Clearing Multiple Characteristic Targets.
Language: Token test, Description of a complex figure, Screening for aphasia in Neuro Degeneration, Phonemic verbal fluency test, Semantic Verbal Fluency test
Learning: Digit span, Two-syllable word test, Block-tapping test courses, Short-Term Memory Binding , Babcock story recall, Rey Auditory Verbal Learning Test
Visual attention: Test for agnosia, Naming test of figures, Visual images of different categories of objects, Baum, Goodenough and Harris tests

Sub-Task 1.2 Evaluation of cortical connectivity network
EEG will be recorded for at least 5 minutes. The following analysis will be performed:
Source power spectrum: Power spectrum analysis and EEG cortical current density time series will be evaluated (PMID:33860614).
Spectral coherence: EEG spectral Magnitude Squared Coherence at electrodes¿ level will evaluate the functional coupling among the brain areas (PMID:20157242).
Connectivity via graph-theory: Intracortical Lagged Linear Coherence will be computed for each hemisphere between all pairs of the available 42 Brodmann areas (PMID:21893527), and used as measure of weight of the graph: ROIs will be the

nodes; coherence¿s values the weights of the edges. Graph parameters (Small world, Characteristic Path Length, Clustering coefficient) will be computed (PMID:19190637).
Complexity analyses: The complexity of brain activity will be studied by entropy measures (Sample, Multiscale, and Approximate Entropy) (PMID:33286988).

Sub-Task 1.3 Evaluation of neuroplasticity and sensorimotor integration
Cortical excitability, sensorimotor integration and cortical plasticity will be investigated by TMS protocols. The resting motor threshold (RMT), Short-latency afferent inhibition (SAI) and effect of paired associative stimulation (PAS) will be assessed recording motor evoked potentials (MEPs) from the right first dorsal interosseous (FDI) muscle.

Subtask 1.4 Evaluation of metabolic biomarkers
The nutritional status will be evaluated by: bioelectrical impedance vector analysis, using an impedance analyser, for body composition; Mini Nutritional Assessment to evaluate risk of malnutrition (PMID:9990575); adherence to Mediterranean Diet by validated questionnaires (PMID:26035442;12826634) and Three-Day Food Diary, instantly capturing after meals/snacks the food/beverage consumed during the day.
Walking ability, speed and dynamic balance will be assessed by: 10-Meter Timed Walk at comfortable and fastest speed, 2-Minute Walk, Five Times Sit-to-Stand, Timed Up and Go. Walking endurance and fatigability will be evaluated by measuring the distance covered in the 6-minute walk test (PMID:12091180). Energy cost of walking, respiratory exchange ratio, VO2 peak, heart and perceived exertion rates will be recorded for cardiovascular and metabolic performance.
Hands and lower limb muscles strength will be measured by handgrip and hand-held dynamometry, with a computer based measuring system.

Experimental design aim 2

Task2 will be organized into 6 Sub-tasks, each one addressing a specific Aim2 Sub-aim.
Experiments will be performed on C57BL/6 mice divided into 4 main groups (n=42 mice/group): i) 2×TS mice undergoing HSV-1 infection at 6 months of age (6m) and 2 cycles of HSV-1 reactivation by thermal stress (the first TS at 8m and the 2nd TS at 9m); ii) 7×TS mice undergoing HSV-1 infection at 1m and 7 cycles of HSV-1 reactivation (1 cycle/month, beginning at 3m and ending at 9m); iii) 2×TS-mock and iv) 7×TS-mock undergoing TS without virus inoculation (PMID:30870531). A fifth experimental group of naïve mice (n=15 enrolled at 1m) will be also included as a further control to evaluate possible effects of TS in the development of AD-like phenotype. All mice will undergo LFP recordings and behavioral tests, then each group will be subdivided in subgroups to be processed for the different analyses on brain tissues.

Sub-task 2.1. To evaluate cognitive functions in 2×TS and 7×TS mice in order to monitor disease onset and progression.
Associative and spatial memory and motor function will be assessed 1 week after the 1st and the 2nd TS (in 2×TS mice) and the 1st, 2nd and 7th TS (in 7×TS and age-matched naïve mice).

Sub-task 2.2. Characterizing changes in brain connectivity in 2×TS and 7×TS mice and correlating them to changes in cognitive performances and to AD hallmarks.
Changes in brain connectivity will be assessed by performing LFP recordings in awake mice using epidurally implanted electrodes placed over the frontal and temporo-parietal cortices of both hemispheres. LFPs will be recorded in 30-min sessions once a week for 3 consecutive weeks starting from the week before the last TS. The global functional coupling of the LFP rhythms will be indexed by TotCoh (PMID:35291824). Brain complexity and functional coupling analyses (with less nodes) will be carried as in Task1. Correlation analyses will be performed to establish a relationship among EEG power, complexity and connectivity indices, AD-dependent changes in cognitive/motor performances (Task. 2.1), molecular indices (Task 2.3) and neuroinflammation (Task 2.4).

Sub-Task 2.3. To evaluate morphological alterations in brain structures.
Morphological analysis will be performed in pyramidal neurons of temporo-parietal and frontal cortices and hippocampus, by using Golgi-Cox staining and Sholl analysis to evaluate dendritic complexity and changes in spine density and morphology (n=5 mice/group) (PMID:34699347). We will also evaluate changes in the expression of extracellular matrix (ECM) and perineuronal net (PN) proteins, such as hyaluronic acid, lecticans, tenascins R (TnR) and C (TnC) and their degrading enzymes, such as matrix metalloproteinases (MMPs) and ADAMs in the selected brain areas by Western blotting (WB, n=8 mice/group) and immunohistochemistry (n=5 mice/group).

Sub-Task 2.4. Characterize the inflammatory response of AD-like mouse brain.
Immunohistochemical analyses in sections of frontal and temporo-parietal cortices and the hippocampus (n=5 mice, same used in subtask 2.3) will be performed in order to detect changes in microglia morphology by IBA-1 or TMEM119 labeling. Alterations in microglia morphology (i.e. amoeboid or hypertrophic shape) will be evaluated by using Sholl analysis. Expression of microglial markers will be also evaluated. In hippocampal and cortical lysates, we will perform WB (8 mice/group, same used for Task 2.3) and ELISA analyses (n=6 mice/group) to assess the level of pro-inflammatory mediators (e.g. interleukin -1 and tumor necrosis factor alpha) and anti-inflammatory cytokines, including transforming growth factor ß and interleukin-10 and nitric oxide. The expression of key genes mediating the inflammatory response will be evaluated by RT² Profiler PCR Array (n=3 mice/group). AD molecular hallmarks (i.e., Aß, pTau, S199, T205, S396) will be also assessed by WB, ELISA and immunofluorescence experiments (n=15 mice/group).

Experimental design aim 3

Sub-task 3.1. Membrane purification and Leukosome synthesis. Healthy C57BL/6 mice will be used to isolate circulating leukocytes from peripheral blood using the MACS automated cell separator. Macrophage polarization will be obtained in vitro through IL-4 treatment as previously described (PMID: 31290914). Commercial kits (Ab-conjugated magnetic beads by Calbiochem) will be used to extract membrane protein fractions from native and activated macrophages to generate native and activated Leukosomes. Purified membrane proteins will be mixed with commercial phospholipids (DPPC, DOPC, DSPC) and cholesterol (labeled with a near infrared fluorescent -NIR- dye for in vitro and in vivo imaging purposes), followed by formulation into Leukosomes through a proprietary microfluidic mixing procedure (Panel H) (PMID: 29512198). Control liposomes will be synthesized using similar procedures with the exception that no membrane proteins will be used. All nanoparticles will be characterized for their structural integrity and uniformity (transmission electron microscopy), chemical (composition and surface charge), and physical (size, stability) features (Panel I). The inter- and intra-cellular trafficking of Leukosomes will be evaluated through confocal microscopy on reconstructed endothelial monolayers and neuronal cells.

Sub-Task 3.2. Determine Leukosomes bio-distribution and brain targeting. To investigate the kinetics of brain targeting and accumulation, and the rates of clearance, animals will be imaged after the tail vein injection of native and activated Leukosomes two days after the last thermal reactivation (10 mice per group: 2×TS and 7×TS). For all time points, the animals will undergo whole body imaging using IVIS lumina optical and fluorescent imager at 0-2-8-24 and 48 hours post injection. The explanted brain, lung, liver, kidney, heart, and spleen of a subset of animals will be imaged ex vivo upon dissection along their major axes. To further quantify accumulation, one third of the organs and tissues will be homogenized in PBS for fluorescence analysis and another third will be snap-frozen in OCT and stored at -80°C for fluorescent immunostaining (Panel M and N).

Sub-task 3.3. Load Leukosomes with dexamethasone (DEX). Encapsulation methods previously described will be used to load DEX (PMID: 33233748). The in vitro release profile of the DEX loaded leukosomes will be investigated by transferring Leukosomes into dialysis membranes and sampling aliquots at 1, 2, 4, 8, 12, 24, and 48h (Panel J). The in vivo

pharmacokinetics and pharmacodynamics will be assessed by processing one third of the explanted organs and tissues (see Sub-Task 3.2), using HPLC as previously shown (PMID: 27213956).

Sub-task 3.4. Evaluate Leukosomes therapeutic effect according to the source of membrane proteins from freshly isolated leukocytes (Native Leukosomes) vs M2 polarized leukocytes (Activated Leukosomes) and to their therapeutic payload (empty or dexamethasone loaded). 7×TS mice will be divided into 4 treatment groups (15 mice each) subjected to: 1) Native Leukosomes + free DEX; 2) DEX loaded Native Leukosomes; 3) Activated Leukosome; 4) DEX loaded Activated Leukosome. Mice will be injected systemically 48 hours after each TS and, 1 week after injection, assessed for cognitive/motor functions as described in Sub-Task2.1 (at the 1st, 2nd and 7th TS). Connectivity indices, AD hallmarks and neuroinflammation will be assessed as in Sub-Tasks, 2.2-2.4. In particular, immunohistochemical analyses in sections of frontal and temporo-parietal cortices and the hippocampus will be performed in order to detect changes in microglia morphology (as described in subtask 2.4). A full spectrum of systemic pro- and anti-inflammatory cytokines (Panel K and L) will be assessed as previously described in our publications (PMID: 31290914) and in subtask 2.4.

Picture to support preliminary data

Figure 1 PNRR Salute LOW RES.pdf

Hypothesis and significance

Although Aß and tau protein accumulation is considered as a key event in the pathogenesis of AD, the precise pathophysiology of this disease remains unclear and no effective treatment to inhibit or even slow its progression has been discovered so far. A critical aspect is that the pathogenesis of AD begins years before the clinical diagnosis of dementia is established. Moving from the hypothesis that early diagnosis might increase the effectiveness of therapeutic interventions, our study will focus on validation of early biomarkers of AD (Aim1). MCI is characterized by measurable cognitive impairment, but not overt dementia. Interestingly, half of MCI patients progresses to AD, and these are the subjects that we expect to identify within our study. The analysis of their neurocognitive profile, and their brain connectivity indices, acquired through EEG, will be evaluated as candidate markers of AD. The rationale is based on the assumption that in a brain undergoing neurodegeneration, alteration in synaptic transmission/plasticity and functional brain connectivity can be measured very early. OU1 has extensively demonstrated that EEG analysis provides remarkable information on both parameters (Figure 1A-B), supporting its use to probe the synaptopathy characterizing early dementia (PMID:35388959). By performing parallel studies on a mouse model of AD-like phenotype (Aim2), we will corroborate the validity of connectivity indices establishing their correlation with functional and molecular hallmarks of AD that cannot be investigated in human tissues. Since neuroinflammation has been identified as crucial to the pathogenesis of AD, we hypothesize that the characterization of the role of neuroinflammation in AD onset and progression could help identify new cellular and molecular targets for the development of alternative/complementary treatments. To this end we selected a mouse model of sporadic AD that, following repeated cycles of HSV-1 reactivations, progressively develops the functional and molecular phenotypes associated to different stages of AD. Our published data (PMID:26487282; PMID:30870531) demonstrated that infected mice exposed to 2 cycles of HSV-1 reactivations showed signs of neuroinflammation and increased brain levels of interleukin 1ß (IL-1ß). Of note, 7 HSV-1 reactivations induced over time also an increment of Aß and pTau levels in some cerebral areas, along with increased proinflammatory cytokine levels, thus contributing to the development of the AD-like phenotype (Figure 1C-G).
In AD, the chronic activation of microglia and its M1 polarization, triggers the release of pro-inflammatory cytokines and chemokines which induce pathological changes leading to neurobehavioral complications. We hypothesize that the inhibition of microglia over-activation and microglia-mediated neuroinflammation could represent an alternative strategy for the treatment of AD. Unfortunately, targeting brain inflammation through a systemic pharmacological approach does not hold ground clinically, because of poor delivery across the blood brain barrier. Our published data extensively demonstrate that leukosomes induce a reduction of inflammation through the favorable modulation of both pro- and anti- inflammatory

genes, as well as the reduction of immune cells infiltration and activation (PMID: 31290914) (Figure 1H-N). As a consequence of leukosome treatment we also observed enhanced tissue repair and return to function (PMID: 28932838), suggesting that leukosomes could also ameliorate the functional activity of the brain.
This represents the rationale and working hypothesis of Aim3 in which we will develop next-generation targeted therapies based on biomimetic anti-inflammatory nanoparticles (PMID:34382379; PMID:27213956). We hypothesize that the selective downregulation of the pathogenic and pro-inflammatory signaling, will enhance tissue preservation, promote neurological recovery, and slow down the progression of AD.

5.5 Methodologies and statistical analyses

Methods of data collection

Aim1. Resting EEG will be performed at eyes closed and opened on subjects seated and relaxed in a sound-attenuated dimply lit room. EEG will be recorded using a DC amplifier (BrainAmp) connected to a 64-channel EEG cap arranged according to the 10-20 international EEG system. Recordings will be online referenced to linked mastoids and the ground electrode will be placed on FPz. For offline analysis, an average reference will be used. Sampling frequency will be at 1000Hz. EEG data will be reviewed and the artefactual activity will be removed manually and through Independent Component Analysis (ICA). TMS will be performed using a 90-mm figure-of-eight shaped coil. EMG activity will be recorded from the right FDI with surface electrodes using a muscle belly-tendon montage (PMID: 25797650). RMT will be defined as the lowest stimulus intensity able to elicit MEP at rest of an amplitude >50 microV in at least 5 of 10 trials (PMID: 25797650). SAI will be assessed using a single-pulse TMS of the M1 which will be preceded by electrical stimulation (ES) of the median nerve at the ipsilateral wrist to the recorded FDI at 25 ms interstimulus interval (ISI). ES (square-wave pulses of 0.2 ms duration) will be applied over the stimulated nerve through a pair of cup electrodes. PAS will be administered by pairing ES of the ipsilateral median nerve to the recorded FDI, with TMS of the contralateral M1 using an ES-TMS ISI of 25 ms. 200 pairs of stimuli will be given, and 15 MEPs will be recorded before and after 0, 10, 20 and 30 minutes from PAS delivery.

Aim2. HSV-1 (F strain) will be inoculated via lip scarification; 1 month later, mice will be subjected to TS (see Task2) raising their body temperature to 40-42°C for 15 min (PMID: 32719795). Behavioral tests will provide the following outcomes: Fear conditioning: freezing time; Radial-arm maze: time spent on locating the escape platform; Novel object Recognition: preference index (i.e., time spent exploring the novel object/time spent exploring both objects); Grid-walking test: number of foot faults/(number of foot faults+number of non-foot-fault steps)×100; Grip strength: forelimb force g/body weight; Locomotor activity: distance travelled in the arena. ANY-MazeTM will be used for scoring. LFP data will be acquired using the Cereplex Direct system. Fluorescent images will be collected with confocal laser scanning system (Ti-E, A1 MP, Nikon). The number of immunoreactive cells will be calculated using NIH ImageJ software. Neuronal and microglia morphology, number of spines/µm and the number of bifurcating nodes in dendritic processes will be analyzed with a Zeiss microscope equipped with Neurolucida. WB will be quantified by using UVItec Cambridge Alliance. Synaptic Plasticity RT2 Profiler PCR Array will be carried out by using ABI Prism 7500 PCR. ELISA analyses will be carried out using Perkin Elmer Victor X4 Plate Reader.

Aim 3. To achieve membrane protein incorporation in the Leukosome lipid bilayer, the aqueous buffer containing the isolated proteins and the ethanol solution containing the phospolipids will be mixed at different flow rates and flow ratios using the NanoAssemblr Ignite platform (Precision Nanosystems). Chemical and physical features of the nanoparticles will be acquired using commercial analytical softwares (Nanoparticle Tracking Analysis, Malvern, Zetasizer Nano, Matter Toledo). Plasma levels of pro- and anti-inflammatory cytokines will be quantified by Quantikine ELISA (R&D Systems Inc). Trafficking dynamics will be analyzed using fluorescent and confocal microscopy (Ti-E, Confocal Head A1 MP, Nikon) for the in vitro, and IVIS lumina (Perkin Elmer) for the in vivo. Accumulation of DEX in tissues will be measured using HPLC

(LC 300 UHPLC System, Perkin Elmer) on explanted and homogenized tissues and organs. Infiltration of immunoreactive cells neuronal and microglia morphology in response to Leukosome treatment will be evaluated as described in Aim2.

Statistic plan

Aim 1. Sample size calculation was based considering 10 categories of interest and effect size 0.25 with significance level 0.05 and statistical power 80%. The software R, version 4.2.0, and the WebPower package using multi-way ANOVA approach were used to determine the sample size of subjects with AD, MCI and HC to be enrolled. A total number of 300 subjects (100 for each group) was calculated to achieve the minimum statistical power (1-ß) of 0.80; alpha-error of 0.05, considering a drop-out equal to 10%.

Aim2. Sample size for Task 2 and Task3.4, indicated in the experimental design section, was calculated by using SYSTAT 10.2 according to results of prior pilot data sets or published studies, including ours, using similar methods or paradigms and taking into consideration the Three Rs guiding principles for ethical use of animals. The following formula has been applied n>2[(zalpha/2+zß)SD/D]2 (i.e., n = mice/group; zalpha/2 = 1.96 with alpha = 0,05 (5%); zß = 0,842 for power=80%; based on pilot or literature data, D=minimal important difference).
Given that more measures are obtained from the same experimental group, the number of samples is calculated based on the test or analysis that needs the greatest number of sample, that is connectivity analysis based on LFP recordings (n=15 mice; TotCoh index, mean±S.D of 0.45±0.1 and ¿ =0.11). Then, a total of 42 mice for the 4 main experimental groups has been estimated to have adequate sample size for i) molecular analyses to detect >20% changes in the expression of different markers by ELISA, immunofluorescence and WB experiments; ii) changes in spine density (n=5 mice; number spines/µm; mean±S.D of 1.07±0.24, ¿ =0.43).

Aim 3. To standardize Leukosome composition, their synthesis will be attained using frozen aliquots of membrane proteins purified from pooled circulating leukocytes (native or activated, see Sub-task 3.1). In order to minimize possible bias, all samples relative to Leukosome synthesis and testing will be randomized and blinded to the operator through the use of keyed codes. Nanoparticles biodistributions will be evaluated using n=10 mice per group (subtask3.2). Based on our extensive experience and preliminary and published data, this will provide adequate power to detect >15% change in nanoparticles accumulation from baseline compared to control through two-group pairwise comparison. Descriptive statistics, ANOVA, and longitudinal models will be employed for biodistribution. PK and therapeutic efficacy experiments with a sample size of n=15 (Subtask3.4), which will provide >90% power to detect >15% reduction in tissue inflammation from baseline based on a two-sided t-test with 5% alpha level and a nonparametric adjustment. This effect size is conservative and reasonable given the large difference in pro- and anti-inflammatory markers observed in our preliminary studies (Panel K and L) (PMID: 31290914). The use of an imaging software (Image J, Living Image® Version 4.5 Software by Perkin Elmer) for the automatic analysis of fluorescence intensity will guarantee uniform image corrections to baseline and the univocal application of quantification criteria to evaluate leukosome trafficking and biodistribution in vitro and in vivo.

Statistical analysis

Statistical analysis will include both uni- and multi-variate procedures, within a parametric approach (given the known nature of data). Data will be first tested for equal variance and normality (Shapiro-Wilk test) and non-parametric approach will be pursued if required. Continuous variables will be described with the following set of measures: number of subjects/samples, mean, standard deviation, range and median. Frequency counts and percentage of subjects within each category will be provided for categorical data. Analysis of variance (ANOVA) and planned post-hoc t-tests with Bonferroni correction for multiple comparisons will be used to evaluate differences among groups. A repeated-measures (RM) ANOVA will be performed to investigate significant factors within and between experimental groups. OU1 will be involved in supervising all statistical analyses using the following statistical softwares: SPSS, Prism, STATISTICA, SYSTAT,

SigmaPlot.

More specifically:

- Correlation analyses will be performed using the Pearson correlation coefficient to establish a relationship among EEG power, connectivity and complexity indices, AD-dependent changes in neuropsychological scales (Subtask 1.1), neuroplasticity and sensorimotor indices (Subtask 1.3) and metabolic biomarkers (Subtask 1.4). Confounding variables (age, sex, smoking, alcohol consumption, employment status, income, marital status, family status, place of residence, polypharmacy, comorbidities) will be considered throughout the analysis.

-Two-way RM ANOVA (group×TS) will be used for analyses of cognitive/motor performance indices at different TS (Sub-Task2.1 and Sub-Task3.4), three-way ANOVA (group×band×time) will be used to evaluate TotCoh. For all the other experimental data, differences among experimental groups will be assessed by one- or two-way ANOVA or Mann Whitney U-test for nonparametric analyses. For experiments with biological replicates, errors will be calculated between replicates. For in vivo experiments, animals will be considered biological replicates. Data from experiments using one biological replicate will be averaged across technical replicates, without calculation of significance.

-The statistical analysis will include ANOVA to perform specific pair-wise comparison among all experimental groups. In case significance is not reached, to perform group comparisons ANOVA will be replaced with non-parametric methods including Kruskal-Wallis or Wilcoxon rank sum tests. Correlation analyses will be performed using the Pearson correlation coefficient to establish a relationship among leukosomes treatment, neuroinflammatory markers, connectivity and complexity indices (Subtask 3.4).

Timing of analysis data

The whole study will last 24 months. Ethical approvals for human subjects and mouse models will be obtained before starting the project.

Aim1. The first month will be assigned for technical set-up. The enrollment of AD, MCI and matched HC is expected to be completed within the end of twenty-second month (months: 2-22). Immediately after the enrolment, each subject will be appropriately evaluated as described in Aim1, during three experimental sessions each lasting 1.5 hours (months: 2-23). To exclude heterogeneity in the enrollment and to evaluate group features of subjects a preliminary data and statistical group analysis will be performed every six months. The analysis of cortical connectivity networks will be conducted along the months 2-23. Correlation of indices obtained in human model will be performed from months 4 to 24.The last month will be assigned for final data statistics, correlation analysis and interpretation of the results (month: 24).

Aim2. Treatment of mice to be assigned to the different experimental groups will start at month 1 with a 1st cohort of about 50% of total mice; a second cohort will be recruited at month 4 to complete enrolment and obtain statistical power for all measures. Raw data of cognitive/motor tests (Task2.1) and LFP/EEG recordings (Task.2.2) as well as brain tissues for morphological/molecular analyses (Tasks 2.3, 2.4) will be obtained by month 4 in 2×TS mice and their respective controls, and by month 9 in 7×TS mice and their respective controls, given the time schedule of HSV-1 infection and reactivation by TS (1 cycle month) to obtain our experimental model of sporadic AD. Behavioral indices will be quantified: in 2×TS mice by month 6 (1st cohort) and analysis completed in all mice by month 10; in 7×TS mice by month 11 (1st cohort) and completed at month 15 (2nd cohort of mice); LFP raw data will be obtained at the same time points of behavioral data and will be subsequently processed and analyzed by OU1 for evaluation of connectivity indices (months 4-16) and correlation analyses with behavioral and molecular indices obtained in the same animals, will be completed by month 18. Morphological analyses and data on inflammatory responses in brain tissue will start at month 4 and will be completed for all mice by

month 16. By the end of the first year, 100% of samples from 2×TS mice and about 50% of samples from 7×TS mice will be analyzed, providing data supporting the experiments on the efficacy of Leukosome targeting and anti-inflammatory treatment (Aim3). By month 24 all data will be processed and correlation with behavioral and connectivity indices will be completed.

Aim3. The synthesis, characterization and testing of Leukosomes and control liposomes will be conducted in parallel with the studies described in Aim1 (patients) and Aim2 (mice). The initial work will consist in the standardization of the manufacturing conditions for all nanoparticles. All formulations will be initially evaluated for a set of chemical (composition, surface charge) and physical (size, stability) features (first 4 months of the study) and then for their pharmacological properties (loading and release kinetics of dexamethasone) from months 4 to 6. The different formulations will be also evaluated in vitro for their ability to traffic between cells (months 6-8) and to accumulate at the target site in vivo (months 9-17). Selected formulations will be studied in vivo (Subtask 3.4) to evaluate amelioration of AD-like phenotype upon administration (months 11-17), to characterize the cellular and molecular effects in the cerebral tissues as well as in distant healthy organs (months 18-24). Assessment of all molecular and connectivity indices following leukosome treatment will start at month 17. All data will be collected by the first half of the second year. Data analyses and correlation of indices will be completed by month 24.

5.6 Expected outcomes

The multidisciplinary approach proposed in this project will provide clinical, neuropsychological, neurophysiological, metabolic and functional outcomes with immediate translational value for the assessment of cognitive disorders. We anticipate that the expected results will offer a new way to characterize and discriminate the cases of MCI and AD. We also anticipate that the pre-clinical testing of the novel therapeutic approach based on biomimetic nanoformulations with inherent anti-inflammatory activity (Activated Leukosomes) will ameliorate AD-like phenotype and ground future translational studies on human subjects.

Specifically, we expect:
1) To characterize MCI and AD patients with respect to neuropsychological, neurophysiological, metabolic, functional and lifestyle domains in comparison with matched HC, in order to identify specific disease-associated phenotypes and to prospectively implement early interventions.
2) To establish the predictive power of EEG-based brain network analyses for MCI to AD conversion by demonstrating, in the human patient, the correlation among connectivity indices and clinical-functional features; and in the mouse model, the cellular and molecular correlates of changes in connectivity and the network alteration occurring at microcircuit level (synaptic contacts, synaptic environment, perineuronal net) at the different stages of disease progression.
3) To characterize phenotypes associated with different stages of AD by taking advantage of a mouse model that allows the assessment of AD molecular hallmarks and cognitive impairment in response to controlled cycles of virus replications within the brain.
4) To highlight in the mouse model of sporadic AD a causal role of neuroinflammation in triggering AD onset and progression and to identify major determinants in the inflammatory pathway, such as microglia activation and local and systemic cytokines.
5) To evaluate the targeting efficiency of Activated vs Native Leukosomes to the areas of brain inflammation and to assess their trafficking kinetics among the different subgroups of cells (endothelia, neurons, microglia, astrocytes) present in the affected brain tissue.
6) To assess the ability of Activated Leukosomes to induce macrophages and glial cells polarization towards anti-inflammatory phenotypes (M2-like). We expect that the interaction between the cell surface receptors of immune cells and the membrane proteins incorporated in the leukosome bilayer, will favor the creation of an anti-inflammatory

microenvironment, boost neural function, and counteract functional impairment.

5.7 Risk analysis, possible problems and solutions

Aim 1. The risk of a poor enrolment will be mitigated by the large outpatient flow of our clinical unit and by public campaigns advertising the new study. In addition, OU2 will also ask to local General Practitioners for referral of any defined or suspected AD and MCI patient in order to speed and improve the recruitment. An additional issue could be the patient drop out before the end of the study since a subject and/or a subject¿s representative has the right to decide withdrawal from the study at any time, for any reason, without prejudice to the subject¿s future medical care. This event was already taken in account over-sizing the number of enrolled patients.

Aim 2. Our published papers have already demonstrated the reproducibility of the mouse model of sporadic AD based on recurrent cycles of experimentally induced HSV-1 replication in the brain. Nevertheless, potential variability in the degree of infection and efficacy of reactivation as well as its impact on AD phenotype will be accurately monitored by post-mortem assessment of viral infection and of AD functional and molecular hallmarks. These data will further corroborate the validity of our mouse model and mitigate the risk of poor representation of human AD phenotypes.
Behavioral tests and LFP recordings might be affected by the novelty of the test apparatus, which can lead to variability of data, mask treatment effect, and consequently lead to misinterpretation. Novelty induced increases in variance will be circumvented by habituation session to the test apparatus. Additionally, sample size for the most variable data (i.e., behavioral and connectivity indices) greatly exceeds the calculated sample based on power analysis (42 vs 15 mice), as a result of the fact that these mice will be divided in subgroups to be used for different molecular analyses.

Aim 3. Our published studies have already identified the critical surface proteins necessary to target endothelial sites of inflammation. If an efficient targeting is not achieved, we will increase the relative abundance of selected targeting proteins by enriching their content through selective isolation of LFA1 and PSGL1. It has been reported that leukocytes have site densities of approximately 100-350 sites/¿m2 LFA1 and 50-200 sites/¿m2 PSGL1, which is enough to guarantee biological activity of these NPs. We will optimize formulation criteria to meet these physiological levels of receptors¿ density of the Leukosomes¿ surface. Although fluorescent techniques to evaluate the biodistribution of Leukosomes have been previously proven and extensively validated, there might be limitations in: 1- precisely determining the comprehensive distribution of injected NPs within the different organs, 2- quantifying the percentage of injected dose accumulated at the site; and 3- discriminating Leukosome signal from the inherent auto-fluorescence of certain tissues. If one of these issues become substantial, Leukosomes biodistribution will be evaluated using HPLC to detect the therapeutic payload or labeling NIR dye.

Our approach is ambitious at both technical and scientific levels, and involves a number of situations where the outcome of a preceding experiment may modify the execution of the subsequent experiment. Orchestration of such an interrelated group of experiments will represent a challenge, since glitches in communication might easily delay tasks or subtasks within the project (or render their design suboptimal). However, we believe we will maintain this risk at a very low level because the OUs have already worked together, have demonstrated the ability to maintain a tight coordination and an open communication during previous studies, have consolidated their expertise in all the techniques described, and are well prepared to deliver the incremental technical improvements over the two-year period.

5.8 Significance and Innovation

From a diagnostic standpoint, brain connectivity and cortical plasticity can be measured early during neurodegeneration.

These parameters along with neuropsychological, metabolic, functional outcomes provide information which can be used to identify and stratify AD progression earlier and more precisely.
From a mechanistic standpoint, neuroinflammation is crucial to the pathogenesis of AD and this study will deepen our understanding of its role in the onset and exacerbation of the disease and will help identify new molecular and cellular pathways to halt or revert disease progression.
From a therapeutic standpoint, targeting brain inflammation pharmacologically does not hold ground clinically because of poor delivery across the blood brain barrier. The development of nanoparticles able to reduce inflammation through the favorable modulation of both pro- and anti- inflammatory signaling, and reduction of immune cells activation could represent a paradigm shift in the treatment of AD.

5.9 Bibliography

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5.10 Timeline / Deliverables / Payable Milestones

Approval by Ethical Committees will be granted before the project starts
Mo 1 Technical set-up (equipment and personnel acquisition)
Mo 2-22: recruitment of MCI, AD and HC subjects and assessment of neuropsychological, neurophysiological, metabolic, functional and lifestyle parameters
Mo 1-4: Functional evaluation and LFP recordings in 50% of 2×TS mice and controls. Leukosome preparation will be set-up.
Mo 1-9: Functional evaluation and LFP recordings in 50% of 7×TS mice and controls. Antiinflammatory properties will be tested.
Mo 6-16: Behavioral indices will be quantified in all mice. LFP data will be processed and analyzed. Morphological analyses and data on inflammatory responses in brain tissue will be completed. Preliminary data on biodistribution and efficacy of leukosome treatment will be obtained
Mo 4-24: Correlation analyses of all indices obtained in human subjects and mice will be performed and completed
Mo 12: Drafting of intermediate report
Mo 24: Drafting of final report

Milestones 12 month

At Month 12, 50% of EEG/LFP data will be collected, analyzed and correlated to the degree of functional impairment in human subjects and mice. Correlation with metabolic, functional and lifestyle domains in humans and molecular/morphological alteration in brain in mice will be also obtained. Optimal formulation of Native and Activated Leukosomes (empty and loaded with DEX) will be completed. An intermediate report will be submitted.

Milestones 24 month

MCI, AD and HC subjects will be characterized as for neuropsychological, neurophysiological, metabolic domains and specific disease-associated phenotypes will be identified. The predictive power for MCI to AD conversion of brain network analyses will be established based on EEG in both human and mice. The role of neuroinflammation and efficacy of Leukosome treatment in AD mice will be demonstrated and correlation with connectivity indices will be established. Final report will be prepared.

Gantt chart

GANTT.tif

5.11 Equipment and resources available

Facilities Available

OU1. IRCCS San Raffaele Pisana has the capability to perform the proposed analyses. The Brain Connectivity Laboratory of IRCCS San Raffaele Pisana is equipped to perform the requested analyses on the EEG data (software and expertise for both human and animal). Furthermore, the following resources are provided:
Laboratory space: 1 room of 30 m2
Office space: 1 room 30 m2
Major equipment for EEG: 5 PC, 4 laptop, 1 digital EEG system
Access to the necessary equipment for nanoparticle formulation and characterization: FACS, Ultracentrifuge, Rotary Evaporator, Nanoassembler, Zeta Sizer, Spectrophotometers, HPLC, transmission electron microscopy.

OU2 has the capability to perform the enrollment and selection of patients and to perform neuropsychological, neurophysiological, and metabolic biomarkers investigation. The following resources are provided: ActiCHamp Plus (BrainAmp Products) 64 channels system and Brain Vision Analyzer software, for EEG recordings; high-power Magstim

200 mono-pulse magnetic stimulators (The Magstim Company, Whitland, Dyfed, UK), connected to figure-of-eight coil for TMS protocols; Digitimer Stimulator, model DS7A (Digitimer Ltd, Welwyn Garden City, UK) for the electrical stimulation, an analog/digital converter (CED 1401 power) interface, managed by Signal 5.0 for EMG recordings; an impedance analyzer (BIA 101 BIVA, AKERN) for bioelectrical impedance vector analysis; portable ergospirometric metabolic system (MetaMax 3b, Cortex Medical, Leipzig, Germany) for the metabolic measurements

Major Equipment at OU3 includes: integrated platform for in vivo multichannel EEG recordings on mice (CerePlex, Blackrock Microsystem); Animal House with laboratories for behavioral tests (Fear Conditioning, Radial-arm maze, NOR, Grid-walking, Grip-strenght, open field), Video Tracking System and Any-Maze for automated data analysis; an IVIS lumina fluorescent imager (Perkin Elmer); Laboratory for molecular biology with ABI Prism 7500 PCR instruments (Applied Biosystems), BioPhotometer gel electrophoresis and blotting devices, Uvitec Gel Documentation systems; Perkin Elmer Victor X4 Plate Reader; Termoblock; Thermal Cycler (Bio-Rad); NanoDrop; Bioruptor sonicator, centrifuges. VT1200S vibratome. Central Lab of microscopy, with two Confocal Laser Scanning System (Ti-E, Confocal Head A1 MP, Nikon, Japan); a fluorescence and optical microscope with Neurolucida and Stereoinvestigator Softwares (MBF Bioscience)

Subcontract

For this study no subcontracts are forecasted

5.12 Desc. of the complementarity and sinergy of secondary collab. researchers

Three secondary collaborators will be enrolled based on their experience and on the know-how needed for the achievement of the project Aim1. The recruitment of these collaborators is driven by the will to integrate new complementary skills in the areas of neurophysiology, functional and metabolic processes. Every secondary collaborator will contribute to the analysis of the data, the statistical analysis and the critical interpretation of the results.
Dr Nicola Loi has wide neurophysiology background and excellent skills in the use of non-invasive neurophysiological techniques. He will perform EEG and TMS recordings, to identify neurophysiological markers of neurodegeneration in MCI compared to AD and HC.
Dr Antonella Cano has a background on innate immunity with specific competence in cell culture and molecular biology techniques. Her research has recently focused on the assessment of the nutritional status through the evaluation of body composition, metabolic rates and dietary intake. She will perform anthropometric measures and body composition analysis. She will also evaluate the nutritional status and the adherence to the Mediterranean Diet of patients.
Dr Lucia Ventura is a clinical research physiotherapist and exercise physiologist. The current goal of her research is the translation of dynamometric and neurophysiological evaluations into the assessment and management of neurological disorders. She will assess the physical capabilities of patients measuring handgrip strength, walking speed, endurance and fatigability, energy cost of walking, fall risk and walking ability, functional lower extremity strength and transitional movements, dynamic stability and balance.

Furthermore, the following figures will be hired:
2 (full time) biomedical engineers for EEG data analyses;
1 (part time) biostatistician for statistical data analysis;
1 (part time) molecular biologist to characterize in vitro functions;
1 (part time) Organic chemist to synthesize nanoparticles;
2 (full time) neurobiologists for experiments on animal models. One will be involved in LFP recordings and behavioral assessments and data analyses and the other will be involved in molecular and morphological analyses on brain tissues.

Along with the single collaborator¿s expertise, the strength of this project relies on the punctual collaboration between OU. The collaborators during regular meetings will share their data and results and help identify potential pitfalls of upcoming experiments. The cooperation among investigators with different expertise will allow the achievement of the project¿s goals.

Exchange traineeship of young researchers from different units and collaborators will also promote a fruitful work experience. Project progress will be assessed monthly within each research group by means of scheduled lab meetings and telematic meetings, coordinated by the project PI. A townhall meeting of all units will be scheduled on a tri-monthly basis, to share achievements and difficulties, to discuss results and manage potential risks or problems.

5.13 Translational relevance and impact for the national health system (SSN)

What is already know about this topic?

The negative results of the clinical trials on AD-modifying therapies, along with laboratory data on animal models, made it clear that available treatments have limited effects. In AD, this is particularly true once neurodegeneration is too advanced, suggesting that to achieve disease modification the treatment should begin earlier. Similarly to cardiac or neoplastic diseases, AD could be optimally treated, but just in its preclinical stage. MCI is generally recognized as a pre-dementia phase, but at an individual level it may or not underlie an AD pathophysiology. It is crucial to develop a test to discriminate with accuracy the MCI due to underlying AD. The EEG could be this first-line tool for the early identification of these subjects, to monitor the progression of their disease, and to evaluate the response to innovative therapies.

Details on what is already know about this topic

Several biomarkers have been proposed for the detection of dementia in order to address the unmet need of early diagnosis. The innovative analysis of brain connectivity from EEG data is a promising tool with widespread availability in the clinical centers, and relatively low cost of execution. It is known that the accuracy of the diagnosis could be increased if EEG biomarkers are associated to neurophysiological, neuropsychological and metabolic biomarkers.
A growing body of evidence suggests neuroinflammation¿s prominent role in AD pathogenesis. In particular, in light of its implications in CNS pathologies, microglia has become an attractive therapeutic target because of its central role in response to adverse tissue events. The significant challenge of accessing CNS tissue to tune microglial activation and pro-inflammatory response urges the development of new therapeutic approaches capable of overcoming the limitations in transport across the blood brain barrier.

What this reasearch adds?

Since AD is a multifactorial disease characterized by the interplay of genetic susceptibility, lifestyle and psychosocial factors, and functional cortical disconnection is already present in early AD, Aim1 will provide a comprehensive set of biomarkers which may support the predictive value of connectivity indices in the conversion process to dementia.
By performing parallel studies on mouse model of AD-like phenotype we aim to characterize the role of neuroinflammation in AD onset and progression, and to identify new targetable molecular pathways or cellular targets. The neurophysiological and functional characterization of early AD stages and the study of neuroinflammatory pattern in animal models could help to advance knowledge of the etiopathology and pathophysiology of AD and to develop new therapeutic approaches. Downregulation of pro-inflammatory signaling by nanotherapeutics targeting activated microglia is expected to counteract neurodegeneration and slow down the progression.

Details on what this reasearch adds

The study will result in the: 1- identification of a set of accessible and affordable neurophysiological, neuropsychological and metabolic biomarkers to characterize more accurately dementia patients and predict the conversion to dementia in the prodromic stage of mild cognitive impairment; 2- characterization of the hallmarks of AD onset and progression in the proposed mouse model to establish the causal effect of inflammation on disease progression and identify new molecular and cellular pathways to be targeted therapeutically; 3- development of a new class of inherently anti-inflammatory biomimetic nanoparticles (Leukosomes) able to modulate immune response systemically and mitigate local microglia activation in early and late AD. The overall value of this research is in the multimodal approach, which will provide insight

into disease-related phenotype with an immediate translational value in terms of implementation of new diagnostic biomarkers and therapeutic approaches.

What are the implications for public health, clinical practice, patient care?

The expected results of this project will potentially offer a new test to discriminate with accuracy the cases of MCI due to AD. These patients would be the ideal candidate to undergo early interventions. This project has the potential to significantly address the management of AD whose costs for medical and social care exceed 40 billions of euros annually. Earlier diagnosis and new treatments are necessary to ensure the sustainability of the Italian National Health System.

Details on what are the implications for public health, clinical practice, patient care

The significant epidemiological impact of AD (8.1/100 of prevalence in Italy, with an incidence of newly diagnosed patients of 109/1000/year) and the constant increase of such rate due to the population ageing in the next 20 years will lead to a progressive increase in healthcare and related social costs. Considering the importance of early diagnosis, prevention and the role of protective lifestyle factors involved in AD, this project is warranted to adequately face this sanitary and socio-economical challenge. The identification of a panel of markers of MCI and early AD will open exciting windows in the early diagnosis and novel treatment to be transferred into daily clinical practice. The multidisciplinary design proposed in this project will provide clinical, neuropsychological, neurophysiological, metabolic and functional outcomes with an immediate translational value in terms of implementation of pharmacological and non-pharmacological approaches to these cognitive disorders.

6 - Budget

Total proposed budget (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1 Staff Salary	122.000,00	122.000,00	not permitted	0,00
2 Researchers' Contracts	591.000,00	0,00	591.000,00	59,10
3a.1 Equipment (Leasing -	22.500,00	7.500,00	15.000,00	1,50
3a.2 Equipment (buying)	25.000,00	15.000,00	10.000,00	1,00
3b Supplies	129.000,00	0,00	129.000,00	12,90
3c Model Costs	30.000,00	0,00	30.000,00	3,00
4 Subcontracts *	0,00	0,00	0,00	0,00
5 Patient Costs	16.000,00	0,00	16.000,00	1,60
6 IT Services and Data Bases	45.000,00	0,00	45.000,00	4,50
7 Travels	28.000,00	0,00	28.000,00	2,80
8 Publication Costs	42.000,00	0,00	42.000,00	4,20
9 Dissemination	28.000,00	0,00	28.000,00	2,80
10 Overheads *	65.380,00	0,00	65.380,00	6,54
11 Coordination Costs	620,00	0,00	620,00	0,06
Total	1.144.500,00	144.500,00	1.000.000,00	100,00

* percentage calculated as average value between all the Operating Units.

Report the Co-Funding Contributor:

Partial salary of the OU Coordinators and principal research collaborators and partial cost of equipment

Budget Justification	
1 Staff Salary	Partial salary of the Coordinators and pricipal collaborators
2 Researchers' Contracts	Contracts for research personnel dedicated to the project
3a.1 Equipment (Leasing - Rent)	Upgrading of actual equipment
3a.2 Equipment (buying)	Upgrading of actual equipment
3b Supplies	Funding for routine laboratory supplies, EEG analyses and commercial kits
3c Model Costs	mouse models, housing and treatment costs

4 Subcontracts	none
5 Patient Costs	Patient costs for clinical analyses
6 IT Services and Data Bases	Data management. Creation of specialized cloud for the data sharing. External Hard disks for local data storing
7 Travels	travel costs for meeting related to the project
8 Publication Costs	Scientific publications in peer-review open access journals
9 Dissemination	Result dissemination and Cost to attend national/international scientific meetings/congresses
10 Overheads	Overhead required by hosting institution
11 Coordination Costs	Cost for DI coordination

Proposed total budget UO1 Institution: Casa di cura San Raffaele Pisana (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1 Staff Salary	45.000,00	45.000,00	not permitted	0,00
2 Researchers' Contracts	215.000,00	0,00	215.000,00	59,88
3a.1 Equipment (Leasing - Rent)	22.500,00	7.500,00	15.000,00	4,18
3a.2 Equipment (buying)	0,00	0,00	0,00	0,00
3b Supplies	48.000,00	0,00	48.000,00	13,37
3c Model Costs	0,00	0,00	0,00	0,00
4 Subcontracts	0,00	0,00	0,00	0,00
5 Patient Costs	0,00	0,00	0,00	0,00
6 IT Services and Data Bases	25.000,00	0,00	25.000,00	6,96
7 Travels	10.000,00	0,00	10.000,00	2,78
8 Publication Costs	12.000,00	0,00	12.000,00	3,34
9 Dissemination	10.000,00	0,00	10.000,00	2,78
10 Overheads	23.450,00	0,00	23.450,00	6,53
11 Coordination Costs	620,00	0,00	620,00	0,17
Total	411.570,00	52.500,00	359.070,00	100,00

Budget Justification	
1 Staff Salary	Partial salary of the OU1 Coordinator
2 Researchers' Contracts	Research contracts for two years
3a.1 Equipment (Leasing - Rent)	Upgrading of already available setup, pc and laptop for data analysis
3a.2 Equipment (buying)	none
3b Supplies	Funding for routine laboratory supplies, EEG analyses and commercial kits, and to perform cellular and molecular biology studies is requested to perform all the proposed research activities
3c Model Costs	none
4 Subcontracts	none
5 Patient Costs	none
6 IT Services and Data Bases	Data management. Creation of specialized cloud for the data sharing. External Hard disks for local data storing
7 Travels	travel costs for meeting related to the project
8 Publication Costs	Scientific publications in peer-review journals
9 Dissemination	Result dissemination and Cost to attend national/international scientific meetings/congresses
10 Overheads	Overhead required by hosting institution
11 Coordination Costs	Cost for DI coordination

Proposed total budget UO2 Institution: Azienda Ospedaliero-Universitaria di Sassari (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1 Staff Salary	42.000,00	42.000,00	not permitted	0,00
2 Researchers' Contracts	240.000,00	0,00	240.000,00	59,97
3a.1 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a.2 Equipment (buying)	25.000,00	15.000,00	10.000,00	2,50
3b Supplies	46.000,00	0,00	46.000,00	11,49
3c Model Costs	0,00	0,00	0,00	0,00
4 Subcontracts	0,00	0,00	0,00	0,00
5 Patient Costs	16.000,00	0,00	16.000,00	4,00
6 IT Services and Data Bases	20.000,00	0,00	20.000,00	5,00
7 Travels	11.000,00	0,00	11.000,00	2,75
8 Publication Costs	20.000,00	0,00	20.000,00	5,00
9 Dissemination	11.000,00	0,00	11.000,00	2,75
10 Overheads	26.180,00	0,00	26.180,00	6,54
11 Coordination Costs	not permitted	not permitted	not permitted	0,00
Total	457.180,00	57.000,00	400.180,00	100,00

Budget Justification	
1 Staff Salary	Partial salary of the OU2 Coordinator
2 Researchers' Contracts	Full-time research contracts for two years
3a.1 Equipment (Leasing - Rent)	none
3a.2 Equipment (buying)	TMS (single module)
3b Supplies	Materials for: EEG recordings and TMS (EEG caps, electrodes, connectors, TMS coils, earphones), Body composition scanning (electrodes, bedsheets), metabolic assessment (oxygen cells, calibration gas bottles, sealed oxygen masks).
3c Model Costs	none
4 Subcontracts	none
5 Patient Costs	Patient costs
6 IT Services and Data Bases	Data management. External Hard disks for local data storing
7 Travels	travel costs for meeting related to the project
8 Publication Costs	Scientific publications in peer-review journals
9 Dissemination	Result dissemination and Cost to attend national/international scientific meetings/congresses
10 Overheads	Overhead required by hosting institution
11 Coordination Costs	none

Proposed total budget UO3 Institution: IRCCS Fondazione Policlinico Universitario A. Gemelli (Euro)

Costs	TOTAL BUDGET	Co-Funding	List of costs proposed for funding to the MOH	Percentage of total proposed to the MOH
1 Staff Salary	35.000,00	35.000,00	not permitted	0,00
2 Researchers' Contracts	136.000,00	0,00	136.000,00	56,49
3a.1 Equipment (Leasing - Rent)	0,00	0,00	0,00	0,00
3a.2 Equipment (buying)	0,00	0,00	0,00	0,00
3b Supplies	35.000,00	0,00	35.000,00	14,54
3c Model Costs	30.000,00	0,00	30.000,00	12,46
4 Subcontracts	0,00	0,00	0,00	0,00
5 Patient Costs	0,00	0,00	0,00	0,00
6 IT Services and Data Bases	0,00	0,00	0,00	0,00
7 Travels	7.000,00	0,00	7.000,00	2,91
8 Publication Costs	10.000,00	0,00	10.000,00	4,15
9 Dissemination	7.000,00	0,00	7.000,00	2,91
10 Overheads	15.750,00	0,00	15.750,00	6,54
11 Coordination Costs	not permitted	not permitted	not permitted	0,00
Total	275.750,00	35.000,00	240.750,00	100,00

Project Code: PNRR-MAD-2022-12376667	**Call section:** Malattie Croniche non Trasmissibili (MCnT) ad alto impatto sui sistemi sanitari e
Applicant Institution: Casa di cura San Raffaele Pisana	**Applicant/PI Coordinator:** Vecchio Fabrizio

Budget Justification

1 Staff Salary	Partial salary of the OU3 Coordinator
2 Researchers' Contracts	2 Full-time research contracts for two years
3a.1 Equipment (Leasing - Rent)	none
3a.2 Equipment (buying)	none
3b Supplies	Materials for: EEG/LFP recordings (electrodes, connectors, surgical tools, resins), immunohistochemical and molecular analyses (antibodies; ELISA Kit; RT2 Profiler¿ PCR Arrays), Golgi staining kit
3c Model Costs	mouse models, housing and treatment costs
4 Subcontracts	none
5 Patient Costs	none
6 IT Services and Data Bases	none
7 Travels	travel costs for meeting related to the project
8 Publication Costs	Scientific publications in peer-review journals
9 Dissemination	Result dissemination and Cost to attend national/international scientific meetings/congresses
10 Overheads	Overhead required by hosting institution
11 Coordination Costs	none

Principal Investigator Data

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Struttura: Laboratorio di Brain Connectivity
Istituzione: IRCCS San Raffaele Roma
Datore/ente di lavoro? Yes
Datore/ente di lavoro SSN? Yes
Nome datore/ente di lavoro non SSN:
Nome istituzione SSN: IRCCS San Raffaele Roma
Tipo contratto: Professore Associato distaccato presso IRCCS/IZS/ISS/Ente SSN (convenzione di clinicizzazione e/o ricerca)

Con l'invio della presente proposta si dichiara che la stessa o parti significative di essa non sono oggetto di altri finanziamenti pubblici o privati e che di conseguenza vi è assenza del c.d. doppio finanziamento ai sensi dell'art. 9 del Regolamento (UE) 2021/241, ossia che non ci sia una duplicazione del finanziamento degli stessi costi da parte di altri programmi dell'Unione, nonché con risorse ordinarie da Bilancio statale.

By submitting this proposal, I declare that no significant part or parts of it are recipient of any other public or private funding and that consequently there isn't any so-called double financing pursuant to art. 9 of Regulation (EU) 2021/241, i.e. that there is no duplication in the financing of the same costs by other Euopean Union programs or any other ordinary resources from the State budget.

Project validation result