Finanziato dall'Unione europea NextGenerationEU | Ministero dell'Università e della Ricerca | Italiadomani | Scuola Normale Superiore

Spoke 8

Biotechnologies and imaging in neuroscience

Mid Term Meeting

15th May 2024

Scuola Normale Superiore

Program

Time	Activity	Institution	Speaker
8.45	Salutation	SNS	Antonino Cattaneo Tommaso Pizzorusso
9.00	M3: Integrated Approach to Nanobody Development: From Biochemical Characterization to In Vivo Validation in Transgenic Disease Models	SNS	Alessandro Cellerino
9.25	M1: Innovative drug design strategies for neurosciences: metalloenzymes, adenosine receptors and intrinsically disordered proteins as targets	UNIFI	Claudiu Supuran
9.55	M2: Integrative Approaches in Rational Theranostic Probe Design and Drug Repurposing: Predictive Modeling and Cellular Validation of Disease Signature Inversion	SNS	Francesco Raimondi
10.15	Coffee break		
10.30	M7: Novel mechanistic approaches for the study of retinal degenerations and Alzheimerâ€™s disease	CNR	Cristina Di Primio
10.55	M5: Morphological rescue in ASD models, Patient-derived iPS brain / SC organoids show successfully axonal regeneration and target validation. Models of diabetic retinopathy	UNIPI	Marco Scarselli
11.15	M6: Preclinical Validation of Biomarkers and Therapeutic Approaches for Creatine Transporter Deficiency and Angelman Syndrome: From Gene Therapy to Novel Administration Methods	SNS /CNR	Laura Baroncelli
11.45	Spoke1 spoke8 collaborative studies on ADVANCED RADIOTHERAPIES IN ONCOLOGY	CNR	Mario Costa
12.10	Spoke 5 activities' presentation	TLS	Roberta Mezzena
12.30	Poster session	from12.30 to 15.00	
13.00	Lunch		
14.00	Administrative session		
15.00	M4: Disease-modyfing therapies for experimental models of human illnesses	SNS	Emanuela Colla
15.25	M8: Patient-derived stem cells and “brain-in-a-dish” cultures: a cellular platform for target validation and drug screening	SNS /CNR/CNR	Gabriele Lagani
15.55	M9: Successful monitoring of neuroplasticity in patients and successful change of neuroplasticity in animal model with optogenetic or metabolic stimulation.	UNIPI	Paola Binda
16.15	Coffee break		
16.30	M10: Definition and validation of novel measures for the non-invasive assessment of sympathetic nerve activity during sleep	IMT	Monica Betta
16.55	M11: Brain-to-body interface and aDBS biomarkers evaluation	SSSA	Silvestro Micera
17.20	Concluding remarks	SNS	Tommaso Pizzorusso

Abstract Book

Poster Presenter: Andrea, Ammara

Institution: UNIFI

Milestone: M1 UNIFI

Poster Title: Development of multi-target directed ligands for the treatment of alzheimer's disease and memory disorders.

Authors: Andrea Ammara, Alessandro Bonardi, Niccolò Paoletti, Simone Giovannuzzi, Alessio Nocentini, Claudiu T. Supuran, Paola Gratteri.

Abstract: Alzheimer's disease (AD), a leading cause of dementia, presents as a chronic, multifaceted neurodegenerative condition that remains largely overlooked and insufficiently treated. While a few medications are available, none of these provides a cure, offering only palliative relief. Given AD's complex nature, we've embraced the multi-target directed ligands (MTDLs) approach to develop innovative hybrids capable of modulating two pivotal protein classes: soluble Epoxide Hydrolases (sEHs, EC 3.3.2.10) and Carbonic Anhydrases (CAs, EC 4.2.1.1). Soluble epoxide hydrolases encompass a group of versatile enzymes that bind to specific epoxy fatty acids (EpFAs), converting them into corresponding diols. EpFAs exhibit intriguing anti-inflammatory and neuroprotective properties, though their effects are swiftly halted by sEH activity. Thus, inhibiting this enzyme holds potential for reinforcing neuroprotection and mitigating amyloid-induced oxidative stress. Conversely, CAs represent a diverse superfamily of metalloenzymes encoded by eight unrelated gene families. Their primary role involves catalyzing the reversible conversion of CO_2 into HCO_3^- and H^+, a vital process in numerous biological functions in both prokaryotes and eukaryotes. Recent research highlights CA modulators as an emerging field, with various studies reporting significant improvements in synaptic activity, spatial learning, and memory in experimental animals through CA activation. We herein propose novel ureido derivatives merging a sEH inhibitory moiety with established CA activator scaffolds. These hybrids were meticulously designed through in silico studies, and their efficacy against sEH and brain CAs was evaluated using fluorescence-based and Stopped-Flow techniques, respectively. The results reveal promising multi-targeting profiles, holding promise for the development of novel agents for AD treatment.

Reference email: andrea.ammara@unifi.it

Poster Presenter: Cecilia, Steinwurzel

Institution: UNIPI

Milestone: M9: UNIPI

Poster Title:

Authors: C. Steinwurzel, G. Pennella, G. Sandini, M.C. Morrone, P. Binda

Abstract: Introduction (800 characters)

Ocular dominance is a basic visual property that shows both developmental plasticity and short-term plasticity in adult humans, where 2h of monocular contrast-deprivation leads to a counterintuitive shift of ocular dominance in favor of the deprived eye. Here we asked whether the same plasticity is elicited by merely changing the utility of monocular signals for visuo-motor control, without changing contrast.

Methods (800 characters)

Participants wore a VR set (head-fixed) with monocular screens connected with two cameras placed above each eye; these monitored the front space and sent their input to the corresponding eye. After a familiarization phase, the input to the dominant eye was delayed by 333 ms, making it useless for visuomotor coordination.

Participants underwent several experimental conditions; in the 'active' condition, they performed a complex visuo-motor task (building blocks). Then, they underwent two different 'passive' conditions where they 1) observed the experiment engaging in the same visuo-motor-task 2) viewed an in-person recording of their own previous active condition.

Results (800 characters)

We found that, after 60 minutes of task performance, ocular dominance (quantified by binocular rivalry dynamics) was systematically shifted in favor of the delayed eye: a similar effect as that produced by monocular contrast-deprivation. The shift was only observed when participants actively engaged in the visuomotor task, not when they passively watched the experimenter performing the same task nor when watching the in-person recording of themselves performing the task.

Discussion (800 characters)

Based on these findings, we suggest that active vision is foundational to weighting sensory information, even at the level of simple visual processes as those setting ocular dominance.

Reference email: cecilia.steinwurzel@med.unipi.it

Poster Presenter: Claudiu, Supuran

Institution: UNIFI

Milestone: M1 UNIFI

Poster Title: Updates

Authors: CT Supuran, P. Gratteri, A. Bonardi, A. Nocentini, A. Ammara, R. Pierattelli, V. Colotta, G. Provensi

Abstract: Not yet available

Reference email: claudiu.supuran@unifi.it

Poster Presenter: Daniela, De Luca

Institution: SSSA

Milestone: M11: SSSA

Poster Title:

Authors: D. De Luca, S. Moccia, L. Lupori, R. Mazziotti, T. Pizzorusso, and S. Micera

Abstract: Optic nerve stimulation holds great potential for visual prostheses, but its effectiveness

depends on the stimulation protocol. This can be optimized to drive the cortical activation towards to a pattern similar to that evoked in response to visual stimuli. It is necessary to characterize the cortical response to define a target cortical activation.

We here propose a convolutional neural network (CNN) to do it exploiting widefield calcium brain images, which allow large-scale visualization of cortical activity with high signal-to-noise ratio.

Reference email: daniela.deluca@santannapisa.it

Poster Presenter: Elena, Montagni

Institution: CNR

Milestone: M7: CNR

Poster Title:

Authors: Elena Montagni, Manuel Ambrosone, Alessandra Martello, Francesco Saverio Pavone, Anna Letizia Allegra Mascaro

Abstract: Atypical sensory processing is a proposed etiological factor underlying the development of behavioral deficits in autism spectrum disorder (ASD). SHANK3 is a postsynaptic scaffolding protein of excitatory synapses, whose deletion or mutation is well-known to cause a rare genetic disorder named Phelan-McDermid syndrome (PMS). Similarly to PMS patients, Shank3B mutant mice display aberrant whisker-independent texture discrimination and reactivity to tactile stimuli. Increasing evidence supports brain network dysfunction as the neurobiological basis for ASDs. Since traveling waves were identified as central features of brain organization, here we developed an all-optical method combining optogenetics and calcium imaging for modulating sensory processing and visualizing the sensory-evoked responses in a mouse model of autism. To this aim, we performed wide-field calcium imaging of the entire dorsal cortex in mice expressing GCaMP7f in excitatory neurons. Mutant mice for the Shank3 gene (Shank3b+/-) were compared to non-mutant littermates (Shank3b+/+). Air puff stimulation of the whiskers was used to evaluate sensory-evoked cortical activity in awake subjects. Light-mediated activation of the inhibitory opsin GtACR2 was employed for modulating the sensory response. Preliminary results show that a single application of optogenetic inhibition was able to modify the spatiotemporal features of the sensory-evoked cortical response.

Reference email: montagni@lens.unifi.it

Poster Presenter: Elena, Chiavacci

Institution: SNS

Milestone: M3: SNS. Integrated Approach to Nanobody Development: From Biochemical Characterization to In Vivo Validation in Transgenic Disease Models

Poster Title: Generation and analysis of new fish transgenic models to test innovative therapies for brain aging

Authors: Chiavacci Elena, Bagnoli Sara, Cellerino Alessandro

Abstract: The turquoise killifish (Nothobranchius furzeri) is the shortest-lived vertebrate animal model that recapitulates all the major features of mammalian brain aging. We firstly generated the BDNF and NGF Knock-out models via CRISPR/Cas9 Knock-out, among the mutations we found in the F0 founders we selected the out-of-frame BDNF delta-26 deletion and NGF +1 insertion. We are now raising the first incrossed F3 clutches of these two Knock-out lines and we will then proceed to characterize them both at molecular and behavioral level. Furthermore, we will then generate a transgenic line expressing a double copy of BNDF, as a proof of principle we already generated a BDNF double copy construct to be injected in zebrafish. Since N. furzeri shows signs of spontaneous age-dependent neurodegeneration reminiscent of Parkinson's disease (PD), we decided to create two transgenic models of the protein alpha-synuclein with the aim of inducing a more aggressive PD-like phenotype. We currently designed two constructs for two different approaches. The first transgene will carry a single point mutation in the endogenous alpha-Synuclein gene recreating the A18T human mutation. The second transgene will consist in the addition of a copy of codon optimized human alpha-Synuclein carrying the A18T mutation, positioned in frame with the endogenous coding sequence resulting in the double expression of the human mutated protein and the wild type N. furzeri alpha-Synuclein.

Reference email: elena.chiavacci@sns.it

Poster Presenter: Federica, Cherchi

Institution: UNIFI

Milestone: M1 UNIFI

Poster Title:

Authors: F. Cherchi*, M. Morozzi*, S. Calenda, M. Venturini, Frulloni L., V. Colotta, D. Catarzi, F. Varano, C. Donati, A.M. Pugliese and N. Galeotti

Abstract: Oligodendrocyte-formed myelin sheaths play important roles in neuronal functions in the Central Nervous System (CNS). Multiple Sclerosis is the most common disabling disease of the CNS, with a progressive neurodegenerative pattern. It is characterized by demyelination of white matter in CNS and apoptosis of oligodendrocytes (OLs). In addition, remyelination processes are hindered due to a failure of the oligodendrocyte precursor cells (OPCs) differentiation into mature OLs. However, remyelination therapies are not in clinical use.

Extracellular adenosine increases during ischemia or inflammation, suggesting adenosine receptors (A1R, A2AR, A2BR, A3R) as valid therapeutic targets in a variety of pathological conditions. A higher density of A2ARs has been found in the brains of MS patients, which appears to correlate with disease severity. In experimental autoimmune encephalomyelitis (EAE), an experimental model of MS, the A2A antagonist SCH58261, administered at an advanced stage, reduces neuroinflammation and demyelination of axons. We previously demonstrated that selective stimulation of A2ARs or A2BRs decreases OPC maturation in vitro by inhibiting potassium currents necessary to their differentiation.

The first purpose of this work was to further explore the functional role of A2AR and A2BR on OPC maturation and on myelin deposition in purified rat OPC cultures by patch clamp recordings. We used selective ligands of these adenosinergic receptors and for the first time the new synthetized dual antagonist, able to simultaneously block A2AR and A2BR receptors, P626 (Ki A2A = 5,73+-0,48 nM, IC50= 5,2 nM; IC50 A2B = 34+-3 nM).

The endogenous agonist adenosine (0.1-50 µM), applied in the presence of A1R and A3R selective blockers DPCPX and MRS1523, respectively (both 500 nM), inhibited ramp-evoked outward current and transient (IA) and delayed rectifier (IKDR) potassium currents evoked by step voltage protocols. These effects were blocked by the dual A2AR/A2BR antagonist P626 (0,1-100 nM).

Our data indicate that A2BR and A2AR are involved in OPC maturation in vitro and that the novel A2AR/A2BR antagonist is efficacious to block this effect.

The second aim of this project was to define the pharmacological profile of the dual A2A/A2B antagonist P626 in reducing symptoms associated with EAE model and to compare its activity to that of the A2A antagonist SCH58261. EAE was induced in C57BL/6 female mice by the administration of MOG35-55 that generates a chronic form of the disease. P626 slightly showed better efficacy than SCH58261 in counteracting neuropathic pain and motor disability, which are recognized as important clinical signs of multiple sclerosis. Therefore, an increase of myelin detected with immunofluorescence on the spinal cord was observed in P626-treated mice. These data reveal the interesting pharmacological role of a new dual A2ARs and A2BRs antagonist, P626, in counteracting EAE associated symptoms and myelin loss.

Reference email: federica.cherchi@unifi.it

Poster Presenter: Gabriele, Lagani

Institution: CNR

Milestone: M8: SNS/CNR. Preclinical Validation of Biomarkers and Therapeutic Approaches for Creatine Transporter Deficiency and Angelman Syndrome: From Gene Therapy to Novel Administration Methods

Poster Title: Inducing Plasticity in Biological Cultured Neural Networks through Tetanic Stimulations with MEAs

Authors: Luca Ciampi, Eleonora Crocco, Ludovico Iannello, Gabriele Lagani, Fabrizio Tonelli, Federico Cremisi, Fabrizio Falchi, Angelo Di Garbo, Giuseppe Amato

Abstract: Multi-Electrode Arrays (MEAs) provide a useful interface for fine-grained recording and stimulation of biological cultured neural networks. Previous work has shown that, by leveraging high frequency stimulations (tetanization), it is possible to induce plasticity in such cultured networks. Our research group has been investigating the possibility to leverage the plastic behavior of biological neurons in vitro to learn to solve simple Artificial Intelligence (AI) tasks, pursuing a different paradigm of Biological Intelligence (BI). In our preliminary investigation, we have developed certain analytic tools that are useful to compare the behavior of cultured networks before and after a tetanization protocol, in order to evaluate the effects of different training protocols in the emergence of Long-Term Potentiation/Depression (LTP/LTD). Our preliminary experiments indicate that changing tetanization protocol and stimulation parameters has an impact on the resulting behavior of cultured networks. These results will hopefully be useful in the future to engineer a specific training protocol to modify the behavior of the networks in a desired and controllable way, towards solving desired AI/BI tasks.

Reference email: gabriele.lagani@isti.cnr.it

Poster Presenter: Gustavo, Provensi

Institution:

Milestone: M1 UNIFI

Poster Title: Dr.

Authors: Alessia Costa, Barbara Rani, Fiorella Casamenti, Patrizio Blandina, Claudiu T. Supuran, Maria Beatrice Passani, Gustavo Provensi,

Abstract: A series of recent observations are shedding light on the involvement of brain carbonic anhydrases (CAs) in neurodegenerative disorders: A higher expression of the CAII isoform was found in the plasma of Alzheimer's Disease (AD) patients when compared to age-matched control subjects. Two CA inhibitors (CAIs) prevented Aβ-induced mitochondrial toxicity and cell death in vitro. Despite these encouraging findings, the impact of CAIs in AD animal models were not investigated. Here we evaluated the expression of the 15 CAs isoforms in the cortex and hippocampus of TgCRND8 mice (a transgenic AD model expressing the human amyloid precursor protein carrying the Swedish and Indiana mutations) at transcriptional level by RT-PCR. Then the efficacy of a chronic treatment with the CA inhibitor acetazolamide (ACTZ) against the cognitive impairments and neuropathological alterations observed in TgCRND8 mice was evaluated. To do so, mice were fed with control or ACTZ-enriched diets (100 and 200 ppm) starting at 6 weeks of age. After 6 or 12 weeks of treatment, animals' cognitive function was evaluated in the social discrimination paradigm. Their brains were then, collected for neurochemical analysis. Our analysis revealed a differential transcription expression of CAs isoforms with some of them up regulated and others downregulated. The pharmacological treatment prevented the memory deficits observed in TgCNRD8 mice at both ages. A reduction in the number of β-amyloid plaques (Aβ1-42), in the expression of the β-amyloid pyroglutamic derivative (Aβ N3pE) Tau phosphorylation (AT8) and astrocytic activation (GFAP) were also observed in ACTZ-fed animals compared to the animals fed with control diet. In conclusion, this study revealed that chronic carbonic anhydrase inhibition prevented both behavioral and neuropathological alterations observed in TgCNRD8 animals, suggesting their role as an innovative target for AD treatment.

Reference email: gustavo.provensi@unifi.it

Poster Presenter: Gustavo, Provensi

Institution: UNIFI

Milestone: M1 UNIFI

Poster Title: Dr

Authors: Alessia Costa, Barbara Rani, Fiorella Casamenti, Patrizio Blandina, Claudiu T. Supuran, Maria Beatrice Passani, Gustavo Provensi,

Abstract: A series of recent observations are shedding light on the involvement of brain carbonic anhydrases (CAs) in neurodegenerative disorders: a higher expression of the CAII isoform was found in the plasma of Alzheimer's Disease (AD) patients when compared to age-matched control subjects. Two CA inhibitors (CAIs) prevented Aβ-induced mitochondrial toxicity and cell death in vitro. Despite these encouraging findings, the impact of CAIs in AD animal models were not investigated. Here we evaluated the expression of the 15 CAs isoforms in the cortex and hippocampus of TgCRND8 mice (a transgenic AD model expressing the human amyloid precursor protein carrying the Swedish and Indiana mutations) at transcriptional level by RT-PCR. Then the efficacy of a chronic treatment with the CA inhibitor acetazolamide (ACTZ) against the cognitive impairments and neuropathological alterations observed in TgCRND8 mice was evaluated. To do so, mice were fed with control or ACTZ-enriched diets (100 and 200 ppm) starting at 6 weeks of age. After 6 or 12 weeks of treatment, animals' cognitive function was evaluated in the social discrimination paradigm. Their brains were then, collected for neurochemical analysis. Our analysis revealed a differential transcription expression of CAs isoforms with some of them up regulated and others downregulated. The pharmacological treatment prevented the memory deficits observed in TgCNRD8 mice at both ages. A reduction in the number of β-amyloid plaques (Aβ1-42), in the expression of the β-amyloid pyroglutamic derivative (Aβ N3pE) Tau phosphorylation (AT8) and astrocytic activation (GFAP) were also observed in ACTZ-fed animals compared to the animals fed with control diet. In conclusion, this study revealed that chronic carbonic anhydrase inhibition prevented both behavioral and neuropathological alterations observed in TgCNRD8 animals, suggesting their role as an innovative target for AD treatment.

Reference email: gustavo.provensi@unifi.it

Poster Presenter: Isabella, Felli

Institution: UNIFI

Milestone: M1 UNIFI

Poster Title: Felli

Authors: Marco Schiavina, Giacomo Parigi, Isabella Felli, Roberta Pierattelli

Abstract: Many currently incurable diseases, such as neurodegenerative diseases, depend on the misfunction of intrinsically disordered proteins (IDPs) or of intrinsically disordered regions (IDRs) of large protein machineries. Some well-known examples include α-synuclein and tau, involved in the onset Parkinson's and Alzheimer's diseases respectively, as well as proteins with repetitive amino acids motifs leading to different neurodegenerative diseases to mention just a few. The lack of efficient drugs against these diseases is partly due to the fact that proteins (or protein regions) lacking a 3D structure are challenging to be studied using traditional structural biology techniques. NMR spectroscopy constitutes a unique tool to study with atomic resolution the structural and dynamic properties of highly flexible disordered proteins. The novel NMR methods developed at CERM ranging from very high to very low fields will be presented focusing on applications to a-synuclein dynamics and interactions.

Reference email: felli@cerm.unifi.it

Poster Presenter: Laura, Baroncelli

Institution: CNR

Milestone:

Poster Title: New neuroimaging tools and therapies for Creatine Deficiency Syndromes

Authors:

Abstract:

Reference email: baroncelli@in.cnr.it

Poster Presenter: Marco, Mainardi

Institution: CNR

Milestone: M8: SNS/CNR. Patient-derived stem cells and “brain-in-a-dish” cultures: a cellular platform for target validation and drug screening

Poster Title: N/A

Authors:

Abstract:

Reference email: marco.mainardi@in.cnr.it

Poster Presenter: Marta, Bianchini

Institution: SSSA

Milestone: M11: SSSA

Poster Title: A Biomimetic Scaffold with Piezoelectric Properties to Enhance Peripheral Nerve Regeneration

Authors: Marta Bianchini, Francesco Iacoponi, Andrea Cafarelli, Silvestro Micera, Leonardo Ricotti and Eugenio Redolfi Riva

Abstract: Peripheral nerve injury causes complete loss of axon continuity, impairing motor and sensory functions. Nowadays, clinical therapies are based on autografts or allografts implantation to avoid denervated muscle atrophy. However, they present limitations, so another possible solution is the manufacturing of a tubular scaffold, called nerve guidance conduits (NGC). To improve and promote nerve regeneration, topographical cues and electrical stimulation (ES) can be integrated into the NGC. Ultrasound (US)-activated piezoelectric nanomaterials could be a wireless alternative solution to ES, having shown promising results in enhancing neural cell functions5. This study aims is to develop an anisotropic scaffold with US-activated piezoelectric nanoparticles.

Reference email: Marta.Bianchini@santannapisa.it

Poster Presenter: Martina, Morozzi

Institution: UNIFI

Milestone: M1 UNIFI

Poster Title:

Authors: F. Cherchi*, M. Morozzi*, S. Calenda, M. Venturini, L. Frulloni, V. Colotta, D. Catarzi, F. Varano, C. Donati, A.M. Pugliese and N. Galeotti

Abstract: Oligodendrocyte-formed myelin sheaths play important roles in neuronal functions in the Central Nervous System (CNS). Multiple Sclerosis is the most common disabling disease of the CNS, with a progressive neurodegenerative pattern. It is characterized by demyelination of white matter in CNS and apoptosis of oligodendrocytes (OLs). In addition, remyelination processes are hindered due to a failure of the oligodendrocyte precursor cells (OPCs) differentiation into mature OLs. However, remyelination therapies are not in clinical use.

Extracellular adenosine increases during ischemia or inflammation, suggesting adenosine receptors (A1R, A2AR, A2BR, A3R) as valid therapeutic targets in a variety of pathological conditions. A higher density of A2ARs has been found in the brains of MS patients, which appears to correlate with disease severity. In experimental autoimmune encephalomyelitis (EAE), an experimental model of MS, the A2A antagonist SCH58261, administered at an advanced stage, reduces neuroinflammation and demyelination of axons. We previously demonstrated that selective stimulation of A2ARs or A2BRs decreases OPC maturation in vitro by inhibiting potassium currents necessary to their differentiation.

The first purpose of this work was to further explore the functional role of A2AR and A2BR on OPC maturation and on myelin deposition in purified rat OPC cultures by patch clamp recordings. We used selective ligands of these adenosinergic receptors and for the first time the new synthetized dual antagonist, able to simultaneously block A2AR and A2BR receptors, P626 (Ki A2A = 5,73+-0,48 nM, IC50= 5,2 nM; IC50 A2B = 34+-3 nM).

The endogenous agonist adenosine (0.1-50 µM), applied in the presence of A1R and A3R selective blockers DPCPX and MRS1523, respectively (both 500 nM), inhibited ramp-evoked outward current and transient (IA) and delayed rectifier (IKDR) potassium currents evoked by step voltage protocols. These effects were blocked by the dual A2AR/A2BR antagonist P626 (0,1-100 nM).

Our data indicate that A2BR and A2AR are involved in OPC maturation in vitro and that the novel A2AR/A2BR antagonist is efficacious to block this effect.

The second aim of this project was to define the pharmacological profile of the dual A2A/A2B antagonist P626 in reducing symptoms associated with EAE model and to compare its activity to that of the A2A antagonist SCH58261. EAE was induced in C57BL/6 female mice by the administration of MOG35-55 that generates a chronic form of the disease. P626 slightly showed better efficacy than SCH58261 in counteracting neuropathic pain and motor disability, which are recognized as important clinical signs of multiple sclerosis. Therefore, an increase of myelin detected with immunofluorescence on the spinal cord was observed in P626-treated mice. These data reveal the interesting pharmacological role of a new dual A2ARs and A2BRs antagonist, P626, in counteracting EAE associated symptoms and myelin loss.

Reference email: martina.morozzi@unifi.it

Poster Presenter: Miriam, Acquafredda

Institution: UNIPI

Milestone: M9: UNIPI

Poster Title:

Authors: Miriam Acquafredda, Laura Biagi, Michela Tosetti, Maria Concetta Morrone, Paola Binda

Abstract: In adult humans, a brief period (2 hours) of monocular deprivation induces a form of homeostatic plasticity. Stimuli in the deprived eye are transiently boosted, shifting perceptual ocular dominance towards the deprived eye and modulating visually evoked fMRI responses. Here we demonstrate that monocular deprivation also produces a functional reorganization of visual processing circuits as revealed by changes in functional connectivity.

Ultra-high field 7T fMRI EPI resting-state sequences (with two TRs, 3000 and 1000 ms) were acquired in 20 normally sighted adult participants, before and after the application of a translucent patch on the dominant eye for two hours. During the acquisitions, participants kept their eyes closed.

Functional connectivity was measured by correlating BOLD signals in each cortical voxel with those in a subcortical “seed” area: ventral (visual) pulvinar and LGN. The correlation was computed after performing temporal filtering (bandpass 0.01 - 0.1 Hz) and nuisance regression (fMRI time-course in a mask covering white matter and CSF voxels).

Comparing functional connectivity before and after monocular deprivation, we found decreased connectivity of early visual areas (V1-V3) with the ventral pulvinar, but unchanged connectivity with LGN.

These results suggest that the ventral pulvinar plays an important role in broadcasting signals that modulate sensory processing and that may implement the homeostatic plasticity of neural function.

Reference email: miriam.acquafredda@unifi.it

Poster Presenter: Nicola, Vanello

Institution: UNIPI

Milestone: M9: UNIPI

Poster Title:

Authors: Santa Sozzi (presenter), Enzo Pasquale Scilingo, Paola Binda, Nicola Vanello

Abstract: Given the brainstem's role in essential physiological functions and various pathological conditions, it is crucial to develop methods and tools for assessing brainstem functional connectivity (FC). However, brainstem nuclei often complicate this estimation because of their strong neuromodulatory influence, acting as confounders, elements of a chain system, and colliders within brain networks, which presents a significant challenge in determining their direct FC. We utilize principal component analysis (PCA) and regularized regression to address the issue of multicollinearity in estimating partial correlations, which helps in investigating connectivity both within the brainstem and between the brainstem and other brain regions. We present discuss and preliminary results on brainstem connectivity from resting state data.

Reference email: nicola.vanello@unipi.it

Poster Presenter: Paola, Binda

Institution: UNIPI

Milestone: M9: UNIPI

Poster Title: Successful monitoring of neuroplasticity in patients and successful change of neuroplasticity in animal model with optogenetic or metabolic stimulation.

Authors: Mirco Cosottini,

Nicola Vanello,

Chiara Magliaro

Lorenzo Cangiano,

Jose Fernando Maya-Vetencourt

Francesco Fornai,

Paola Lenzi

Abstract:

Reference email: paola1binda@gmail.com

Poster Presenter: Santa, Sozzi

Institution: UNIPI

Milestone: M9: UNIPI

Poster Title: Methodological issues and possibilities of the brainstem fcMRI

Authors: Santa Sozzi, Enzo Pasquale Scilingo, Paola Binda, Nicola Vanello

Abstract: Given the brainstem's role in essential physiological functions and various pathological conditions, it is crucial to develop methods and tools for assessing brainstem functional connectivity (FC). However, because of the strong neuromodulatory influence of the brainstem nuclei, they can act as potential confounders, elements of a chain system, and colliders within the brain networks, making the estimation of their direct FC a significant challenge. In this work, we propose a partial correlation approach exploiting principal component analysis (PCA), and regularized regression to address the multicollinearity issue and mitigate the effects of mediator regions. We discuss preliminary results on brainstem-to-brainstem and brainstem-to-brain connectivity from resting-state data.

Reference email: santa.sozzi@phd.unipi.it

Poster Presenter: Sara, Bagnoli

Institution: SNS

Milestone: M3: SNS. Integrated Approach to Nanobody Development: From Biochemical Characterization to In Vivo Validation in Transgenic Disease Models

Poster Title:

Authors: Elena Chiavacci, Sara Bagnoli, Alessandro Cellerino

Abstract: The turquoise killifish (Nothobranchius furzeri) is the shortest-lived vertebrate animal model that recapitulates all the major features of mammalian brain aging. We firstly generated the BDNF and NGF Knock-out models via CRISPR/Cas9 Knock-out, among the mutations we found in the F0 founders we selected the out-of-frame BDNF delta-26 deletion and NGF +1 insertion. We are now raising the first incrossed F3 clutches of these two Knock-out lines and we will then proceed to characterize them both at molecular and behavioral level. Furthermore, we will then generate a transgenic line expressing a double copy of BNDF, as a proof of principle we already generated a BDNF double copy construct to be injected in zebrafish.

Since N. furzeri shows signs of spontaneous age-dependent neurodegeneration reminiscent of Parkinson's disease (PD), we decided to create two transgenic models of the protein alpha-synuclein with the aim of inducing a more aggressive PD-like phenotype. We currently designed two constructs for two different approaches. The first transgene will carry a single point mutation in the endogenous alpha-Synuclein gene recreating the A18T human mutation. The second transgene will consist in the addition of a copy of codon optimized human alpha-Synuclein carrying the A18T mutation, positioned in frame with the endogenous coding sequence resulting in the double expression of the human mutated protein and the wild type N. furzeri alpha-Synuclein.

Reference email: sara.bagnoli@sns.it

Poster Presenter: Sara, Calenda

Institution: UNIFI

Milestone: M1 UNIFI

Poster Title:

Authors: Sara Calenda, Daniela Catarzi, Costanza Ceni, Giulia Vagnoni, Flavia Varano, Federica

Cherchi, Chiara Donati, Lucia Frulloni, Nicoletta Galeotti, Martina Morozzi, Anna

Maria Pugliese, Vittoria Colotta.

Abstract: Multiple Sclerosis (MS) is a demyelinating disease characterized by neurodegeneration, neurological symptoms, and cognitive impairment. Adenosine and its A2A and A2B receptor (R) subtypes are involved in the pathogenesis of MS and their blockade proved to favor remyelination processes. Oxidative stress plays an important role in promoting MS, and antioxidants such as Edaravone (EDA) and lipoic acid (LA) have been shown to decrease inflammation and demyelination in animal MS models. This project aims to find novel multitarget compounds capable of blocking adenosine A2AR and A2BR and possessing antioxidant activity as they might be more effective than single-target derivatives. Thus, potent A2AR/A2BR antagonists belonging to our thiazolo[5,4-d]pyrimidine series were hybridized with EDA and LA to give the desired compounds. Binding studies at human (h) A1, hA2A, hA3, and hA2BR are in progress, and available results show that some compounds possess high hA2AR/hA2BR affinity and selectivity. Antioxidant studies (DPPH assay) were performed on selected compounds, showing a high radical scavenging activity, better than EDA and comparable to ascorbic acid. Pharmacological studies are in progress on selected EDA-thiazolopyrimidine hybrids to evaluate their efficacy in cellular and animal MS models

Reference email: sara.calenda@unifi.it

Poster Presenter: Simonetta, Lisi

Institution: SNS

Milestone:

Poster Title:

Authors: Simonetta Lisi, Angela Rachel Bitonti, Stefano Guglielmo, Ajesh Jacobs, Martina D'Assoro, Gabriele Ugolini and Antonino Cattaneo

Abstract: An scFv recognizing p-Tau has been selected by P.I.S.A. (Post-translational Intracellular Silencing Antibodies) technology. PISA screening works by exploiting yeast L40 strain co-transformed with antigen-bait/antibody-prey couples, in which the antigen-bait is fused to the modifying enzyme, so that it installs the PTM on the adjacent antigen (tethered catalysis). The prey is a scFv binding to the PTM, fished out from a co-transformed library. A positive interaction between one of the scFv in the library (prey) and the bait activates transcription of the HIS3 gene allowing survival on selective media and of the β-gal gene allowing the cells to become blue in a β-galactodidase assay. The bait used for the selection was a chimeric fusion protein in which the Microtubules Binding Domain (MTBD) and the C-Terminal Domain (CTD) of human Tau (amino acids 244-441) was fused to the DNA binding protein LexA and to GSK3β S9A, a constitutively active kinase: LexA-MTBD-CTD-GSK3β S9A. The bait was challenged with a human SPLINT library of scFvs. We initially picked 360 clones, which underwent β-gal assay and BSTNI fingerprint. 157 clones were then tested in a secondary screening using the initial bait LexA-MTBD-CTD-GSK3β S9A or an unrelated bait, the LexA-Synuclein or the LexA-GSK3β S9A bait. One scFv was confirmed to selectively bind a phospho epitope of the screening bait. To identify the epitope, we performed in vivo epitope mapping (IVEM), which consist in challenging the isolated scFv with shorter versions of the initial bait. First, we generated two version of the initial bait one expressing just the microtubules binding domain (MTBD) of Tau (LexA-MTBD- GSK3β S9A-HA) and one expressing only the C-Terminal domain (CTD) (LexA-CTD-GSK3β S9A-HA), and found that the epitope was located in the MTBD. We than subdivided the MTBD in other shorter versions made of the individual R domain, or combinations of them. The epitope resides in the R1+R2 of the MTBD. Mutational analysis of the some phospho residues combined with bioinformatics epitope prediction indicates that the scFv epitope involves S289 and S316.

Reference email: simonetta.lisi@sns.it