UNIVERSITÀ DEGLI STUDI DI GENOVA

AREA RICERCA, TRASFERIMENTO TECNOLOGICO E TERZA MISSIONE

SERVIZIO PER IL TRASFERIMENTO TECNOLOGICO E DELLE CONOSCENZE

SETTORE VALORIZZAZIONE DELLA RICERCA, TRASFERIMENTO TECNOLOGICO E RAPPORTI CON LE IMPRESE

IL RETTORE

Vista la Legge 9 maggio 1989, n. 168 - Istituzione del Ministero dell'Università e della ricerca scientifica e tecnologica e ss.mm.ii;

Visto lo Statuto dell'Università degli Studi di Genova;

Visto il Regolamento Generale di Ateneo;

Visto il Regolamento di Ateneo per l'Amministrazione, la Finanza e la Contabilità;

VISTA la legge 7 agosto 1990, n. 241 recante "Nuove norme in materia di procedimento amministrativo e di diritto di accesso ai documenti amministrativi" pubblicata sulla Gazzetta Ufficiale n. 192 del 18/08/1990 e s.m.i.;

VISTO il Decreto del Presidente della Repubblica 28 dicembre 2000, n. 445 (Disposizioni legislative in materia di documentazione amministrativa) e s.m.i.;

VISTO il Decreto Direttoriale MUR n. 341 del 15/03/2022 di emanazione di un Avviso pubblico per la presentazione di Proposte di intervento per la creazione di "Partenariati estesi alle università, ai centri di ricerca, alle aziende per il finanziamento di progetti di ricerca di base" nell'ambito del Piano Nazionale di Ripresa e Resilienza, Missione 4 "Istruzione e ricerca" – Componente 2 "Dalla ricerca all'impresa" – Investimento 1.3, finanziato dall'Unione europea – NextGenerationEU";

VISTO il Decreto Direttoriale MUR n. 1553 dell'11/10/2022 di concessione del finanziamento del progetto Codice identificativo PE00000006, Acronimo MNESYS, Titolo "*A multiscale integrated approach to the study of the nervous system in health and disease*", registrato alla Corte dei Conti il 23/11/2022 al n. 2948 e relativi allegati;

CONSIDERATO che l'Università degli Studi di Genova è leader dello Spoke 6, dal titolo "*Neurodegeneration, trauma and stroke*";

CONSIDERATO che gli Spoke possono emanare - nell'ambito dei limiti e con le modalità previste dall'Avviso - "bandi a cascata" finalizzati alla concessione di finanziamenti a soggetti esterni per attività coerenti con il progetto approvato;

VISTA la delibera della seduta del 27 settembre 2023 con cui il Consiglio di Amministrazione dell'Università degli Studi di Genova ha approvato l'emanazione del bando a cascata per organismi di ricerca nell'ambito del Progetto MNESYS - "*A multiscale integrated approach to the study of the nervous system in health and disease* - PNRR M4C2 per lo Spoke 6;

VISTO il Decreto del Direttore Generale n. 5418 del 14 novembre 2023 di nomina del Responsabile

del Procedimento;

VISTO il Decreto del Rettore n. 5439 del 14 novembre 2023 e il Decreto Rettorale n. 5474 del 15 novembre 2023 di emanazione del Bando a cascata per il finanziamento di proposte di intervento per le attività di ricerca svolte da Organismi di Ricerca nell'ambito del programma di ricerca PE MNESYS "*A multiscale integrated approach to the study of the nervous system in health and disease*", per lo Spoke 6 dal titolo "*Neurodegeneration, trauma and stroke*", nell'ambito del PNRR, Missione 4, Componente 2, Investimento 1.3 – finanziato dall'Unione europea – NextGenerationEU (CUP D33C22001340002);

CONSIDERATO che alla data di scadenza per la presentazione delle proposte progettuali, fissata entro e non oltre il giorno 14 dicembre 2023, per la **Tematica Q – "Dynamics of neuronal calcium, sodium and mitochondria membrane potential in rodent models of neurodegenerative diseases"** era pervenuta a mezzo PEC all'indirizzo air3@pec.unige.it la seguente proposta:

PROPONENTE: Università degli Studi di Napoli "Parthenope"
TITOLO PROPOSTA: IHDECINPAM – Ion homeostasis dysregulation and epigenetic changes in neurons of Parkinson's disease models

TENUTO CONTO che la Responsabile del procedimento, Ing. Patrizia Cepollina, ha ritenuto ricevibile, ammissibile e conforme la proposta sopra citata;

CONSIDERATO che nel Bando è previsto che la valutazione di merito tecnico-scientifico dei progetti pervenuti sia affidata ad una Commissione composta da almeno tre esperti esterni al Partenariato, indipendenti e competenti dell'Area tematica dello Spoke;

VISTO il Decreto Rettorale n. 6114 del 20 dicembre 2023 con cui è stato emanato l'Avviso di manifestazione di interesse per la costituzione di un albo di esperti indipendenti a supporto della valutazione di merito dei progetti PNRR presentati sui bandi a cascata del progetto MNESYS – A multiscale integrated approach to the study of the nervous system in health and disease;

VISTO l'Estratto del Verbale della Riunione del 12 febbraio 2024 del Comitato Scientifico del programma di ricerca MNESYS "*A multiscale integrated approach to the study of the nervous system in health and disease*" che ha approvato la "Rosa di Candidati" per le Commissioni di Valutazione dei Bandi a cascata sul Programma MNESYS;

VISTO il Decreto del Rettore n. 855 del 20 febbraio 2024 con cui è costituito l'Albo a supporto delle valutazioni dei progetti presentati in risposta al bando pubblico per la selezione di proposte progettuali da finanziare nell'ambito delle attività di ricerca dello Spoke n. 6 di cui al programma di "*A multiscale integrated approach to the study of the nervous system in health and disease*" – MNESYS, a valere sulle risorse del Piano Nazionale di Ripresa e Resilienza (PNRR), Missione 4 "Istruzione e Ricerca", Componente 2 "Dalla ricerca all'impresa", linea di Investimento 1.3 "Creazione di Partenariati Estesi alle università, centri di ricerca, alle aziende per il finanziamento di progetti di ricerca di base";

VISTO il Decreto del Rettore n. 1121 del 5 marzo 2024 con cui è stata nominata la Commissione di

valutazione delle proposte pervenute in risposta al bando a cascata di cui al D.R. n. 5439 del 14 novembre 2023, indicato nelle premesse del presente decreto;

ACQUISITO il verbale della Commissione di Valutazione della seduta del 16 aprile 2024 (Prot. n. 37982 del 07/05/2024);

VISTO il Decreto del Rettore n. 2297 del 10 maggio 2024 con cui è stata approvata la graduatoria di merito per la Tematica Q – "Dynamics of neuronal calcium, sodium and mitochondria membrane potential in rodent models of neurodegenerative diseases", di cui al bando a cascata di cui al Decreto del Rettore n. 5439 del 14 novembre 2023, indicato nelle premesse del presente decreto;

TENUTO CONTO che in data 14 maggio 2024 è stata inviata all'Università degli Studi di Napoli "Parthenope" la comunicazione con prot. n. 41380 in cui si rendevano noti gli esiti della procedura e si richiedeva la documentazione propedeutica all'adozione del provvedimento di ammissione del finanziamento;

VISTO che in data 21 maggio 2024 con prot. n. 44362 la documentazione richiesta è stata ricevuta dall'Università degli Studi di Genova che l'ha ritenuta conforme a quanto previsto nel bando a cascata di cui al Decreto del Rettore n. 5439 del 14 novembre 2023 e il Decreto Rettorale n. 5474 del 15 novembre 2023 , indicato nelle premesse del presente decreto,

DECRETA

ART. 1

L'ammissione a finanziamento del progetto IHDECINPAM – Ion homeostasis dysregulation and epigenetic changes in neurons of Parkinson's disease models per la **Tematica Q – "Dynamics of neuronal calcium, sodium and mitochondria membrane potential in rodent models of neurodegenerative diseases"** con Soggetto proponente l'Università degli Studi di Napoli "Parthenope" – come rappresentato negli Allegati B e C alla proposta presentata con domanda di partecipazione prot. n. 74583 del 14 dicembre 2023.

ART. 2

L'entità dell'agevolazione concessa, a fondo perduto, ammonta a 150.000 euro complessivi come rappresentati nell'allegato C alla proposta presentata con domanda di partecipazione prot. n. 74583 del 14 dicembre 2023. L'agevolazione è pari al 100% dei costi di progetto trattandosi di attività di ricerca fondamentale per Organismi di Ricerca. L'agevolazione è concessa a valere sui fondi PNRR - Programma "*A multiscale integrated approach to the study of the nervous system in health and disease*" – MNESYS Codice PE00000006 a valere sulla Missione 4, Componente 2, Investimento 1.3, ai sensi del Decreto di concessione n. 1553 dell'11 ottobre 2022, registrato alla Corte dei Conti il 23/11/2022 n. 2948, iscritto al Bilancio di Ateneo sul progetto UGOV 100009-2022-TF-PNRR-PE_MNESYS_BAC_DINOGMI.

ART. 3

Le attività, come indicate dettagliatamente nell'Allegato B alla domanda di finanziamento, dovranno

essere avviate a partire dalla data di sottoscrizione del Contratto e concluse entro e non oltre 12 mesi, affinché siano rendicontate in tempo utile per consentire la chiusura del Programma PE MNESYS, il cui termine è attualmente previsto al 31 ottobre 2025.
Potrà essere valutata e concessa una sola proroga in presenza di ritardi dovuti a circostanze eccezionali e non dipendenti da scelte del Beneficiario esclusivamente nel caso in cui il MUR, a sua volta, proroghi il termine del Programma MNESYS.

ART. 4

Il presente atto sarà pubblicato sul sito UniGe https://unige.it/progetti-finanziati-dal-pnrr e laddove la normativa vigente lo richiede.
Il documento informatico originale sottoscritto con firma digitale sarà conservato presso l'Area Ricerca, Trasferimento Tecnologico e Terza Missione.

ALLEGATI:

Allegato B – Proposta progettuale
Allegato C – Piano economico-finanziario

IL RETTORE
Prof. Federico DELFINO
(documento firmato digitalmente)

Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA
MNESYS
Università di Genova

ANNEX B

PE00000006
"A multiscale integrated approach to the study of the nervous system in health and disease"

MNESYS

SPOKE N. 6

Research proposal

Topic addressed by the project
(with reference to Annex 2)
'Q': **Dynamics of neuronal calcium, sodium and mitochondria membrane potential in rodent models of neurodegenerative diseases.**

Title of the project
IHDECINPAM - **Ion homeostasis dysregulation and epigenetic changes in neurons of Parkinson's disease models**

- Name of the PIs' host institution for the project: University of Naples 'Parthenope'
- Name of the Principal Investigators (PIs): Ezia Guatteo
- Proposal duration in months: 12

- Name and qualification of the Principal Investigator (PI): Ezia Guatteo, Associate professor of Physiology (BIO-09)
- Name and qualification of the co-Principal Investigator (PI): Rosaria Meccariello, Full professor of Experimental Biology (BIO-13)
- Name and qualification of the components the research team

ROLE IN THE PROJECT	*NAME*	*SURNAME*	*DEPARTMENT*	*QUALIFICATION*	*YOUNG (under 40 al 31.12.2023)*	*F/M*
Principal Investigator	*Ezia*	*Guatteo*	*Medical and Movement Sciences and wellbeing*	*Associate Professor*		*F*
co-Principal Investigator (coPI)	*Rosaria*	*Meccariello*	*Medical and Movement Sciences and wellbeing*	*Full Professor*		*F*
Component	*To be recruited*		*Medical and Movement Sciences and wellbeing*	*Post-doc*		
Component	*To be recruited*		*Medical and Movement Sciences and wellbeing*	*Post-doc*		
Component	*To be recruited*		*Medical and Movement Sciences and wellbeing*	*Post-doc*		

ABSTRACT (limit 2000 characters, spaces and line breaks included)

Parkinson's disease (PD) is characterized by dopaminergic neuron loss within the substantia nigra of the midbrain, causing dopamine depletion in dorsal striatum and other brain regions receiving dopaminergic innervation. A combination of genetic and epigenetic factors have been put forward to explain neuronal degeneration but we are still far from understanding the precise mechanisms responsible for neuronal demise. Here we take advantage of a mouse PD model overexpressing human tyrosinase, yielding to accumulation of neuromelanin and degeneration of catecholaminergic neurons, and of a mouse model of dopaminergic dysfunction with reduced midbrain expression of miR-218, a microRNA controlling a group of targets associated with PD and dopamine release. Both models display alterations in dopaminergic neurons' electrical activity and neurotransmission. Main aim of the present project is to unravel some aspects of the genetic manipulation consequences that may participate, together with ageing, to degeneration of dopaminergic neurons. Particularly, we will investigate *a)* the dysregulation of cytosolic calcium homeostasis and of Ca2+-activated membrane conductance; *b)* epigenetic modifications in the two rodent models, such as histone tail landscape or in the global DNA methylation and hydroxy-methylation status or changes in the epigenetic eraser and writer enzymes; *c)* the effects of brain tissue exposure to degradable or non-degradable plastic debris capable to interfere with [Ca2+]i; *d)* alterations in components of the endocannabinoid system, recently reported modified in synuclein-based PD models. We believe our project will provide precise information on dopaminergic neuron functional and molecular modifications related to specific genetic manipulations.

RESEARCH PROPOSAL
Sections (a) and (b) should not exceed 4 pages. References do not count towards the page limits.

Section a. State-of-the-art and objectives

Parkinson's disease (PD) is characterized by progressive degeneration of the pigmented dopaminergic neurons located in the substantia nigra pars compacta (SNpc) of the midbrain, leading to decrease of dopamine content within basal ganglia and other brain areas, ultimately causing typical motor and non-motor symptoms of the disease (Singh et al., 2016). Dopaminergic neuron pigmentation is due to neuromelanin (NM) accumulation with age (Fedorow et al., 2005), occurring in primates (human and non-human) but not in rodents (Marsden, 1961). Within the brain, neuromelanin is mainly formed by non-enzymatic oxidation of dopamine (DA), whereas melanin synthesis in peripheral tissue (skin) requires the enzyme tyrosinase (Tyr) (although tyrosinase expression at low levels has been demonstrated within the brain and SNpc, Miranda et al., 1984; Greggio et al., 2005). It has been largely demonstrated that NM accumulation directly correlate with dopaminergic cell death in PD (Hirsch et al., 1988, Hirsch et al., 1989) however, the cellular mechanisms involved in NM-related dopaminergic (DAergic) neuron degeneration have been understudied due to the lack of animal models accumulating NM. In this regard, we have recently obtained from our collaborator prof. Vila a transgenic mouse model overexpressing human Tyr (hTyr) in the brain, which displays intense NM accumulation inside cathecolaminergic neurons and progressive degeneration of the pigmented neurons. We have characterized electrophysiological properties (unpublished) of DAergic neurons in midbrain slices obtained from 15-17 months old mice and found an altered firing activity of the surviving neurons, associated to changes in a calcium-activated potassium conductance. The latter result may be either a direct effect of NM granules inside neurons or secondary to endogenous α-synuclein deposition triggered by NM accumulation, as recently confirmed in non-human primates and rats injected with adeno-associated-viral vectors encoding hTyr (AAV-hTyr, Chocarro et al., 2023; Carballo-Carbajal et al., 2019) into SNpc. In line with this concept, α-synuclein binds and activate SERCA, thus controlling cytosolic Ca^{2+} concentration ([Ca^{2+}]i; Betzer aet al., 2018; Kovacs et al., 2021).

The presence of proteinaceous deposits containing α-synuclein, termed Lewy bodies and Lewy neurites (LB, LN) is a hallmark of PD brain (Spillantini et al., 1997) and a rat model overexpressing human α-synuclein encoding gene (hSNCA, Nuber et al., 2013), morphological and functional alterations within the dopaminergic circuit exist, including bi-directional changes of the electrical activity of DAergic neurons, that resulted hypo-excitable at early stages (Krashia et al., 2019) and hyper-excitable in advanced phases of disease progression (Ledonne et al., 2022). Such altered firing correlated with changes of the large conductance calcium-dependent potassium current. Finally, in another synuclein-based animal model of PD (rats that received pre-formed α-syn fibrils by intracerebral injection into the midbrain), similar alterations of firing in dopaminergic neurons have been reported, possibly associated to alterations of the same calcium-dependent potassium conductance (Tozzi et al., 2021).

Objective 1: Based on our observation of altered firing activity and of the calcium-activated potassium conductance in hTyr DAergic neurons, here we aim at unravelling if cytosolic Ca^{2+} ([Ca^{2+}]i) dysregulation occurs in DAergic neurons in hTyr animals vs. controls, at two time points (2-4 and 6-8 months old), in order to highlight if different degree of NM accumulation, exerts detrimental actions on DAergic neurons by inducing cytoplasmic Ca^{2+} dyshomeostasis. We will test the ability of hTyr DAergic neurons to buffer Ca^{2+} ion increase following membrane depolarization and/or activation of ionotropic calcium-permeable membrane channels as well as the ability to release Ca^{2+} ions from endoplasmic reticulum in response to activation of metabotropic glutamate receptors (Guatteo et al., 2007; Cucchiaroni et al., 2010).

Objective 2: According to what reported in the midbrain of non-human primates and rats who received AAV-hTyr, we expect that NM accumulation would trigger deposition of α-synuclein and synucleinopathy also in our hTyr transgenic mice. We will evaluate α-synuclein deposition at two time points (4 and 8 months old mice).

Next, to gain further insights on the role of $[Ca^{2+}]i$ dysregulation in DAergic neurons function perturbation, we will investigate $[Ca^{2+}]i$ dynamics in another rodent model in which the levels of miR-218, a microRNA controlling a group of targets associated with PD and DA release at synapses (Dunn et al., 2017), have been lowered. We have recently reported that intrinsic excitability and neurotransmitter release of native SNpc DAergic neurons of adult mice (2 months old) knocked-out for miR-218 are heavily impaired (Pulcrano et al., 2023). Particularly, reducing the expression of miR-218 at very low levels (KO of both isoforms) causes hyperexcitability of DAergic neurons and a reduction of the Ca^{2+}-activated K^+ conductance, that limits the action potential afterhyperpolarization phase.

Objective 3: We will ascertain if knocking-out miR-218 from mouse genome causes alterations of $[Ca^{2+}]i$ in SNpc DAergic neurons which in turn would affect Ca^{2+}-activated K^+ conductances. We will perform the same pharmacological manipulations described for hTyr DAergic neurons to highlight a putative role of miR-218 in controlling neuronal machinery that regulate $[Ca^{2+}]i$ homeostasis. Finally, we will also ascertain if miR-218 controls α-synuclein deposition, by performing IHC to highlight LB and LN.

As PD is influenced by a combination of genetic and environmental factors, there is an increasing interest in the identification of epigenetic changes in PD pathogenesis. In the brain, DNA methylation and hydroxy-methylation status, post translational modifications of the histone landscape and the production of non-coding RNA (i.e., Long-non-coding antisense RNA or circular RNA) are crucial for neuronal differentiation, development, and aging, synaptic function, dendrite morphogenesis or axon guidance; in PD epigenetic changes can affect the production of α-Syn itself (Meccariello et al in press). Hence the need to investigate the changes in the epigenetic erasers and writers in our experimental models.

Objective 4: Is the accumulation of α-syn related to modifications in histone tail landscape or in the global DNA methylation and hydroxy-methylation status? Western blot, ELISA, or IHC will be used in hTyr overexpressing animal model.

Objective 5: Is the accumulation of α-syn related to changes in the epigenetic eraser and writer enzymes (e.g, DNA methyltransferases (DNMTs), Histone deacetylases (HDACs), ten eleven translocation (TET))? Gene expression analysis will be carried out in animal models.

Among environmental factors capable to induce epigenetic effects, pollutants are gathering interest in PD pathogenesis (Schaffner and Kober 2022). In a world largely contaminated plastic, neurotoxic effects of plastic debris in the nano- or micrometer range deserve particular interest due to the ability of these substances to bypass the biological barriers, included the blood-brain-brain barrier, thus causing neuroinflammation and neurotoxicity (Lamparelli et al., 2023, Santoro et al Curr. Neuropharmacol. manuscript under the second round of revision).

Objective 6: Are degradable or non-degradable plastic debris capable to interfere with [Ca2+]i or α-syn production and accumulation in DAergic neurons in both physiological or pathological conditions? We'll carry out ex vivo incubations of freshly collected brain slices with biodegradable (e.g., fluorescent Poly(Lactic Acid) plastic debris) and non-degradable plastics (e.g., fluorescent polystyrene particles) to assess [Ca2+]i, α-syn production and the epigenetic effects as in objectives 4 and 5.

Endocannabinoid system is one of the main signalling system in biological systems and it is both epigenetically modulated and capable to affect the expression rate of the epigenetic machinery (Meccariello et al., 2020). Its impairment is notably linked to neuroinflammation, neurodegeneration and neurological disorders (Chianese et al., 2018). Recently, the time-course of alterations in components of the endocannabinoid system after viral-mediated α-syn overexpression has been reported in rat, revealing that the system is impacted by α-syn overexpression (Kelly et al., 2022). Hence the endocannabinoid system may represent a suitable biomarker in PD pathology.

Objective 7: Is the endocannabinoid system (e.g., the receptors CB1, CB2 and TRPV1, the biosynthetic/hydrolysing enzymes NAPE-PLD, MAGL, DAGL and FAAH) affected in our hTyr

overexpressing and miR-218 KO experimental models? Western blot, qPCR or IHC will be carried out in both the proposed experimental models.

Section b. Methodology

Animals. We have established in our animal facility a colony of hTyr overexpressing mice. Heterozygous male and female are bred and pups are genotyped by ear punch tissue collection. Mice will be used at 2-4 and 6-8 months of age, as NM accumulation is progressive. Experimental mice with reduced expression of miR218 will be obtained according to (Pulcrano et al., 2023).

Midbrain slice preparation. Brain tissue will be prepared according to recently published procedures (Ledonne et al., 2022; Pulcrano et al., 2023). Briefly, horizontal slices (thickness 250 or 300 μm) containing the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA), will be cut using a NMDG-based solution containing (in mM): 92 NMDG, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 0.5 CaCl2, and 10 MgSO4, saturated with 95% O2–5% CO2 (pH 7.3 with 18% HCl). After decapitation, the brain will be rapidly removed from the skull, and a tissue block containing the midbrain immersed in the NMDG-based aCSF at 2-4 °C. Horizontal slices (250 μm) will be cut using a vibratome (Leica VT1200S, Leica Microsystems, Wetzlar, Germany). Slices will be maintained in NMDG-based aCSF at 33.0 ± 0.5°C for 15 min before adding increasing volumes of a sodium-spike solution (2M NaCl in NMDG-based aCSF) every 5 min for 40 min, then were transferred in a HEPES-based aCSF for long-term storage, containing (in mM): 92 NaCl, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 2 CaCl2, and 2 MgSO4 (pH 7.3). After 1h of recovery, slices will be transferred in the recording chamber and perfused at 2.5–3.0 mL/min with aCSF (33.0 ± 0.5°C) containing (in mM): NaCl 126, NaHCO3 24, glucose 10, KCl 2.5, CaCl2 2.4, NaH2PO4 1.2 and MgCl2 1.2 (95% O2–5% CO2, pH 7.3).

Calcium Imaging and electrophysiology. Whole-cell patch clamp recordings in combination with microfluorometry will be conducted using a Multiclamp 700B amplifier (Molecular Devices) using pipettes (2–5 MΩ) filled with (mM): 145 K-gluconate, 0.1 CaCl2, 2 MgCl2, 10 HEPES, 0.75 mM EGTA, 0.25 Fura-2, 2 ATP-Mg2+ and 0.3 GTP-Na+ (pH 7.3, 280 mOsm). SNpc neurons will be visualized in transmitted light using infrared differential interference contrast (BX51WI Olympus) and infrared video-cameras (Evolve Photometrics, Arizona) and identified as tightly-packed, medium-sized (diameter > 20 μm) cells adjacent to the medial terminal nucleus of the accessory optic tract. They will be selected by the presence, in voltage-clamp (Vhold = −60 mV), of a prominent Ih current in response to hyperpolarizing voltage steps (−80 to −120 mV) and a transient outward current after a brief application of DA (30–100 μM). Measurements of intracellular free calcium concentration ([Ca2+]i) will be performed as described previously (Freestone et al., 2009; Guatteo et al., 2004). Cells will be illuminated using a monochromator-based system (Till Photonics) to provide 340 and 380 nm excitation wavelengths. Fluorescence ratios will be converted to [Ca2+]i using the standard calibration procedure (Grynkiewicz et al., 1985).

IHC. Procedures to highlight α-synuclein deposits in the two mouse models will be performed in accordance with those described in (Carballo-Carbajal et al., 2019; Chocarro et al., 2023).

Western blot, qPCR, ELISA, and IHC will be performed according to (Marino et al., 2023)

Section c. Available instrumentations and resources

-Animal care facility, with veterinarian service and staff, be on call (located in 200 square meters)

-1 fully equipped set-up for patch-clamp electrophysiology combined to CCD camera and Polychrome IV monochromator for microfluorimetric measurements;

- 1 vibratome for brain slice preparation;

- Confocal and fluorescence microscope for IHC experiments in midbrain slices.

- Equipment to perform Western blot, qPCR, gene expression analysis of epigenetic eraser and writer enzymes (e.g, DNA methyltransferases (DNMTs), Histone deacetylases (HDACs), ten eleven translocation (TET)).

- Western blot, ELISA, or IHC to investigate modifications in histone tail landscape or in the global DNA methylation and hydroxy-methylation status.

Section d. GANTT diagram

Project duration (Months)												
OBJECTIVE	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
APPROVAL OF THE LICENCE FOR ANIMAL USE FOR THE PROJECT												
Objective 1												
Objective 2												
Objective 3												
Objective 4												
Objective 5												
Objective 6												
Objective 7												
DATA DISSEMINATION												

Curriculum vitae PI (max. 2 pages)

PERSONAL INFORMATION

Guatteo, Ezia

Researcher unique identifier(s) **ORCID**: 0000-0001-6338-1793; **Scopus Author ID**: 6603264401

Date of birth: June, 18th, 1968

Nationality: Italian

URL for web site: https://motorie.uniparthenope.it/motorie/organigramma/16308

- **EDUCATION**

1997 PhD in Human Physiology, Faculty of Medicine, Department of Human Physiology, University of Milan La Statale, Milan, Italy (PhD supervisor Prof. G. Miserocchi).

1993 *Esame di Stato* (National qualification as Biologist), University of Pavia, Pavia, Italy.

1992 Master degree, Faculty of Mathematical, Physical and Natural sciences, School of Biology, Department of Biochemistry and General Physiology, University of Milan La Statale, Milan, Italy (Supervisor Prof. Enzo Wanke).

- **CURRENT POSITION(S)**

2016 –present Associate Professor, University of Naples Parthenope, Department of medical and movement sciences and wellbeing, Naples, Italy.

2016 –present Leading Researcher of the Microfluorometry Unit, Santa Lucia Foundation IRCCS, Experimental Neurology, Laboratory, Rome, Italy (Formal agreement *Accordo Quadro di collaborazione Scientifica* between University of Naples Parthenope and Santa Lucia Foundation IRCCS, 2021-2028).

- **PREVIOUS POSITIONS**

2006 – 2016 Tenured Leading Researcher (E1 level), Microfluorometry Unit, Santa Lucia Foundation IRCCS, Rome, Italy.

1998 – 2006 Tenured Researcher (E level), Experimental Neurology Laboratory, Santa Lucia Foundation IRCCS, Rome, Italy.

1996-1998 Post-doc position (CoCoCo contract), Experimental Neurology Laboratory, Santa Lucia Foundation IRCCS, Rome, Italy.

1994-1995 Visiting Research Fellow at University of Washington, Department of Neurosurgery, Seattle, WA, USA.

- **FELLOWSHIPS AND AWARDS**

2017 Academic qualification for Full Professorship of Physiology 05/D1 (ASN, 2017-2028).

2007 Invited visiting scientist at University of Auckland, Department of Physiology, Auckland, New Zealand (2 months).

1994 – 1995 Scholarship, School of Medicine/ Department of Neurosurgery/University of Washington/ Seattle/ WA, USA

- **SUPERVISION OF GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS (if applicable)**

2020 – 2023 Tutor of 2 PhD Students, Department of Medical and movement sciences and wellbeing, University of Naples Parthenope, Naples, Italy.

2017 External tutor of 1 PhD student, Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy.

2019-2023 Supervisor of 7 Master students, Department of Medical and movement sciences and wellbeing, University of Naples Parthenope, Naples, Italy.

- **ORGANISATION OF SCIENTIFIC MEETINGS (if applicable)**

2023 Co-organiser of the symposium 'Walking through misfolded proteins in movement disorders' 20th national meeting of the Italian Society of Neuroscience (SINS) September 14-17, Turin, Italy. Symposium N. 8_5.

2019 Organizer of the international workshop 'Functions and dysfunctions of the dopaminergic system in murine models of brain disease' Department of Medical and movement sciences

and wellbeing, University of Naples Parthenope, Naples, Italy.

- **INSTITUTIONAL RESPONSIBILITIES (if applicable)**

2017- Faculty member, College of Teachers of the PhD program in Motor Sciences and Sport Activities, University of Naples 'Parthenope', Naples, Italy.

2016 – Graduate Student Advisor (n.103 students, bachelor degree), Department of Medical and movement sciences and wellbeing, University of Naples Parthenope, Naples, Italy.

2023 – Member, Faculty Quality Assurance Committee for LM47/68, University of Naples Parthenope/ Department of Medical and Movement Sciences and Wellbeing/ Naples, Italy.

2023- Member, Faculty Committee 'Commissione Paritetica docenti-studenti', University of Naples Parthenope/ Department of Medical and Movement Sciences and Wellbeing/ Naples, Italy (DDD n. 18 del 18-04-2023).

2023-2027 Member, Faculty Commettee 'Comitato Unico di Garanzia' (CUG) per le pari opportunità (DR n.467).

2023 Co-Organizer, Internal Seminar against gender violence (CUG activity), University of Naples Parthenope, Naples, Italy: '*Dialoghi in FormAzione, IMMAGINARI VIOLENTI, Corpo, Estetica e Linguaggi nel femminile'*, November 28th.

2018 – 2023 Member, 'Bandi Competitivi Attivi' Committee, University of Naples Parthenope, Naples, Italy.

- **REVIEWING ACTIVITIES (if applicable)**

2015- Review Editor, Frontiers in Cellular Neuroscience, Frontiers in Systems Neuroscience Frontiers in Molecular Neuroscience (Molecular Neuroscience Archive and Neuroplasticity and Development).

2019 – Reviewer, Journal of Neurochemistry, Neuroscience.

2017 – Scientific Evaluation research project, University of Foggia (DR 449), Foggia, Italy.

2022 – Evaluator, PhD thesis University of Milano-Bicocca, Milan, Italy.

2015- Evaluator, PhD thesis University of Malaga, Campus de Teatinos, 29071-Málaga, Spain.

- **MEMBERSHIPS OF SCIENTIFIC SOCIETIES (if applicable)**

2017 – Member, italian society of physiology (SIF)

2022- Member, Research Network '*Aligning Science Across Parkinson's*' [ASAP-020505] through the Michael J. Fox Foundation for Parkinson's Research (MJFF).

1997-2010 Member, Society for Neuroscience, Washington, DC 20005, USA (SFN)

2010-2016 Registered at National Order of Biologists, N. 062984.

- **MAJOR COLLABORATIONS (if applicable)**

-Prof. J. Lipski, dopaminergic system dysfunction in Parkinson's disease animal models, Department of Physiology, University of Auckland, New Zealand;

-Dr. Gian Carlo Bellenchi, miRNA controlling the dopaminergic system development and function, CNR, Naples, Italy.

- Dr. Patrizia Longone, calcium dynamics in spinal motorneurons exposed to agents related to amyotrophic lateral sclerosis, Santa Lucia IRCCS Foundation, Rome, Italy.

- **CAREER BREAKS (if applicable)**

Maternity leaves (Low dated 30/12/71, n. 1204), 10 months (09/2001-02/2002 and 11/2005-04/2006).

Appendix: All current grants and on-going and submitted grant applications of the PI (Funding ID)

Mandatory information (does not count towards page limits)

Current grants (Please indicate "No funding" when applicable):

Project Title	*Funding source*	*Amount (Euros)*	*Period*	*Role of the PI*	*Relation to current proposal*
Clinical efficacy of pharmacological treatments targeting energy metabolism on motor function in Parkinson's Disease patients	Ministry of Health (RF-2021-12374979)	20.000,00 (my experimental section)	12/2022-12/2025	Component	Similar experimental approach but focused on other genetic murine models of Parkinson's disease
Mir-218 as biomarker of dopaminergic function during development and of depressive syndrome	Ministry of University and Research DM 737	29.667,80	2021-2023	PI	Allowed to obtain part of results on which present proposal is based for a larger and more comprehensive investigation
Unveil the role of miR-218 in the onset of Parkinson's disease	American Parkinson Disease Association	**Submitted Pre-proposal** (ID application #: 1276614)		Component	To validate miR-218 knockout mice as Parkinson's disease murine model, based on appearance of non-motor symptoms of the disease

Curriculum vitae Co-PI (max. 2 pages)

PERSONAL INFORMATION

Meccariello, Rosaria
Researcher unique identifier(s): ORCID**:** orcid.org/0000-0003-2174-1118**;** SCOPUS ID: 6602795788

Date of birth: August 24, 1972

Nationality: Italian

URL for web site: https://www.uniparthenope.it/Portale-Ateneo/organigramma/1203

- **EDUCATION**

2004 PhD in Comparative Endocrinology (Cycle XV), University of Padua, Italy
PhD Supervisor: Prof Riccardo Pierantoni.

1999 Advanced training abroad, School of Biological Sciences, University of Manchester, UK, Supervisor/Host: Prof. Ian D. Morris.

1999 *Esame di Stato* (National qualification as Biologist), University of Naples Federico II, Naples, Italy.

1997 Master Degree in Biological Sciences, University of Naples Federico II, Naples Italy (110/110 cum laude).

- **CURRENT POSITION**

2022 –present Full Professor of Experimental Biology (BIO/13), Department of Medical and Movement Sciences and wellbeing, University of Naples Parthenope, Naples Italy.

- **PREVIOUS POSITIONS**

2010 – 2021 Associate Professor of Experimental Biology (BIO/13), Department of Movement Sciences and wellbeing, University of Naples Parthenope, Naples Italy.

2005 – 2009 Assistant Professor of Experimental Biology (BIO/13), Department of Movement Sciences and wellbeing, University of Naples Parthenope, Naples Italy.

2004 Post-doc fellowship (GEAR), University of Naples Federico II, Naples Italy (Regione Campania, POR 2000/2006, misura 3.13, d.r. n. 759 del 5/3/2004).

2003 Research fellowship (ex art. 2222), Department of Experimental Medicine, University of Campania L. Vanvitelli, Naples Italy.

1999-2002 Research fellowship, Department of Experimental Medicine, University of Campania L. Vanvitelli, Naples, Italy.

- **FELLOWSHIPS AND AWARDS**

2006 President's Prize in recognition of the best oral communication at the 23rd Conference of Comparative Endocrinologists, Manchester, UK.

2008 Commentary on Endocrine News (April 2008, pp22-23), for the manuscript Meccariello et al., 2008, Endocrinology 149, 2149-2158.

- **SUPERVISION OF GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS**

2020 – 2022 Supervision of 1 research fellow (PRIN 2017, Modulation and disruption of endocannabinoid system in the control of spermatogenesis and novel molecular markers of sperm quality).

2014 – 2015 Supervision of 1 research fellow (2010-2011 PRIN-MIUR, Turmoil exerted by endocrine disruptors in vertebrates: emerging aspects in the induction of obesity and reproductive activity alteration).

2008 – 2019 Supervisors of 12 master students (University of Naples Parthenope and University of Naples Federico II, Naples, Italy) and 2 Trainees (150 hours) degree course in Biological Science, University of Naples Parthenope, Naples Italy.

- **INSTITUTIONAL RESPONSIBILITIES**

2022 – 2025 President of the Degree Course in Movement Sciences, University of Naples Parthenope, Naples, Italy.

2018 – 2022 Member of the Research and Third Mission Commission of the Department of Movement

Sciences and wellbeing, University of Naples Parthenope, Naples Italy.

2014 – present Faculty member, College of Teachers of the PhD program in Motor Sciences and Sport Activities, University of Naples 'Parthenope', Naples, Italy.

2013 Faculty member, College of Teachers of the PhD program in Motor Sciences, University of Naples 'Parthenope', Naples, Italy.

2001 – 2012 Faculty member, College of Teachers of the PhD program in Human movement and Health Sciences, University of Naples 'Parthenope', Naples, Italy.

2007 – 2009 Representative of researchers in the Faculty Council of Movement Sciences, University of Naples Parthenope, Naples Italy.

2005 – 2013 Delegate of the Faculty of Movement Sciences for Guidance and Tutoring, University of Naples Parthenope, Naples Italy.

- **REVIEWING ACTIVITIES**

2023 European Research Council's ERC, Synergy Grant evaluation (n.1 project).

2023 Scientific Evaluation of grant proposal for the National Science Centre Poland (n.1 project).

2019 Scientific Evaluation of grant proposal, for The Health Research Board (HRB) (n.1 project).

2012 Scientific Evaluation of grant proposals (PRIN) for "Ministero dell'Istruzione, dell'Università e della Ricerca Scientifica" (MIUR), Italy (n.2 full projects and n.2 preliminary proposal).

2020 – 2023 Associate Editor: Frontiers in Toxicology, Section *Developmental and Reproductive Toxicology*.

2019 – 2023 Section Board Member: *International Journal of Environmental Research and Public Health.*

2019 – 2023 Topical Advisory Panel Member: International Journal of Molecular Sciences.

2000 – 2023 Reviewer for over 40 indexed journals.

2013 – 2023 Guest editor of n.15 editorial projects (Special issues) for the IJMS, IJE, Frontiers in Endocrinology- Frontiers in neurosciences, Frontiers in Toxicology.

- **MEMBERSHIPS OF SCIENTIFIC SOCIETIES**

2022–present Member of the Council of the European Society for Comparative Endocrinology (ESCE).

2003 present Associazione Italiana di Biologia e Genetica Generale e Molecolare (A.I.B.G.).

- **MAJOR COLLABORATIONS**

Silvia Fasano and Riccardo Pierantoni, Department of Experimental Medicine, University of Campania L. Vanvitelli, Naples Italy (epigenetics, endocannabinoid system in health and disease, neuroendocrine modulation of the HPG axis).

Antonietta Santoro and Andrea Viggiano, University of Salerno, Italy (Neuroinflammation, neurogenesis, neurotoxicity, environmental pollutants/**e**ndocrine disruptors and health).

Francesca Felicia Operto, University of Magna Graecia, Catanzaro, Italy (Neuroinflammation, cannabinoids and neurological disorders).

Laura N. Vandenberg, School of Public Health and Health Sciences, University of Massachusetts – Amherst, Amherst, MA, USA (Neurotoxicity, environmental pollutants/endocrine disruptors and health).

Sean Richards, Department of Biological and Environmental Sciences, University of Tennessee-Chattanooga, 615 McCallie Ave., Chattanooga, TN 37403 USA (Neurotoxicity, environmental pollutants/endocrine disruptors and health).

Cited References

Singh A, Mewes K, Gross RE, DeLong MR, Obeso JA, Papa SM. Human striatal recordings reveal abnormal discharge of projection neurons in Parkinson's disease. Proc Natl Acad Sci U S A. 2016 Aug 23;113(34):9629-34. doi: 10.1073/pnas.1606792113. Epub 2016 Aug 8. PMID: 27503874; PMCID: PMC5003232

Fedorow H, Tribl F, Halliday G, Gerlach M, Riederer P, Double KL. Neuromelanin in human dopamine neurons: comparison with peripheral melanins and relevance to Parkinson's disease. Prog Neurobiol. 2005 Feb;75(2):109-24. doi: 10.1016/j.pneurobio.2005.02.001. PMID: 15784302.

Marsden CD. Pigmentation in the nucleus substantiae nigrae of mammals. J Anat. 1961 Apr;95(Pt 2):256-61. PMID: 13767161; PMCID: PMC1244469.

Miranda M, Botti D, Bonfigli A, Ventura T, Arcadi A. Tyrosinase-like activity in normal human substantia nigra. Gen Pharmacol. 1984;15(6):541-4. doi: 10.1016/0306-3623(84)90212-x. PMID: 6441736.

Greggio E, Bergantino E, Carter D, Ahmad R, Costin GE, Hearing VJ, Clarimon J, Singleton A, Eerola J, Hellström O, Tienari PJ, Miller DW, Beilina A, Bubacco L, Cookson MR. Tyrosinase exacerbates dopamine toxicity but is not genetically associated with Parkinson's disease. J Neurochem. 2005 Apr;93(1):246-56. doi: 10.1111/j.1471-4159.2005.03019.x. PMID: 15773923.

Hirsch E, Graybiel AM, Agid YA. Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. Nature. 1988 Jul 28;334(6180):345-8. doi: 10.1038/334345a0. PMID: 2899295.

Hirsch EC, Graybiel AM, Agid Y. Selective vulnerability of pigmented dopaminergic neurons in Parkinson's disease. Acta Neurol Scand Suppl. 1989;126:19-22. doi: 10.1111/j.1600-0404.1989.tb01778.x. PMID: 2575832.

Chocarro J, Rico AJ, Ariznabarreta G, Roda E, Honrubia A, Collantes M, Peñuelas I, Vázquez A, Rodríguez-Pérez AI, Labandeira-García JL, Vila M, Lanciego JL. Neuromelanin accumulation drives endogenous synucleinopathy in non-human primates. Brain. 2023 Dec 1;146(12):5000-5014. doi: 10.1093/brain/awad331. PMID: 37769648; PMCID: PMC10689915.

Carballo-Carbajal I, Laguna A, Romero-Giménez J, Cuadros T, Bové J, Martinez-Vicente M, Parent A, Gonzalez-Sepulveda M, Peñuelas N, Torra A, Rodríguez-Galván B, Ballabio A, Hasegawa T, Bortolozzi A, Gelpi E, Vila M. Brain tyrosinase overexpression implicates age-dependent neuromelanin production in Parkinson's disease pathogenesis. Nat Commun. 2019 Mar 7;10(1):973. doi: 10.1038/s41467-019-08858-y. PMID: 30846695; PMCID: PMC6405777.

Betzer C, Lassen LB, Olsen A, Kofoed RH, Reimer L, Gregersen E, Zheng J, Calì T, Gai WP, Chen T, Moeller A, Brini M, Fu Y, Halliday G, Brudek T, Aznar S, Pakkenberg B, Andersen JP, Jensen PH. Alpha-synuclein aggregates activate calcium pump SERCA leading to calcium dysregulation. EMBO Rep. 2018 May;19(5):e44617. doi: 10.15252/embr.201744617. Epub 2018 Mar 29. PMID: 29599149; PMCID: PMC5934765.

Kovacs G, Reimer L, Jensen PH. Endoplasmic Reticulum-Based Calcium Dysfunctions in Synucleinopathies. Front Neurol. 2021 Oct 20;12:742625. doi: 10.3389/fneur.2021.742625. PMID: 34744980; PMCID: PMC8563702.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997 Aug 28;388(6645):839-40. doi: 10.1038/42166. PMID: 9278044.

Nuber S, Harmuth F, Kohl Z, Adame A, Trejo M, Schönig K, Zimmermann F, Bauer C, Casadei N, Giel C, Calaminus C, Pichler BJ, Jensen PH, Müller CP, Amato D, Kornhuber J, Teismann P, Yamakado H, Takahashi R, Winkler J, Masliah E, Riess O. A progressive dopaminergic phenotype associated with neurotoxic conversion of α-synuclein in BAC-transgenic rats. Brain. 2013 Feb;136(Pt 2):412-32. doi: 10.1093/brain/aws358. PMID: 23413261; PMCID: PMC3572936.

Krashia P, Cordella A, Nobili A, La Barbera L, Federici M, Leuti A, Campanelli F, Natale G, Marino G, Calabrese V, Vedele F, Ghiglieri V, Picconi B, Di Lazzaro G, Schirinzi T, Sancesario G, Casadei N, Riess O, Bernardini S, Pisani A, Calabresi P, Viscomi MT, Serhan CN, Chiurchiù V, D'Amelio M, Mercuri NB. Blunting neuroinflammation with resolvin D1 prevents early pathology in a rat model of Parkinson's disease. Nat Commun. 2019 Sep 2;10(1):3945. doi: 10.1038/s41467-019-11928-w. Erratum in: Nat Commun. 2019 Oct 14;10(1):4725. PMID: 31477726; PMCID: PMC6718379.

Ledonne A, Massaro Cenere M, Paldino E, D'Angelo V, D'Addario SL, Casadei N, Nobili A, Berretta N, Fusco FR, Ventura R, Sancesario G, Guatteo E, Mercuri NB. Morpho-Functional Changes of Nigral Dopamine Neurons in an α-Synuclein Model of Parkinson's Disease. Mov Disord. 2023 Feb;38(2):256-266. doi: 10.1002/mds.29269. Epub 2022 Nov 9. PMID: 36350188.

Tozzi A, Sciaccaluga M, Loffredo V, Megaro A, Ledonne A, Cardinale A, Federici M, Bellingacci L, Paciotti S, Ferrari E, La Rocca A, Martini A, Mercuri NB, Gardoni F, Picconi B, Ghiglieri V, De Leonibus E, Calabresi P. Dopamine-dependent early synaptic and motor dysfunctions induced by α-synuclein in the nigrostriatal circuit. Brain. 2021 Dec 16;144(11):3477-3491. doi: 10.1093/brain/awab242. PMID: 34297092; PMCID: PMC8677552.

Guatteo E, Carunchio I, Pieri M, Albo F, Canu N, Mercuri NB, Zona C. Altered calcium homeostasis in motor neurons following AMPA receptor but not voltage-dependent calcium channels' activation in a genetic model of amyotrophic lateral sclerosis. Neurobiol Dis. 2007 Oct;28(1):90-100. doi: 10.1016/j.nbd.2007.07.002. Epub 2007 Jul 10. PMID: 17706428.

Cucchiaroni ML, Viscomi MT, Bernardi G, Molinari M, Guatteo E, Mercuri NB. Metabotropic glutamate receptor 1 mediates the electrophysiological and toxic actions of the cycad derivative beta-N-Methylamino-L-alanine on substantia nigra pars compacta DAergic neurons. J Neurosci. 2010 Apr 14;30(15):5176-88. doi: 10.1523/JNEUROSCI.5351-09.2010. PMID: 20392940; PMCID: PMC6632752.

Dunn AR, Stout KA, Ozawa M, Lohr KM, Hoffman CA, Bernstein AI, Li Y, Wang M, Sgobio C, Sastry N, Cai H, Caudle WM, Miller GW (2017) Synaptic vesicle glycoprotein 2C (SV2C) modulates dopamine release and is disrupted in Parkinson disease. Proc Natl Acad Sci USA 114: E2253–E2262.

Pulcrano S, De Gregorio R, De Sanctis C, Volpicelli F, Piscitelli RM, Speranza L, Perrone-Capano C, di Porzio U, Caiazzo M, Martini A, Giacomet C, Medina D, Awatramani R, Viggiano D, Federici M, Mercuri NB, Guatteo E, Bellenchi GC. miR-218 Promotes Dopaminergic Differentiation and Controls Neuron Excitability and Neurotransmitter Release through the Regulation of a Synaptic-Related Genes Network. J Neurosci. 2023 Nov 29;43(48):8104-8125. doi: 10.1523/JNEUROSCI.0431-23.2023. PMID: 37816598; PMCID: PMC10697421.

Meccariello R, Bellenchi GC, Pulcrano S, D'Addario SL, Tafuri D, Mercuri NB, Guatteo E. Neuronal dysfunction and gene modulation by non-coding RNA in Parkinson's disease and synucleinopathies.Neuronal

dysfunction and gene modulation by non-coding RNA in Parkinson's disease and synucleinopathies. Frontiers in Cellular Neuroscience, in press (Accepted Dec 7, 2023)

Schaffner SL, Kobor MS. DNA methylation as a mediator of genetic and environmental influences on Parkinson's disease susceptibility: Impacts of alpha-Synuclein, physical activity, and pesticide exposure on the epigenome.Front Genet. 2022 Aug 19;13:971298. doi: 10.3389/fgene.2022.971298. eCollection 2022.

Lamparelli EP, Marino M, Szychlinska MA, Della Rocca N, Ciardulli MC, Scala P, D'Auria R, Testa A, Viggiano A, Cappello F, Meccariello R, Della Porta G, Santoro A. The Other Side of Plastics: Bioplastic-Based Nanoparticles for Drug Delivery Systems in the Brain. Pharmaceutics. 2023 Oct 28;15(11):2549. doi: 10.3390/pharmaceutics15112549.

Santoro A, Marino M, Vandenberg LN, Szychlinska MA, Lamparelli EP, Scalia F, Della Rocca N, D'Auria R, Pastorino GMG, Della Porta G, Operto FF, Viggiano A, Cappello F, Meccariello R. PLASTAMINATION: outcomes on the central nervous system and reproduction Curr. Neuropharmacol. manuscript under the second round of revision

Meccariello R, Santoro A, D'Angelo S, Morrone R, Fasano S, Viggiano A, Pierantoni R. The Epigenetics of the Endocannabinoid System. Int J Mol Sci. 2020 Feb 7;21(3):1113. doi: 10.3390/ijms21031113.

Chianese R, Coccurello R, Viggiano A, Scafuro M, Fiore M, Coppola G, Operto FF, Fasano S, Laye S, Pierantoni R, Meccariello R. Impact of Dietary Fats on Brain Functions. Curr Neuropharmacol. 2018;16(7):1059-1085. doi: 10.2174/1570159X15666171017102547.

Kelly R, Bemelmans AP, Joséphine C, Brouillet E, McKernan DP, Dowd E. Time-Course of Alterations in the Endocannabinoid System after Viral-Mediated Overexpression of α-Synuclein in the Rat Brain. Molecules. 2022 Jan 14;27(2):507. doi: 10.3390/molecules27020507.

Guatteo E, Bengtson CP, Bernardi G, Mercuri NB. Voltage-gated calcium channels mediate intracellular calcium increase in weaver dopaminergic neurons during stimulation of D2 and GABAB receptors. J Neurophysiol. 2004 Dec;92(6):3368-74. doi: 10.1152/jn.00602.2004. Epub 2004 Jul 7. PMID: 15240766.

Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem. 1985 Mar 25;260(6):3440-50. PMID: 3838314.

Marino M, D'Auria R, Mele E, Pastorino GMG, Di Pietro P, D'Angelo S, Della Rocca N, Operto FF, Vecchione C, Fasano S, Pierantoni R, Viggiano A, Meccariello R, Santoro A. The interplay between kisspeptin and endocannabinoid systems modulates male hypothalamic and gonadic control of reproduction *in vivo*. Front Endocrinol (Lausanne). 2023 Oct 12;14:1269334. doi: 10.3389/fendo.2023.1269334. PMID: 37900144; PMCID: PMC10602894.

ALLEGATO C

TABELLA COSTI PERSONALE STANDARD				COSTO DEL PERSONALE
FASCIA DI COSTO /LIVELLO	**NUMERO SOGGETTI**	**COSTO ORARIO vedi nota**	**MONTE ORE**	
Basso	**0**			- €
Medio	**1**	48 €	275	13.200 €
Alto	**1**	73 €	230	16.790 €
TOTALI	**2**		**505**	**29.990 €**

COSTO ORARIO: si deve far riferimento al Decreto Interministeriale n. 116 del 24/1/2018

ALLEGATO C

BUDGET DI PROGETTO	COSTO DEL PERSONALE	OVERHEAD	Costi per servizi di Consulenza Specialistica	Costi per licenze direttamente imputabili al progetto	Costi per materiali e attrezzature direttamente imputabili al progetto	Costi per altre tipologie di spese direttamente imputabili al progetto	COSTO TOTALE
	29.990 €	4.498,50 €			40.000,00 €	75.511,50 €	150.000,00 €