Plan Overview

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Title: Interindividual and sex differences in vulnerability to developing depression due to stress: The bidirectional role of the gut-brain axis (GUT-BRAINinDEPRE).

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Project abstract:

The aims of this project fall within the research-oriented modality and address depression as a major public health challenge, in accordance with the thematic priorities of the State Plan for Scientific and Technical Research and Innovation (2021–2023). Depression is a highly prevalent mental disorder whose burden has increased substantially, particularly following the COVID-19 pandemic (1). It profoundly affects daily functioning, interfering with the ability to work, sleep, study, eat, and enjoy life, and is associated with an increased risk of physical comorbidities, including cardiovascular and metabolic diseases. From an aetiological perspective, stress, particularly psychosocial stress, is a well-established risk factor for depression. However, the neurobiological mechanisms underlying stress-induced depressive disorders remain incompletely understood (2).

Building on previous research investigating the bidirectional interactions between stress-induced microglial priming and neurogenic dysfunction, the present project seeks to advance knowledge of the neurobiological mechanisms underlying stress-induced depression, particularly the bidirectional communication along the microbiota–gut–brain axis, with special emphasis on sex-related and interindividual differences. Depression is more prevalent in women (6%) than in men (4%) (1). Furthermore, not all individuals exposed to stress develop depression, reflecting substantial variability in stress susceptibility and resilience. Identifying the neurobiological mechanisms underlying these differences could contribute to the development of more targeted preventive and therapeutic strategies, improved diagnostic approaches, and enhanced prediction of depression risk through the integration of molecular, social, environmental, and behavioural data.

The long-term objective of this research is to identify novel biomarkers of vulnerability to depression and develop new strategies for its prevention and treatment, particularly in the context of psychosocial stress.

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Interindividual and sex differences in vulnerability to developing depression due to stress: The bidirectional role of the gut-brain axis (GUT-BRAINinDEPRE).

Data Collection

What data will you collect or create?

The project will generate experimental data from preclinical mouse models investigating the bidirectional interactions between the gut microbiota and the brain in response to social defeat stress (SDS), with particular emphasis on sex differences and individual susceptibility or resilience to stress.

Experimental groups will include:

- **SDS experiments (males and females):** Control and SDS-exposed mice. Stressed animals will also be classified as resilient (SDS-R) or susceptible (SDS-S) according to their behavioural responses.
- **Microbiota manipulation experiments (males):** Germ-free mice, animals subjected to antibiotic-induced microbiota depletion, and recipients of faecal microbiota transplantation (FMT) from control or SDS-exposed donors, including resilient and susceptible profiles.
- **Neurogenesis manipulation experiments (males and females):** Control and SDS-exposed mice, with or without temozolomide (TMZ) administration, to investigate the contribution of adult hippocampal neurogenesis-associated cell proliferation to stress-related alterations.
- **The main datasets will include:**
- **Behavioural data:** Social interaction, exploratory behaviour and saccharin preference.
- **Physiological and hormonal data:** Body weight, food and water intake, organ weights and circulating corticosterone concentrations.
- **Neurobiological data:** Hippocampal neurogenesis, cell proliferation and survival, microglial density and morphology, and selected molecular and inflammatory markers.
- **Gut microbiota data:** Bacterial taxonomic composition, relative abundance and microbial diversity obtained through 16S rRNA gene sequencing.
- **Intestinal barrier data:** Intestinal permeability and tight junction protein expression, including occludin and claudin-4 (analyses ongoing).
- **Statistical datasets:** Group comparisons, sex-related differences, susceptibility and resilience profiles, and exploratory mediation analyses integrating behavioural, neurobiological and microbiota variables.

The project will produce raw and processed experimental datasets, behavioural recordings, microscopy images, sequencing data, quantitative measurements, statistical outputs and associated experimental metadata.

How will the data be collected or created?

Behavioural tests:

Assessment of hedonic behaviour using the saccharin preference test (SPT), social behaviour using the social interaction test (SIT), and exploratory activity. Behavioural evaluations will be conducted in control and SDS-exposed mice of both sexes, as well as in animals subjected to microbiota manipulation or TMZ treatment.

Immunohistochemistry and immunofluorescence:

Assessment of hippocampal neurogenesis (DCX), cell proliferation and survival (BrdU, IdU and CldU), and microglial density and morphology (Iba1), using light and fluorescence microscopy.

Cell quantification and morphological analyses:

Quantification of hippocampal cell populations and microglial morphological parameters using image analysis and stereological software.

Gut microbiota analyses:

Faecal samples were collected for bacterial DNA extraction and quality assessment. Validated DNA samples were aliquoted in 40 µL of DNase-free sterile water and shipped at room temperature to Novogene Europe (Cambridge, United Kingdom). The V3–V4 regions of the 16S rRNA gene were sequenced using primers 341F and 805R on the Illumina MiSeq platform (2 × 300 bp paired-end sequencing). Bioinformatic analyses were conducted to assess microbial diversity, taxonomic composition and relative abundance.

Intestinal permeability and barrier integrity (ongoing):

Intestinal permeability is being assessed by oral administration of FITC-dextran (FD4), followed by fluorescence measurements in blood samples. Intestinal tissue samples are also being analysed to evaluate tight junction proteins, including occludin and claudin-4.

Molecular studies:

Assessment of selected neuroinflammatory markers and hippocampal proteins using immunoassays, Western blotting and other molecular techniques, as applicable.

Hormonal determinations:

Measurement of corticosterone concentrations in blood samples using commercially available enzyme immunoassay kits.

Experimental microbiota and neurogenesis manipulation:

Data were generated using germ-free and antibiotic-treated animals and faecal microbiota transplantation (FMT). Additional experiments involving pharmacological inhibition of cell proliferation using TMZ have been conducted in male and female mice, with subsequent neurobiological and intestinal analyses ongoing.

Statistical analyses:

Differences between experimental groups will be analysed using Student's *t*-tests, one-way and factorial analysis of variance (ANOVA), as appropriate to the experimental design. Repeated-measures ANOVA will be used for longitudinal measurements, and appropriate post hoc comparisons will be performed when justified. Sex-dependent effects and interactions between stress exposure and experimental treatments will also be examined. Additionally, K-means clustering will be used to identify stress susceptibility and resilience profiles, while exploratory mediation analyses will assess potential relationships among gut microbiota composition, hippocampal neurogenesis, microglial alterations and behavioural outcomes.

Documentation and Metadata

What documentation and metadata will accompany the data?

- Videos from animals' behavioural tests.
- Microphotographs taken from immunohistochemistry and confocal studies. Scanned hippocampal images for microglia studies.
- Documents and metadata related to experimental protocols, animal characteristics and experimental groups, sample collection and processing, gut microbiota sequencing (16S rRNA), behavioural and neurobiological assessments, intestinal permeability measurements, and statistical analyses
- All the data collected from behavioural analyses, immunocytochemical studies, cellular quantifications and molecular studies will be organised in Excel data sheets, separated in experimental groups (control; Social Defeat Stress) and organized by sex and individual differences (SDS-R; SDS-S).
- Research data alongside files generated from analysis (SPPS / R/ Excel) will be stored for 10 years from the completion date of the studies at the University of Malaga, within a password protected server (GoogleDrive) and y two separated external hard disk. Estimates that the volume of data will exceed 2 TB.

Ethics and Legal Compliance

How will you manage any ethical issues?

All procedures follow the current regulations on the use of animals in research and have been approved by the Ethics Committee of the University of Malaga (CEUMA 85-2025-A) and by the Directorate General for Agricultural and Livestock Production of the Andalusian Regional Government (01/06/2026/112 Registered in the ALURES platform under identification number NTS-ES-701062,). All persons involved in the project who will be working with research animals have the relevant accreditations to do so.

This project involves no personal or sensitive data. All experiments comply with ethical regulations and were approved by the University of Málaga (CEUMA 85-2025-A) and the Regional Government of Andalusia. Although not required, data protection principles are observed.

How will you manage copyright and Intellectual Property Rights (IPR) issues?

The data generated under this project will be owned by the University of Málaga. The Agencia Estatal de Investigación (AEI), Ministerio de Ciencia e Innovación (Spain), which funds the project (PID2023-151537OB-I00 /10.13039/501100011033), will be acknowledged in all scientific outputs derived from the project.

Any results with potential for intellectual property protection will be safeguarded through appropriate patent or licensing mechanisms. When results are

published in scientific journals, they will be subject to licensing or editorial policy restrictions.

Data sharing will require the data use agreement from the Principal Investigators (Dr. Carmen Pedraza, mdpedraza@uma.es; Dr. Margarita Pérez

(marper@uma.es) and the investigators who have participated in the collection and/or processing of the data concerned.

Storage and Backup

How will the data be stored and backed up during the research?

All data will be stored in the cloud (Google Drive). Simultaneously a backup of the data will be stored in two hard drives kept in different (guarded by the two
principal investigators of the project)
All data is stored on Google Drive and backed up weekly onto two external hard drives kept in secure locations by the PIs. Video, image, and spreadsheet data are protected via restricted access and passwords. The Google Drive account used is managed through our institutional account at the University of Málaga.

How will you manage access and security?

The access to the cloud data depends on sharing authorization and password, which will be available only to authorized people with research purposes only.

Selection and Preservation

Which data are of long-term value and should be retained, shared, and/or preserved?

All the research data will be kept for 10 years in our servers and in the cloud (GoogleDrive).
All research data will be preserved for a minimum of 10 years. Non-essential data (e.g., pilot images) may be excluded. Datasets will be made publicly
available in RIUMA following publication.

What is the long-term preservation plan for the dataset?

All the research data will be kept for 10 years.

Data Sharing

How will you share the data?

All data resulting from the development of this project will be available in scientific communications presented at conferences and in manuscripts that will be published in peer-reviewed scientific journals. As far as possible, they will be open access or, once the data have been presented, they will be made availablebto the public through the institutional repository (RIUMA). In addition, they will be disseminated to society through the participation of research and work teams in science dissemination activities.
This project aligns with the Open Science principles promoted by the AEI and Horizon Europe, ensuring data will be as open as possible, as closed as necessary[CP1] .

- Proteomic dataset 1: https://dx.doi.org/10.24310/riuma.26238

- Proteomic dataset 2: https://dx.doi.org/10.24310/riuma.26229

- Deposited publications:

1. Sex-specific hippocampal trajectories induced by peripubertal and adult acute stress: proteomic profile, microglial alterations, neurogenic dysregulation, and depression-like behaviors.
RIUMA https://hdl.handle.net/10630/48091
Data sharing: https://hdl.handle.net/10630/46075

2. From Juvenile Stress to Adult Depressive-Like Behavior: A Systematic Review of Biomarkers, Developmental Timing, and Sex Differences.
RIUMA https://hdl.handle.net/10630/46554

3. Differential effects of acute, chronic, and social defeat stress on microglial morphology in the basolateral and central amygdala, and the medial and lateral habenula.
RIUMA: https://hdl.handle.net/10630/47884
Data sharing:
https://static-content.springer.com/esm/art%3A10.1007%2Fs00429-026-03172-y/MediaObjects/429_2026_3172_MOESM1_ESM.pdf
https://static-content.springer.com/esm/art%3A10.1007%2Fs00429-026-03172-y/MediaObjects/429_2026_3172_MOESM2_ESM.pdf
https://static-content.springer.com/esm/art%3A10.1007%2Fs00429-026-03172-y/MediaObjects/429_2026_3172_MOESM3_ESM.docx
Please note that the RIUMA links provided correspond to deposited publications and proteomic datasets. Additionally, the microglial data are stored in a password-protected Google Drive folder. Access credentials are available upon request.

Data will be available post-publication through open-access repositories, under embargo beforehand. Requests require PI approval and a data use agreement.

Are any restrictions on data sharing required?

The use of the data may only be used for scientific purposes or for the dissemination of science.

Responsibilities and Resources

Who will be responsible for data management?

The project coordinators (Carmen Pedraza and Margarita Pérez-Martín)
Data management is overseen by the PIs, Carmen Pedraza and Margarita Pérez-Martín. The University of Málaga provides IT and data services to ensure secure storage, access, and long-term preservation.

What resources will you require to deliver your plan?

The IT services and general library of the University of Malaga currently have all the necessary resources to meet the requirements of this research data management plan.