

# **DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO**

**42° Cycle**

**Project proposal for a PhD scholarship (with no financial support from Sapienza)**

**Title of the research:** *Molecular and neuro-glial mechanisms of stress-induced resilience to adolescent alcohol exposure: role for inositols in modulating cognitive dysfunction*

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**Tutor (internal to the Teachers' Board if supervisor belongs to an external Institution; include E-mail):** **to be identified**

**Host Institution:** Instituto de Investigación Biomedica de Málaga, IBIMA-Plataforma Bionand/ Università di Roma Sapienza

## **Summary (max 500 words)**

Adolescence represents a critical developmental period during which the brain is particularly vulnerable to environmental insults such as alcohol exposure and stress. Ethanol consumption during this phase has been shown to induce long-lasting alterations in synaptic plasticity, neuroinflammation, and intracellular signalling, ultimately leading to persistent cognitive deficits, especially affecting memory, as well as inducing persistent anxiety-like behaviors. In addition, there is a sex-dependent response to the harmful effects of alcohol, being the female animals capable of coping with alcohol-imposed cognitive deficits in a more efficient way, although the anxiety response was similar in both sexes. Recent findings by Verheul-Campos et al. demonstrated that in male animals, adolescent alcohol exposure causes extensive remodeling of the hippocampal phosphoproteome, involving key signaling pathways and transcriptomic machinery. Notably, prior exposure to stress was found to attenuate or even reverse both molecular and behavioral impairments, suggesting the activation of adaptive resilience mechanisms. Females showed a partial protection from alcohol thanks to a sex-dependent remodelling of mitochondrial metabolic machinery. Despite these findings, the underlying cellular and molecular processes involved in resilience to alcohol effect remain largely unclear.

This project aims to investigate the mechanisms through which stress modulates the long-term effects of adolescent alcohol exposure in a sex-dependent manner, with a particular focus on neuro-glial interactions, intracellular signaling, and epigenetic regulation. In addition, the project will explore the potential role of the natural inositols D-Pinitol (DPIN) and D-chiro-inositol (DCI) in modulating these processes and promoting resilience. Both DPIN and DCI have a profound impact on oxidative stress, neuroinflammation and neurodegeneration thanks to its action on the insulin signaling pathway and the modulation of CDK5 kinase, conferring them a neuroprotective profile in neurodegeneration.

The project will initially focus on the establishment and extension of an in vivo model of adolescent alcohol exposure. In order to enhance ethanol intake and better mimic voluntary consumption patterns, animals (adolescent male and females) will be exposed to a

combined ethanol–fructose paradigm, which has been shown to promote increased alcohol consumption and resulted in neuroinflammation and cognitive impairment. In addition to the hippocampus, which represents a well-established target region in this context, the study will extend to the prefrontal cortex, a key brain area involved in executive functions and cognitive control, and the amygdala, a key area for emotional behavior, in order to determine whether alcohol-induced alterations and stress-mediated modulation are conserved across different neural circuits.

In parallel, a comprehensive in vitro approach will be implemented to investigate the cellular and molecular mechanisms underlying these phenomena. Human-derived astrocytes and microglial cells will be employed as primary models to study glial reactivity and neuroinflammatory responses under controlled experimental conditions. Cells will be exposed to low concentrations of ethanol to mimic chronic exposure, as well as to pro-inflammatory stimuli designed to reproduce stress-related conditions comparable to those induced by social defeat in vivo. Both single cultures and co-culture systems will be established in order to evaluate the bidirectional interactions between astrocytes and microglia, which are known to play a central role in the regulation of neuroinflammation and synaptic function. Subsequently, neuronal components will be incorporated, including hippocampal and cortical neurons, allowing the development of more complex co-culture systems in which neuron–glia interactions can be directly assessed. This model will allow to study the effects of DPIN and DCI as potential modifiers of alcohol-induced harms.

In an initial phase, analyses will be performed across both experimental paradigms, assessing the expression of pro-inflammatory cytokines and key proteins involved in inflammatory pathways, including TLR4, calcium-dependent signaling (Ca<sup>2+</sup>), insulin, and endocannabinoid systems.

Subsequently, once all in vivo and in vitro samples have been collected, advanced high-throughput analyses will be conducted, including phosphoproteomics, proteomics, metabolomics, and epigenetic profiling, with the aim of identifying convergent molecular signatures, in order to characterise the molecular mechanisms underlying neuroinflammation and memory deficits induced by alcohol exposure and reversed by stress. In vitro studies will help to determine whether DPIN and/or DCI might be potentially beneficial for protecting against alcohol-induced damage.

### **Pertinent Publications of the proponent (last 5 years)**

- ◆ Medina-Vera, D., Navarro, J.A., Tovar, R., Rosell-Valle, C., Gutiérrez-Adán, A., Ledesma, J.C., Sanjuan, C., Pavón, F.J., Baixeras, E., **Rodríguez De Fonseca, F.**, Decara, J., **2021**. Activation of PI3K/Akt Signaling Pathway in Rat Hypothalamus Induced by an Acute Oral Administration of D-Pinitol. *Nutrients* 13, 2268. <https://doi.org/10.3390/nu13072268>
- ◆ Medina-Vera, D., Navarro, J.A., Rivera, P., Rosell-Valle, C., Gutiérrez-Adán, A., Sanjuan, C., López-Gamero, A.J., Tovar, R., Suárez, J., Pavón, F.J., Baixeras, E., Decara, J., **Rodríguez De Fonseca, F.**, **2022**. D-Pinitol promotes tau dephosphorylation through a cyclin-dependent kinase 5 regulation mechanism: A new potential approach for tauopathies? *Br. J. Pharmacol.* 179, 4655–4672. <https://doi.org/10.1111/bph.15907>
- ◆ Medina-Vera, D., López-Gamero, A.J., Verheul-Campos, J., Navarro, J.A., Morelli, L., Galeano, P., Suárez, J., Sanjuan, C., Pacheco-Sánchez, B., Rivera, P., Pavon-Morón, F.J., Rosell-Valle, C., **Fonseca, F.R.D.**, **2024**. Therapeutic Efficacy of the Inositol D-Pinitol as a Multi-Faceted Disease Modifier in the 5×FAD Humanized Mouse Model of Alzheimer’s Amyloidosis. *Nutrients* 16, 4186. <https://doi.org/10.3390/nu16234186>

- ◆ Medina-Vera, D., Martín-Chaves, L., Sánchez-Marín, L., Díaz-Ottaviano, M., Gavito, A.L., Popova, O., Sánchez-Quintero, M.J., Rodríguez-Capitán, J., **Rodríguez De Fonseca, F.**, Jiménez-Navarro, M.F., Serrano, A., Pavón-Morón, F.J., **2025**. “Maladaptive stress-coping behavior in CX3CR1-deficient mice: Impact of adolescent stress and alcohol exposure on neuroimmune responses and inflammation.” *Neuropharmacology* 275, 110503. <https://doi.org/10.1016/j.neuropharm.2025.110503>
  
- ◆ Pacheco-Sánchez, B., Verheul-Campos, J., Vargas, A., Tovar, R., Rodríguez-Pozo, M., Navarro, J.A., López-Gamero, A.J., Baixeras, E., Serrano-Castro, P.J., Suárez, J., Sanjuan, C., Rivera, P., **Rodríguez De Fonseca, F.**, **2025**. Early Oral Administration of D-Chiro- Inositol Reverses Hippocampal Insulin and Glutamate Signaling Deficits in the 3×Tg Humanized Mouse Model of Alzheimer’s Disease. *Nutrients* 17, 3024. <https://doi.org/10.3390/nu17183024>
  
- ◆ Serrano, M., Saumell-Esnaola, M., Ocerin, G., García Del Caño, G., Soria-Gómez, E., Mimenza, A., Puente, N., Bonilla-Del Río, I., Ramos-Uriarte, A., Reguero, L., Christie, B.R., **Rodríguez De Fonseca, F.**, Rodríguez-Arias, M., Gerrikagoitia, I., Grandes, P., **2025**. Omega-3 Fatty Acids Mitigate Long-Lasting Disruption of the Endocannabinoid System in the Adult Mouse Hippocampus Following Adolescent Binge Drinking. *Int. J. Mol. Sci.* 26, 5507. <https://doi.org/10.3390/ijms26125507>
  
- ◆ Verheul-Campos, J., Sanchez-Marín, L., López, Y., Gavito, A.L., Grandes, P., Serrano, P., Guericagoitia, I., Estivill-Torrús, G., Rodríguez-Arias, M., Miñarro, J., Pavón-Morón, F.J., Suárez, J., Serrano, A., **De Fonseca, F.R.**, **2025**. Prior restraint stress counteracts memory deficits associated with adolescent alcohol exposure by targeting both the hippocampal endocannabinoid and glutamatergic systems. *Drug Alcohol Depend.* 276, 112891. <https://doi.org/10.1016/j.drugalcdep.2025.112891>