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International PhD PPU-IMAGINE Proposal

Call 2023/2024

PhD PROPOSAL IDENTIFICATION

PhD Project title	Reelin in synaptic plasticity and autism
Project Acronym	ReelinASD
Project Keywords	Autism Reelin synapse plasticity behavior

LABORATORY PRESENTATION

Laboratory Team Name	Genetic and development of cerebral cortex
Laboratory Director full name	PIERANI Alessandra
Department IP	NA
Doctoral school	ED562
University	University Paris Cité
Laboratory Telephone	(014) 275-4567
Laboratory website	https://www.institutimagine.org/en/alessandra-pierani-25

PhD PROPOSAL

PhD Supervisor full name	Billuart Pierre
PhD Supervisor position	INSERM CRHC
PhD Supervisor email	pierre.billuart@inserm.fr
Number of ongoing PhD students supervised by the Supervisor	0

PhD Proposal abstract (1000 characters maximum)

Autism spectrum disorders (ASD) include a heterogeneous group of pervasive developmental disorders characterised by alterations in communication, socialisation, and by the presence of repetitive behaviours. ASD has a strong genetic component and hundreds of risk genes have been associated with the disease. Multiple genes encoding synaptic proteins have been found mutated in ASD and synaptopathy has emerged as one of the major pathophysiological mechanisms involved in the disease. Mutations or deletions in Reelin gene (Reln) have been associated not only with cortical malformations (MCD) but also with ASD, schizophrenia and epilepsy. In mouse, reduction of Reln expression in the heterozygous Reeler mutant (HRM) lead to decreased synaptic plasticity together with altered behavior. Our project relies on structure/function analyses of human Reln variants in mouse by in vivo complementation experiments in

order to better understand the pathophysiology of the synaptopathies.

PhD Proposal (10 000 characters maximum)

Reelin (Reln) is a secreted protein well known for its function in neuronal migration during cerebral cortex development. Its loss of function in mice is responsible for the Reeler phenotype characterized by cerebellar atrophy and laminar disorganization in the cerebral cortex. In human, Reln mutations or gene deletion have been reported in patients with cortical malformations (MCDs: lissencephaly, pachygyria…), Autism Spectrum Disorder (ASD), temporal lobe epilepsy and also schizophrenia [1]. Beside its function in the prenatal brain, Reln plays important roles in the postnatal and adult cortex, promoting the maturation of dendrites, synaptogenesis, synaptic transmission and plasticity, thus modulating the formation and function of synaptic circuits [2]. These postnatal roles are also associated to a switch in gene expression from Cajal-Retzius cells (CRs) to inhibitory neurons. The CRs population is transitory and a subtype is known to completely disappear in the first two postnatal weeks in the mouse cortex by apoptosis in an activity-dependent manner [3,4]. The kinetic of CRs elimination is delayed in the hippocampus until adulthood [5]. By genetically maintaining them alive beyond these temporal windows, we found that CRs survival is associated with an increased sensitivity to kainate-induced seizures [5,6] together with enhanced dendritic spines of cortical and hippocampal pyramidal neurons. Whether these non-cell autonomous modifications are due to neurotransmission or paracrine effects remains unknown.

Heterozygous Reeler mutation (HRM) does not alter cortical lamination nor the cerebellum but leads to alterations in behavior (Paired Pulse Inhibition and Cued Fear Conditioning) [7]) and in synaptic plasticity [8], a common phenotype found in ASD patients with various genetic conditions. In addition, rare variants in the Reln gene have been described in patients with autism [1]. The most damaging variants according to bioinformatic predictions are all concentrated in the central domain important for binding to its receptors, VLDLR and ApoER2. Previous work showed that Reln enhances LTP in hippocampal slices, an effect dependent on both VLDLR and ApoER2 [9]. Therefore, we expect that Reln variants in ASD alter binding to its receptors and lead to enhancement or suppression of LTP. We have recently worked with functionally modeling Reln variants in patients with MCDs using in vitro and in vivo models [10].

Aim1: Consequences of Autism associated Reelin mutations on synaptic plasticity

In this context we will first study whether some of these mutations in the central domain also have effects on receptors bindings, on synaptic plasticity and model the ones found in ASD [1] (and unpublished data from Thomas Bourgeron (Coll.), Institut Pasteur). To do so, we will concentrate supernatants from cell culture expressing Reln variants and incubate hippocampal slices with those to monitor the LTP enhancement. In addition to these in vitro experiments, we will implement canulations in hippocampus and perfuse HRM with these supernatants to rescue or not the synaptic plasticity and learning deficits observed in those animals. The HRM mouse line is already available in our animal facility and the biochemistry facility at the IPNP Institute will produce the supernatants from transfected cell lines with Reln mutant plasmids. Canular implantations in these animals will be done under stereotaxic injection to perfuse the supernatant in adults.

Aim2: Contribution of Cajal-Retzius cells and inhibitory neurons to synaptic plasticity in hippocampus

In parallel we will implement a new conditional model by introducing a floxed allele of Reln in HRM and induce in postnatal the loss of the second allele. We expect to enhance the HRM phenotypes associated to developmental alterations by removing the second allele in juvenile or adult stages using tamoxifen injection in the Cre-ERT2 recombinase background. Since there is a time dependent switch of Reln expression after birth, this model will allow to study the contribution of CRs and interneurons to the Reln postnatal functions. Alternatively, we will perform stereotaxic injections of AAV-Cre virus in the hippocampus of HRM/lox mice using specific promoters to target one cell type or the other.

Regardless of the two aims and thanks to the animal (Coll. Stephanie Moriceau) and electrophysiological (Coll. Sorana Curia) facilities at Imagine, we will be able to analyze both the

behavior and the synaptic plasticity in treated animals between 3 to 5 months Hippocampal slices will be recovered from controls and treated mice to record TBS (Theta-Burst Stimulation) induced LTP from CA1. Finally, we will inject biocytin in pyramidal neurons to reconstruct their neuronal morphologies.
Altogether, this project will bring new knowledge on postnatal Reln functions in various cell types and shed light on how various Reln mutations lead to distinct neurodevelopmental disorders (NDDs).

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Laboratory's best 5 publications

Moreau, Matthieu X., Yoann Saillour, Vicente Elorriaga, Benoît Bouloudi, Elodie Delberghe, Tanya Deutsch Guerrero, Amaia Ochandorena-Saa, et al. « Repurposing of the Multiciliation Gene Regulatory Network in Fate Specification of Cajal-Retzius Neurons ». Developmental Cell 58, no 15 (7 août 2023): 1365-1382.e6.

Riva, Martina, Stéphanie Moriceau, Annunziato Morabito, Elena Dossi, Candela Sanchez-Bellot, Patrick Azzam, Andrea Navas-Olive, et al. « Aberrant Survival of Hippocampal Cajal-Retzius Cells Leads to Memory Deficits, Gamma Rhythmopathies and Susceptibility to Seizures in Adult Mice ». Nature Communications 14, no 1 (18 mars 2023): 1531. https://doi.org/10.1038/s41467-023-37249-7. Ramezanidoraki, Nasim, Driss Ouardi, Margaux Le, Stéphanie Moriceau, Mahboubeh Ahmadi, Elena Dossi, Danae Rolland, et al. « Activation of the PI3K/AKT/MTOR Pathway in Cajal–Retzius Cells Leads to Their Survival and Increases Susceptibility to Kainate-Induced Seizures ». International Journal of Molecular Sciences 24, no 6 (11 mars 2023): 5376. https://doi.org/10.3390/ijms24065376. Riva, Martina, Sofia Ferreira, Vera P. Medvedeva, Frédéric Causeret, Olivia J. Henry, Charles-Joris Roux, Céline Bellesme, et al. « Functional Characterization of RELN Missense Mutations Involved in Recessive and Dominant Forms of Neuronal Migration Disorders ». Preprint. Neuroscience, 25 mai 2021. https://doi.org/10.1101/2021.05.25.445586. Riva, Martina, Ioana Genescu, Chloé Habermacher, David Orduz, Fanny Ledonne, Filippo M Rijli, Guillermina López-Bendito, et al. « Activity-Dependent Death of Transient Cajal-Retzius Neurons Is Required for Functional Cortical Wiring ». ELife 8 (31 décembre 2019): e50503. https://doi.org/10.7554/eLife.50503.

Expected profile of the candidate

Expertises in mouse genetics, stereotaxic surgery, electrophysiology and behavioral studies.