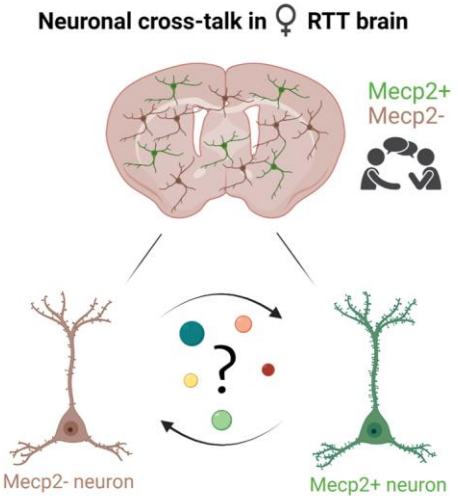
Study of cell-to-cell crosstalk in Rett syndrome: how *Mecp2* mutated neurons influence adjacent wild-type ones

Mutation in the X-linked *MECP2* gene cause a variety of neurological disorders, including Rett syndrome (RTT), a severe neurodevelopmental disorder characterized by progressive loss of motor, verbal, cognitive and social skills, and the development of breathing abnormalities, seizures and stereotypes (Katz et a., 2016).
MECP2 encodes for the transcriptional regulator methyl-CpG-binding protein 2 (MeCP2), which is ubiquitously expressed but it reaches the highest levels in neurons, which represent the cell population mainly affected in RTT. Indeed, RTT neurons present reduced soma size, less complex dendritic branching and morphological and functional synaptic alterations.
RTT patients are predominantly females, which are heterozygous for *MECP2* mutations. Due to X chromosome inactivation, they display cellular mosaicism, characterized by a mixture of cells expressing the *MECP2* mutant allele ($Mecp2^-$) and those expressing the wild-type allele ($Mecp2^+$), the ratio of which can alter RTT phenotypes. Thus, in the heterozygous brain, neurons expressing the mutant or the wild-type *MECP2* allele are in a rich cross-talk to each other. Considering that *MECP2* mutations have an impact not only on cells expressing the mutant allele, but also on adjacent cells expressing the wild-type one, the RTT phenotypes result from both cell-autonomous and non-cell-autonomous disruptions. By molecular profiling mosaic neurons from *Mecp2* heterozygous female mice, it has been reported a transcriptional deregulation of genes in $Mecp2^+$ neurons that in part overlaps with that of $Mecp2^-$ neurons, revealing non-cell autonomous changes (Johnson et al., 2018). Accordingly, dendritic spine density and width are similarly affected in $Mecp2^-$ and $Mecp2^+$ neurons, and soma size of $Mecp2^+$ neurons in the heterozygous brain is reduced compared to neurons from WT brain (Belichenko et al., 2009). In addition, electrophysiological parameters are under the influence of cell autonomous and non-cell autonomous mechanisms as well (Asgarihafshejani et al., 2019).
Although collectively all these evidences point to the occurrence of non-cell autonomous mechanisms between $Mecp2^+$ and $Mecp2^-$ neurons, to date the identity of the molecules involved in the communication between $Mecp2^+$ and $Mecp2^-$ neurons has been only marginally investigated.
Our preliminary data demonstrate that $Mecp2^-$ neurons exert detrimental effects on synapses of WT neurons by secreted molecules, suggesting the release of synaptotoxic factors and/or the lack of beneficial ones. The characterization of the putative molecules secreted by RTT neurons might highlight novel therapeutic targets for this disorder, for which a cure is still lacking.
This project aims to fill this gap of knowledge by studying the mechanisms by which *Mecp2* null neurons affect WT ones, with the final goal to uncover and validate the role of the molecules and pathways involved. The PhD student will conduct the majority of experiments on primary neuronal cultures prepared from transgenic animal models of RTT, and he/she will characterize the defects in WT neurons caused by molecules secreted by null neurons. Molecular biology techniques, combined with electrophysiological and imaging approaches will provide a complete overview of the induced phenotypes. Transcriptomic and metabolomics approaches will address the molecules by which *Mecp2* null neurons affect WT cells, and by bioinformatics tools the student will select a panel of putative molecules. The relevance of the identified molecule will be tested in the RTT animal model, by studying its expression in the brain of heterozygous mice along development. Finally, by using genetic (lentivirus expressing or silencing the genes of interest) and/or pharmacological (agonist or antagonist for the target pathways) tools, he/she will perform additional experiments to validate the role of the identified molecules and pathways.



- *To characterize the defects caused by Mecp2 null neurons on healthy ones*
- *To identify by -omic strategies the molecules released by Mecp2 null neurons and possibly involved in the occurrence of defects in wild-type neurons*
- *To validate by genetic and/or pharmacological tools the role of selected molecules*