REPORT CATEGORY 1A: Visit by the applicant to another laboratory

Applicant: Maria Laura BERTOLDI

Project: Contribution of MeCP2 and synaptic activity in hippocampal structural plasticity

Host Laboratory: Laboratory for Neurometabolism,
Center for Metabolism and Obesity Research (CMOR)
The Johns Hopkins University School of Medicine
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Methyl citosine binding protein-2 (MeCP2) is a chromosomal structural protein involved in the regulation of gene expression. Alterations in the levels of MeCP2 have been linked to neurodevelopmental disorders (Rett Syndrome) and learning disorders suggesting that this protein plays an important role in neurodevelopment (Chahrour et. al 2007, Smrt et. al 2007). Recent work indicates that the deletion of MeCP2 in adult animals also plays neurologic defects suggesting that MeCP2 could play a role in the maintenance of synaptic connections in the adult nervous system (McGraw et.al 2011).

The hypothesis of my Ph.D. thesis is that the lack of MeCP2 alters the expression of molecules involved in the formation and maturation of circuits, generating long-term defects in synaptic structure and function. These defects constitute the neurobiological basis of some of the behavioural alterations present in these disorders. In particular, focussing in the hippocampal circuitry, we aim to understand how defects generated by the lack of MeCP2 alter the function of the circuit to cause failures in synaptic function. In this context, the proposed aim to develop during my short visit was to define mechanisms by which the lack of MeCP2 interferes with processes of activity-dependent structural plasticity. The **specific aims** to develop were:

1. To analyze the response of MeCP2 mutant animals to paradigms of activity (seizures induced by kainic acid administration) on the refinement of hippocampal circuit mossy fibers-CA3 (IPT), including the processes of adult neurogenesis and axonal growth.

2. To examine how synaptic activity in the absence of MeCP2, affects the expression of genes involved in connectivity, particularly members of the class 3 semaphorin family and neurotrophins (i.e. BDNF).

My studies were focused in analyzing the infrapiramidal tract (IPT) in the hippocampus. The IPT is formed by granule cell axons (mossy fibers) that travel below the pyramidal cell layer of CA3 and synapse on the basal dendrites of CA3 pyramidal neurons (Fig. 1). In the adult hippocampus, the IPT suffers dynamic changes in size in response to neuronal activity.

These processes together with adult neurogenesis constitute important events for pre-synaptic structural plasticity of the hippocampus.

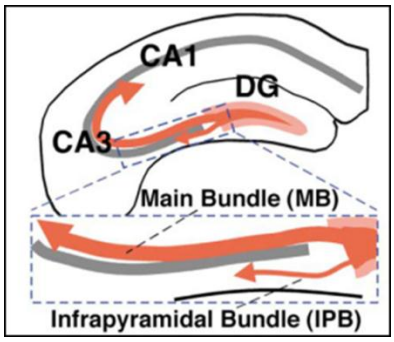

FIGURE 1: Schematic diagram of the two bundles that comprise the mossy fiber pathway. The main bundle (MB) and the Infrapyramidal bundle o tract (IPB or IPT). Both exit the dentategyrus (DG), with the MB traveling above and the IPT below the pyramidal cell layer.

Through immunohistochemical techniques (IHC) using antibodies against calbindin, sinaptoporin and PSA-NCAM, I found that the volume of the IPT tract increases significantly 2 weeks after kainic-induced seizures in wild type mice but not in MeCP2 mutant mouse models (Fig. 2 A, B).

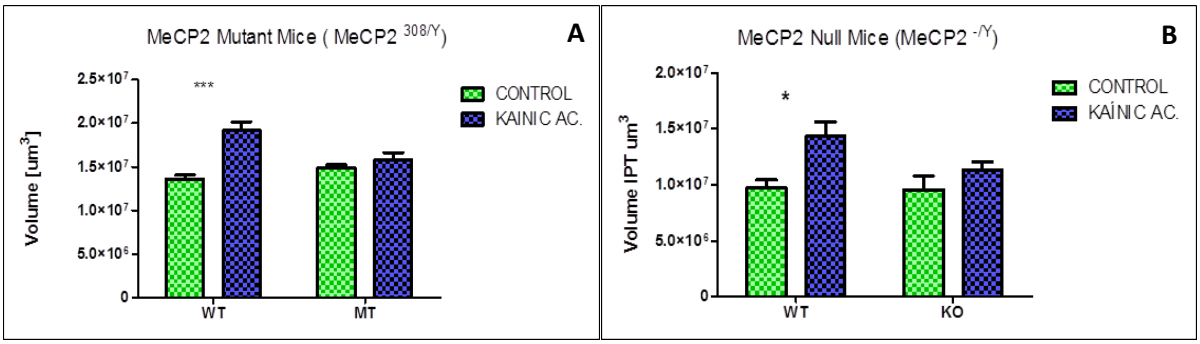

FIGURE 2: Changes in the volume of IPT in response to kainic-induced seizures. Seizure episodes lead to a significantly bigger volume of the IPT in MeCP2 wild tipe mice comparated to MeCP2 mutant (A) and knockout (B) mice during the second week after seizure activity (*** p< 0.001, *p< 0.05, 2way ANOVA, Bonerroni post-test).

Considering that the severity and duration of seizures were similar among the WT and MeCP2 mutant mice, our results suggest that MeCP2 may play a role in the plastic IPT response to neuronal activity. One of these responses is the generation of new neurons from the dentate gyrus. For this, I also learned the technique and set the conditions to measure neurogenesis using BrDU injections and IHC in my experimental model. This technique will allow me to assess whether there are defects in neurogenesis in presence of MeCP2 mutations.

Regarding aim 2, I used micro dissection techniques and real time RT-PCR to explore the expression levels of BDNF and members of the Class 3 Semaphorins in hippocampus from WT and MeCP2 mutant mice after kainic-induced seizures. BDNF is a neurotrophic factor that together with the family of class 3 semaphorins, play an important role in the dynamic changes

of the IPT. Through the experiments mentioned above, I found that in wt animals, the expression of BDNF was up-regulated at both 6hrs and 2 weeks after exposure to the paradigm of activity, concomitant with the peak of IPT growth, while BDNF expression was only increased after 6 hours of kainic injection in MeCP2 mutant animals (Fig.3). This finding suggests that the sustained increase in the expression of BDNF may play a role in the dynamic growth of IPT in response to neuronal activity. These results provide information about the molecular mechanisms through which MeCP2 would regulate hippocampal structural plasticity. More studies will be conducted to get an insight into these mechanisms.

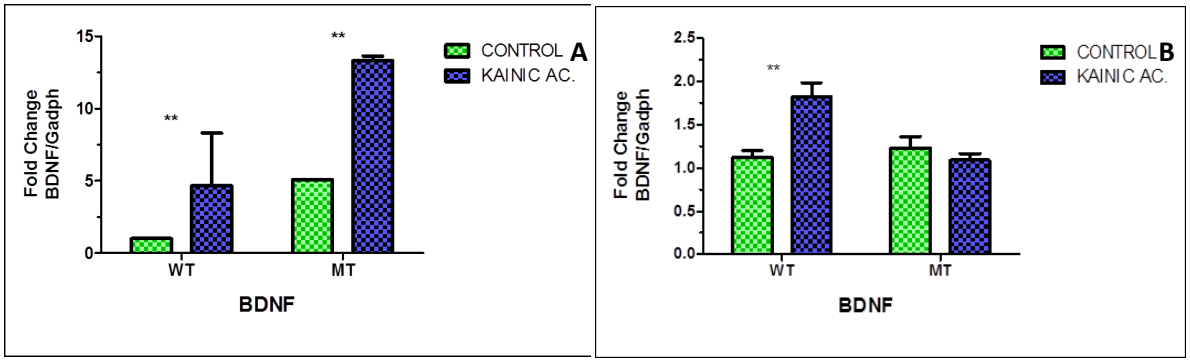

FIGURE 3: Expression of the neurotrophic factor BDNF after exposure to the paradigm of activity. BDNF expression levels after 6 hours (A) and 2 weeks (B) kainic-induced seizures in MeCP2 Mutant Mice (MeCP2 $^{308/Y}$). In wt mice, the expression of BDNF was significantly up-regulated at both 6hrs and 2 weeks after seizure episodes comparated to MeCP2 mutant mice where the BDNF expression was only increased after 6 hours of kainic injection (** $p < 0.01$, 2way ANOVA, Bonerroni post-test).

One of the advantages of working at the host lab was that I had access to a large number of animals for my experiments. Also, I had the opportunity to work with two different animal models that carry MeCP2 mutations and make comparisons. Moreover, I was able to bring tissues back to my home lab in order to complete some studies. For these reasons, having the opportunity to work at the Johns Hopkins University represented an important progress in the experimental part of my thesis, allowing to get significant results in a short amount of time.

In addition this short stay abroad was a great professional and personal experience that will definitely impact my career in a positive way. I was able to successfully overcome the challenge to communicate my ideas and discuss experiments in a language that is not my own; I had the opportunity to participate in seminars with experts of the neuroscience field, learn new techniques, as well as generate new hypotheses. Therefore, I am extremely grateful to the International Society for Neurochemistry for this opportunity that will help me to take on future scientific challenges more confidently.

Maria Laura Bertoldi