CAEN AWARD (**Category 1: Research A**. *Visit by the Applicant to Another Laboratory*)**.**
Recipient: Dr. Nisrin EL MLILI from University Abdelmalek Essaadi, Faculty of Sciences-Tetouan-Morocco.

RE: Visit for the Prof.Francisco Olucha laboratory report (from 15 April to 18 of June).

I am very grateful to CAEN for considering me the suitable candidate for this award and giving me the opportunity to visit the Prof. Francisco Olucha and to establish with him a solid collaboration.

Prof. Francisco Olucha from the Faculty of Medecine-University of Valencia-Spain. He is an expertise in Neuroanatomy, studying the role of Nucleus incertus and Relaxin3 in emotion and cognition. I have been working during 2 months thanks to CAEN award in his laboratory to plan our collaboration and to benefit from his experience in the Neuroanatomy domain and to bring to our laboratory new technical and conceptual expertise (a large behavioral test battery) that are essential for the progress of this project and other projects that we lead in our laboratory.

Background: The amygdala is a key structure in normal emotional processing. Specific lesions in the primate amygdala lead to disruption in emotional processing resulting in the Klüver-Bucy syndrome. That is characterized by different emotional changes as loosing fear, oral exploration, tame and psychic blindness. The parallel symptoms as a whole led (Hetzler and Griffin, 1981) to propose the Klüver-Bucy symdrome as an animal model of autism.

The role of the amygdala has become evident in two models of autism, the valproic acid VPA prenatal exposure and the knockout mice for oxytocin. (Markram, Rinaldi, Markram, 2007; Markram et al.,2008). Very interestingly, autistic patients display significant lower levels of oxytocin than healthty controls (Modahl et al., 1998). In addition abnormalities in oxytocyn receptor and epigenetic hipermetilation of its regulatory element have been observed in autistic patients (Gregory et al., 2009). The oxytocin receptor is a Gq coupled receptor that activates the phospholipase C pathway resulting in erk1/2 phosphorylation (Gimpl and Fahrenholz, 2001).

The regular function of the amygdala can be modulated by ascending brainstem projections. One new pathway that can be used as a therapeutic target is the ascending GABAergic pathway that uses the peptide relaxin3 as neuromodulator and has its origin in the nucleus incertus of the pontine tegmentum just caudal to the raphe nucleus. The nucleus incertus display a widespread pattern of ascending connections to emotion and cognitive processing areas such as dorsal and median rafe, midline intralaminar thalamic nuclei, large areas of the hypothalamus, the medial septum, ventral and caudal hippocampus, medial prefrontal cortex and the **amygdala.**

Another important finding found in parallel to the anatomic studies was the discovery that the nucleus incertus synthetizes relaxin3 (RLN3), a peptide of the insulin

superfamily that is specifically synthetized in this nucleus (Burazin et al., 2002). Soon after the discovery of RLN3, it was found that the former orphan G-protein coupled receptor GPCR135 was the specific receptor for RLN3.

Aims: To identify a signaling cascade through which RLN3 receptor is acting. Our hypothesis is that RLN3 is able to activate erk1/2 phosphorylation and the inhibition of synthesis of cAMP in the amygdala. Therefore, our goal is to analyze the phosphorylation of ERK1 / 2 after intracerebrally injection of relaxin 3 in the nucleus incertus. Once is demonstrated that relaxin 3 is able to activate the phosphorylation of ERK1 / 2 and inhibit cAMP synthesis in the amygdala we will study the implication of the RLN3-erk-cAMP pathway in controlling emotional and behavioral function in different model of autism.

Achieved objectives:

a- **Experiments done during my stay in the Dr.Francisco Olucha Lab:** Our method of work at the beginning was to use an ex-vivo model of amygdala slices; it was expected to incubate them in Krebs solution containing 0.5 uM of RLN3 at different times. Because of the big quantities of RLN3 needed to carry out this experiment (that was not available), Dr. Francisco and I decided to use in vivo experimental model. Rats were anesthetized using halotane and a microdialysis guide was implanted in the amygdala. After 1 week of recovery, animals were perfusing with vehicle or RLN3 (3 rats per group). Fifteen minutes after the injection, animals were scarified and brain areas were separated and saved immediately at -80 Cº and then were transported in dry ice to our laboratory in Tétouan-Morocco for western blots analysis of phosphorylation of ERK1/2 in the amygdala.

   N.B: Before implanting microdialysis probe in the amygdala, we spent a lot of time setting up the ex-vivo experimental method of slices. We also tried to inject directly (without using the microdialysis probe) the RLN3 or vehicle into the amygdale of anesthesied rats, but the results were not very interesting.

b- **Experiments done in our laboratory in Tétouan-Morocco:** Phosphorylation of ERK1/2 in amygdala and hyppocampus (as control) areas were analysed in Tétouan using western blot methods. Results show a clear tendency in increased phosphorylation of ERK1/2 in the amygdala of rats injected with RLN3 compared with rats injected with vehicle. In hippocampus we could not find any differences.

c- **Experiments planned for the next period:** In order to increase the number of animals "N", other animals will be operated and injected with relaxin 3 in the laboratory of Dr. Francisco Olucha (planed in December, 2011), the brain areas then will be transported to our laboratory for further analysis of ERK1/2 phosphorylation by western blots and obtaining statistically significant results. Once we demonstrate the increased of ERK1 / 2, we will buy the Elisa kit to check whether content of cAMP is decreased.

Other objectives achieved from the stay:

- In addition to carrying out the project, our goal was to maintain with Prof. Olucha a long and solid collaboration. This goal has been attended, we have planned a second series of animals to complete the project and have requested a scholarship to send one of our PhD-students for a short stay in his laboratory.

During my stay in Prof.Olucha lab I have also learned some behavior tests (as the labyrinth to 8 arms) that will be very useful for another project in which we are working in our laboratory in Morocco.

- **Paper in preparation in collaboration with Prof. Francisco Olucha**:

Insulin Receptor Substrate type 1 is required for dendritic arborization of hippocampal neurons", sent now to Neurosci Lett.

- **Picture with Prof. Olucha in his laboratory:**

Prof. Francisco Olucha (left) and me (right)

References:

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