Final report: CAEN-ISN Category 1A Visit by the applicant to another laboratory

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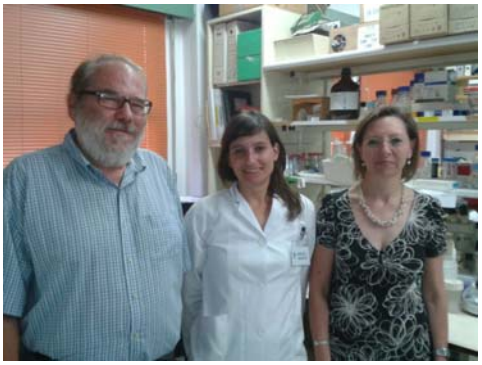
Host Lab: Neuroactive steroids, Cajal Institute, Madrid, Spain.



from left: Luis Miguel García-Segura, Ph.D (PI); Mariana Astiz Cadenas, Ph.D; Maria Ángeles Arevalo, Ph.D.

Outcome and benefit of the award:

Cajal institute is the oldest investigation centre in neurobiology in Spain. The researcher in charge of the host group, Prof. Luis Miguel García-Segura, has been interested in the study of the mechanisms of action of steroids on neurons and glia for over 20 years so he has a great experience in this area. His group, have described many neuroprotective effects of gonadal hormones such as estradiol; and selective estrogen-receptor modulators (SERMs) in the brain and the modulation of steroids synthetic pathway in pathologic situations such as different brain injuries, including acute inflammation (García-Ovejero et al., *Brain Res Brain Res Rev.* 2005 48(2):273-86; Arévalo et al., *Biochim Biophys Acta*. 2010 1800:1106-12).

On the other hand, our lab in Argentina has been working for more than seven years in a project which investigates the effects of low doses of pesticides using Wistar rats as model. We observed that the brain was especially vulnerable to those toxins residues that are unfortunately present in our day-life environment as food and water contaminants (Astiz et al., *Ecotoxicol Environ Saf*. 2009 72:2025-32; Astiz et al., *Neurochem Int*. 2012 61:1231-41). The molecular mechanisms involved in the observed neurotoxic effects are still far to be understood, but among others it has

been suggested that some pesticides behave as endocrine disruptors (McKinlay et al., *Environ. Int.* 2008 34, 68-83).

Before being award by ISN-CAEN fellowship, I had been working for 16 months in Prof. Garcia-Segura's laboratory financed by the Spanish government. During this period we have obtained enough results to publish two articles related to the proinflammatory effect of the insecticide dimethoate and its interference with the steroidogenic process (Astiz et al., *Applied Toxicol. Pharmacol.* 2013 (in press); Astiz et al., *Neurotoxicity Res.* 2013 (in press)). I am really grateful to ISN-CAEN award and to Prof. García-Segura, to have had the opportunity to continue my work in this group because little more time was necessary to finish similar experiments with another widely used pesticide, the herbicide glyphosate (GFS).

GFS is a widely used herbicide all over the world. Widespread use has generated pests with adaptive resistance, so nowadays the fields should receive higher doses of this pesticide to cope with them. This fact unfortunately increase the levels of pesticide residues in food and water for human consumption, that is why we are very interested on demonstrating possible deleterious effects in brain which could be related with the increased incidence in neurodegenerative diseases (Barlow et al., *Neurotoxicology* 2005 26, 63-75; Le Couteur et al., *Biomed Pharmacother*. 1999 53, 122-30; Patel et al., *Brain Res.* 2006 1081, 9-18).

As we expected, the new results (briefly summarized later) showed that the sub-chronic treatment with GFS established a proinflammatory status in brain and interfere with the local synthesis of neurosteroids and probably with the neuroprotective mechanisms triggered by estradiol both in neurons and glia. We proposed that the chronic exposure to GFS residues in food for human consumption could be able to induce damage and reduce the efficiency of damage-repair mechanisms in central nervous system (CNS), leading to greater susceptibility to neurodegenerative processes.

Summary of results:

Previous studies have been shown that the administration of GFS to male rats, at a very low dose and during a sub-chronic period, increased the oxidation of lipids and proteins, reduced the levels of antioxidants and impaired mitochondrial function in various brain regions (Astiz et al., *Ecotoxicol Environ Saf*. 2009 72:2025-32; Astiz et al., *Neurochem Int*. 2012 61:1231-41).

In the experiments performed during ISN-CAEN financial support, we have assessed in C57Bl/6 adult male mice, whether sub-chronic (5 weeks) intoxication with a low dose of GFS (6 mg/Kg, i.p.) affects the expression of inflammatory and steroidogenic molecules and the reactivity of microglia in the hippocampus and striatum under basal conditions and after an immune challenge caused by the systemic administration of lipopolysaccharide (LPS; 5 mg/Kg, i.p.) for 24 hs. So we have 4 experimental groups: a group treated 5 weeks with the pesticide and LPS vehicles (VEH/VEH), a group treated 5 weeks with the pesticide (VEH/GFS), a group treated with the pesticide vehicle and LPS (LPS/VEH) and the group treated with the pesticide for 5 weeks and LPS (LPS/GFS).

The hippocampus and the striatum were selected for this study since they are extremely sensitive to inflammation and oxidative damage (Cerbai et al., *PLoS One* 2012 7:e45250), alterations in these brain structures are associated with important cognitive, affective and neurological disorders in humans (Karen et al., *Neurotoxicology* 2001 22, 811-7; McDaniel and Moser, *Neurotoxicol. Teratol*. 2004 26, 407-15; Ramos et al., *Neurotox Res.* 2006 9, 285-90).

GFS *per se* did not increase the mRNA levels of inflammatory mediators such as tumor necrosis factor α (TNFα), interleukin (IL) 6, interferon-γ-inducible protein 10 (IP10) and IL 1 β, neither in the hippocampus nor in the striatum. The pesticide did not increase the number of astrocytes (GFAP + cells) and microglia (Iba 1 + cells); however the proportion of Iba1 immunoreactive cells with reactive phenotype in the dentate gyrus of the hippocampus was significantly increased. In addition, we observed that the expression of the steroidogenic protein StAR (mitochondrial cholesterol transporter) and the expression of estrogen receptor beta were increased after the treatment with the pesticide in the hippocampus. This may affect steroidogenesis and steroid signaling in this brain region.

As it was previously described, the i.p administration of LPS provoked, 24 hs later, a significant increase in the mRNA levels of IL1β, TNFα, IL6 and IP 10 and a significant increase in the proportion of microglia with reactive phenotype in the hippocampus and the striatum. We observed a different immune response of the hippocampus and the striatum to LPS. Thus, LPS significantly increased the number of microglia cells in the hilus of the dentate gyrus but not in the striatum. TNFα mRNA levels increased 14.4 fold in the hippocampus versus 4.5 fold in the striatum. In contrast, LPS induced a more prominent increase in IL1β mRNA levels in the striatum (4.8 fold) than in the hippocampus (1.9 fold). LPS also caused increased mRNA levels of steroidogenic

proteins such as estrogen receptors (ERα and ERβ), cholesterol transporters (TSPO and StAR) and the first enzyme of the steroidogenic pathway (P450ssc) in the hippocampus. In the striatum LPS slightly increase the expression of ERα, TSPO and aromatase, the enzyme involved in the synthesis of estradiol (comparisons between VEH/VEH vs LPS/VEH).

Some of the effects of LPS were amplified in the animals treated with GFS. LPS administration resulted in significantly higher levels of IP10 in the hippocampus and IL6, TNFα and IP10 in the striatum in the animals previously exposed to GFS compared to the animals previously exposed to the GFS vehicle (comparisons between LPS/VEH vs LPS/GFS).

These findings indicate that a sub-chronic period of administration of a low dose of GFS, comparable to the levels of the pesticide present as residues in food, causes a proinflammatory status in the brain and enhances the neuroinflammatory response to LPS with regional specificity. The differential response of hippocampus and striatum to GFS and LPS could be due to regional differences in cellular composition and neuron-glia communication.

Conclusions and future plans:

In summary, our findings indicate that a sub-chronic period of administration of GFS, even at a very low dose similar to those present as residues in food, alters the steroidogenic pathway and potentiates the neuroinflammatory response of the striatum (and in a lesser extent of the hippocampus) to a subsequent inflammatory challenge. These neuroimmune and neuroendocrine alterations caused by this herbicide could increase the vulnerability to develop behavioral and cognitive deficits after a continued exposure through years.

We plan to publish these results in the near future and after my return to the home lab in Argentina continue with the collaborative relationship with Prof. García-Segura lab.