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During my visit to Dr. Schousboe´s laboratory I performed several experiments relevants for my PhD thesis. The main focus of our work in Argentina is prenatal stress and I am currently studying the effect of this early insult on the glutamatergic system. Prenatally stressed animals show high anxiety-like behaviour, alterations in early motor development and propensity to self-administer drugs. In our laboratory, we have demonstrated that NMDA and metabotropic type III receptors are increased in corticostriatal areas of prenatally stressed rats concomitantly with morphological studies that show a reduction of neural processes, decreased number of synapses and astroglial hypertrophy. Glutamate is one of the most important excitatory neurotransmiters of the nervous system, it is synthesized in the brain from the tricarboxylic acid (TCA) cycle and plays a predominant role in the interaction between astrocytes and neurons.

Taken together these results show that the glutamate metabolism and cellular uptake might be impaired in these adult animals as a consequence of the insult received early in life. In order to test this hypothesis we suggested a series of experiments to perform at Dr Schousboe´s laboratory since he and his group are world known leaders in glutamate metabolism and have the equipment and the expertise needed to carry out this part of my project.

During my stay I was able to fulfill most of the proposed work plan. We measured the expression level of GLT-1 and GLAST transporters and glutamine synthetase (GS) by western blot, we also evaluated the ^{13}C label of glutamate, GABA, glutamine and other metabolites with mass espectrometry and the total amount of this same compounds by HPLC.

Methods

Animal model.

Wistar rats were obtained from highly inbred rats (University of Buenos Aires). Day 1 of pregnancy was designated when spermatozoa were found in the vaginal smear. Pregnant females were randomly assigned to prenatal stress (PS) and control (C) group, and were then individually housed with access to food and water under controlled light and temperature. Control group was left undisturbed in the home cage. Prenatal stress group rats were placed individually in a plastic transparent restrainer fitted closely to body size for three 45 min periods per day (9 and 12 A.M. and 5 P.M.) between the 14^{th} and the 21^{st} days of pregnancy. On the day of parturition litter characteristics were recorded and 10 pups were left in the nest during lactation. After weaning the offsprings were separated by sex and left in the home cage until the day of the experiment.

MS- HPLC evaluation

At postnatal day (PND) 14, 28 and 60 the offspring received an intraperitoneal injection of 1,3-^{13}C-glucose and 15 minutes later the rats were sacrificed. Brain areas, prefrontal cortex (FCx), striatum (Str), hippocampus (Hpc) and mescensephalon (Msc), were dissected and processed to measure newly synthesized glutamate by mass spectrometry (MS) and total glutamate content with high-performance liquid chromatography (HPLC). All the samples were lyophilised in order to take them to Copenhagen.

We measured the percentage of monolabelled ^{13}C-glutamate and other compounds in samples of C and PS rats in FCx and Hpc at 14, 28 and 60 PND using a mass spectometer. We also run the samples with a HPLC to measure the total content of glutamate in the whole brain area.

Western Blot analysis.

C and PS rats at PND 14, 28 and 60 were decapitated and four brain areas were dissected: FCx, Str, Hpc and Msc. Each area was homogenized and processed for standard WB protocol. Samples were loaded on 10% acrilamide gels and the blotting was perform to detect 3 different proteins: GLT-1, GLAST and Glutamine Synthetase. For quantification, the membranes were developed with ECL substrate and pictures of the inmunolabel bands were taken in a dark image station box.

Results

We found no difference between C rats and PS for the amount of monolabel ^{13}C- glutamate (Fig. 1), glutamine, GABA and alanine for all ages and brain areas analysed. These results indicate that this particular prenatal insult do not modify glutamate synthesis metabolism. This is in accordance with the fact that glutamate total content evaluated by HPLC does not show differences between treatments.

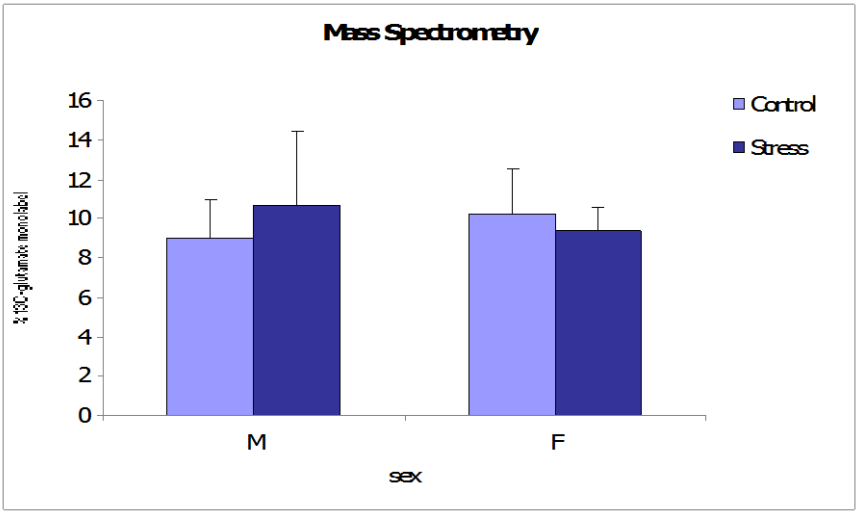

Figure 1. % of ^{13}C- glutamate monolabel of adult male (M) and female (F) rats.

We performed western blot analysis to evaluate the amount of glutamate specific glial transporters protein and so far we have found an increased level of GLT-1 in Hpc for PS male rats at PND 60 (Fig. 2) and an increased of GLAST protein in FCx of PS females at the same PND. And we also observed a decreased GLAST protein in FCx of 28 PND PS male rats. Some of the samples were taken back to our lab in Argentina and we are at present, re-evaluating some brain areas and ages to increase the number of samples and perform a precise statistic analysis.

A)

GLT-1 -

B)

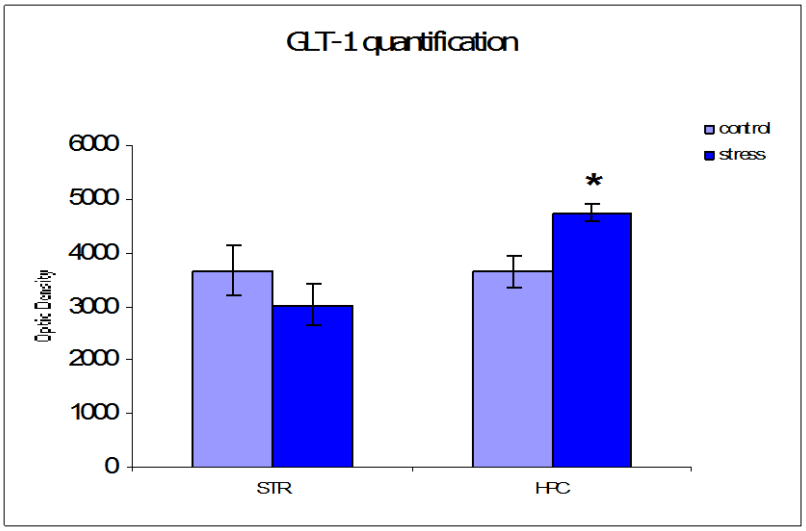

Figure 2. A) Representative western blot image showing GLT-1 inmunolabel. B) Westren blot quantification for GLT-1 immunolable in STR and HPC of adult males. OD ± SE, $p < 0.05$.

This period in Copenhagen laboratory allow me to obtain important data for my PhD work in a very short time. I had the opportunity to learn about and use the mass espectrometer and HPLC equipment by myself. Therefore, I have acquired a great experience with modern equipment and detailed knowledge on classical techniques that we use on a daily basis. We have now add new information on glutamate system to our prenatal stress model that would allow us to publish original data about the effects of this early insult and the consequences in the adult brain.