FULL REPORT

ISN CAEN Award – August 2016 round

Visit by the applicant to another laboratory - Category 1A

ROLE OF ADENOSINE A2A RECEPTORS IN THE CONTROL OF SYNAPTIC PLASTICITY DYSFUNCTION IN A MODEL OF SYSTEMIC PRENATAL HYPOXIA-ISCHEMIA

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Advisor: Rodrigo A. Cunha, PhD. Center for Neuroscience and Cell Biology (CNC), University of Coimbra (UC), Coimbra – Portugal.

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Dear Dr. Caroline Rae,

I was awarded a CAEN grant (category 1A) in order to support my visit to Dr. Rodrigo Cunha's laboratory at the Center for Neuroscience and Cell Biology (CNC) of the University of Coimbra in Portugal in 2017.

During this visit, I was very glad to meet Dr. Paula Canas, a very kind and helpful host who planned all the logistic in advance and provided the necessary material. Dr. Paula welcomed me on my arrival and introduced me to the members of the group and collaborators from CNC. She was also very supportive along all the experiments I performed and techniques that I learned.

The general goal of my visit was to explore neurochemical, eletrophysiological and behavioral alterations related to hippocampal activity in a model of prenatal hypoxia-ischemia (HI) in rats.

Along my stay, I was able to perform several surgeries of induction of prenatal HI using Wistar rats on the 18^{th} day of gestation. Briefly, the animals were anesthetized with isofluorane, had the uterine horns exposed and the uterine and ovarian blood flux was obstructed for 45 minutes (HI group). After this period, the horns were repositioned, the rat was sutured and gestation proceeded. The pups were born at term, without any intervention. We also generated sham operated rats by the exposure of uterine horns with no artery clamping (SH group).

After the pups reached the 42 postnatal days, they were submitted to the behavioral evaluation, which comprised 3 days of tests, in the following sequence: open field, object displacement and modified Y-maze tests. The animals were sacrificed 24 hours after the last day of behavioral test and the hippocampi were used for electrophysiological and neurochemical assays.

Transverse slices from the dorsal hippocampus were used in electrophysiology recordings. The dendritic spine density and sensitivity to synaptic input was determined on the basis of input/output (I/O) curves in which the field excitatory post-synaptic potentials (fEPSP) slope was plotted versus the stimulus intensity. Long-term potentiation (LTP) was induced by high-frequency stimulation train and depotentiation (depot) was induced by a low frequency stimulation train applied 60 min after LTP induction.

A subset of hippocampi was submitted to synaptosomal preparation, which consisted in the homogenization of samples followed by a series of centrifugation steps, generating enriched fractions of synaptic proteins. The samples were analyzed through western blot technique or plated in order to perform immunocytochemical staining. In western blot we analyzed the content of synaptic vesicles proteins. The immunocytochemical assay provided the visualization of the synaptic vesicle transporters and their colocalization with A_{2A} receptors.

Concerning the analysis of spontaneous locomotor activity in open field, we did not observe differences in the total distance traveled by SH and HI

animals. There were also no differences in relation to anxiety parameters, such as the distance and time in the center zone of the arena (Fig 1).

Animals from HI group, however, presented deficits in learning and memory tests. In object displacement test, it was possible to notice that SH animals were able to differentiate the displaced object from the familiar one. Although the number of visits to each object was similar, SH animals explored the displaced object longer than the familiar one. HI rats, however, presented similar number of visits and percentage of exploration time in both objects (Fig 2).

Y-maze modified test confirmed the memory deficits elicited by HI through the analysis of the percentage of distance traveled in each zone (start, other and novel). While SH animals traveled longer distances in the novel zone, HI group presented similar rates in the three arms of the maze. We did not observe any difference between the groups concerning the percentage of entries and time spent in each of the three regions (Fig 3).

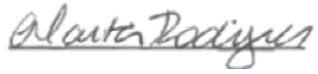
In order to gather information about the effect of prenatal HI on synaptic plasticity, we performed extracellular electrophysiological recordings in hippocampal slices. Both groups exhibited similar I/O curves and similar amplitude of LTP. Likewise, the amplitude of depotentiation was also not affected by the insult (Fig 4).

The synaptosomes provided relevant information about the content of a series of proteins associated with synapses namely: synaptophysin, SNAP 25, syntaxin, VGLUT1 (glutamate vesicular transporter 1) and VGAT (GABA vesicular transporter) besides the postsynaptic density protein PSD 95. We did not observe differences concerning the levels of synaptophysin, SNAP 25, syntaxin and VGLUT. However, HI group presented higher levels of VGAT. In HI group it was also found a significant reduction of PSD 95 (Fig 5).

With the aim of deepening our knowledge on these markers, we performed the immunolabeling of plated synaptosomes preparations. We measured the density of glutamatergic terminals (VGLUT as a marker) and GABAergic terminals (VGAT as a marker). Synaptophysin was used as a marker for synaptosomes, since in this preparation it is possible to have significant glial contamination. We also analyzed the presence of $A_{2A}R$ in glutamatergic and GABAergic terminals. We did not find significant differences between the groups in any of the comparisons (Fig 6).

In summary, although some questions of the initial project could not be answered yet, as the modulation of $A_{2A}R$, this experience helped to clarify some notions of synaptic function in the model I have been studying during the PhD course. It was surely a remarkable period of intense exchange of knowledge with very competent colleagues. I thank ISN for this opportunity.

Regards,



Marta Rodrigues, M. Sc.

FIGURES

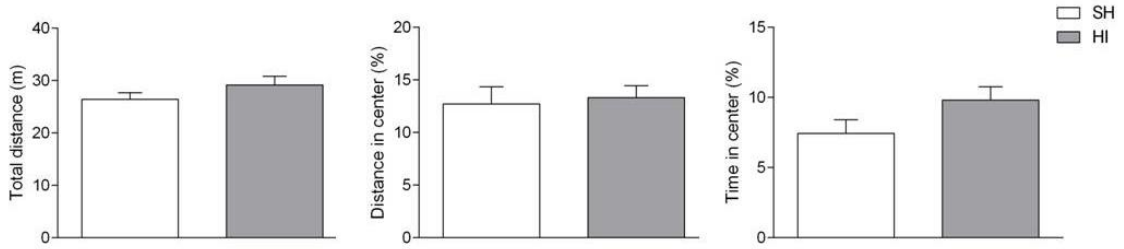


Figure 1 – Analysis of spontaneous locomotor activity in open field. HI animals did not present significant differences in relation to the total distance traveled and to the percentage of distance and time in the center of the arena. Data expressed as means±SEM; n= 11-12/group.

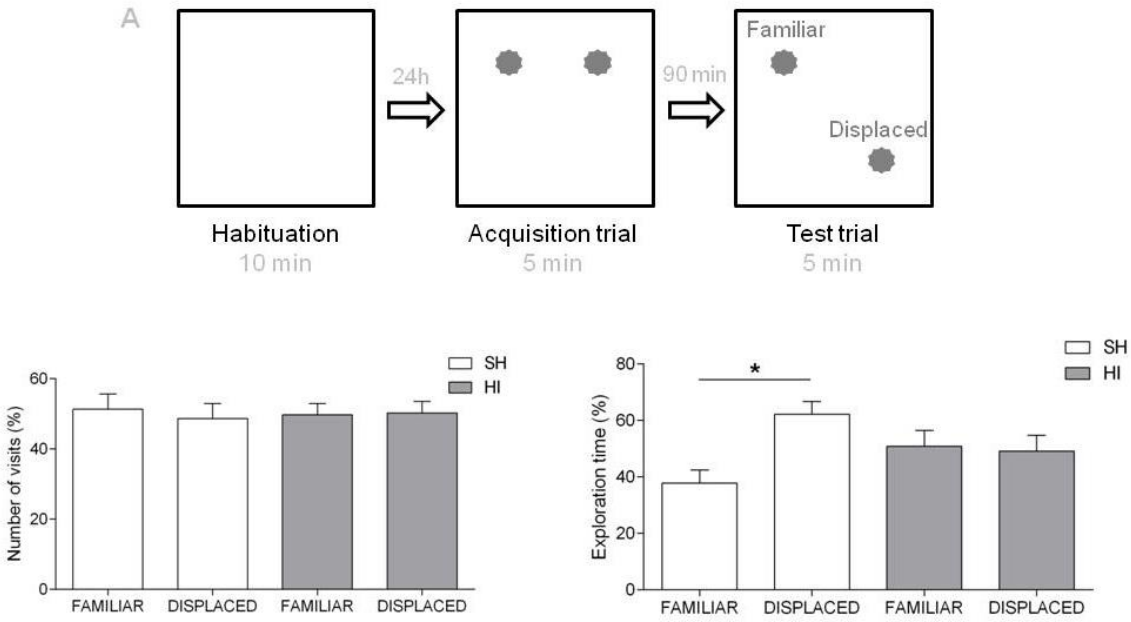


Figure 2 – Evaluation of learning and memory in object displacement test. The protocol included a session of habituation followed by the acquisition trial, when the animal was exposed to two identical objects for 5 minutes. After a 90 min-interval, the rat was submitted to the test trial, in which one of the objects was positioned in the same place where it was during acquisition and the other one was placed in a new position. There were no differences in the number of visits to each object. However, the analysis of exploration time revealed that SH animals were able to differentiate the displaced object in relation to the familiar one whereas HI group presented similiar percentages of exploration. Data expressed as means±SEM; **p<0,001; n= 7-11/group.

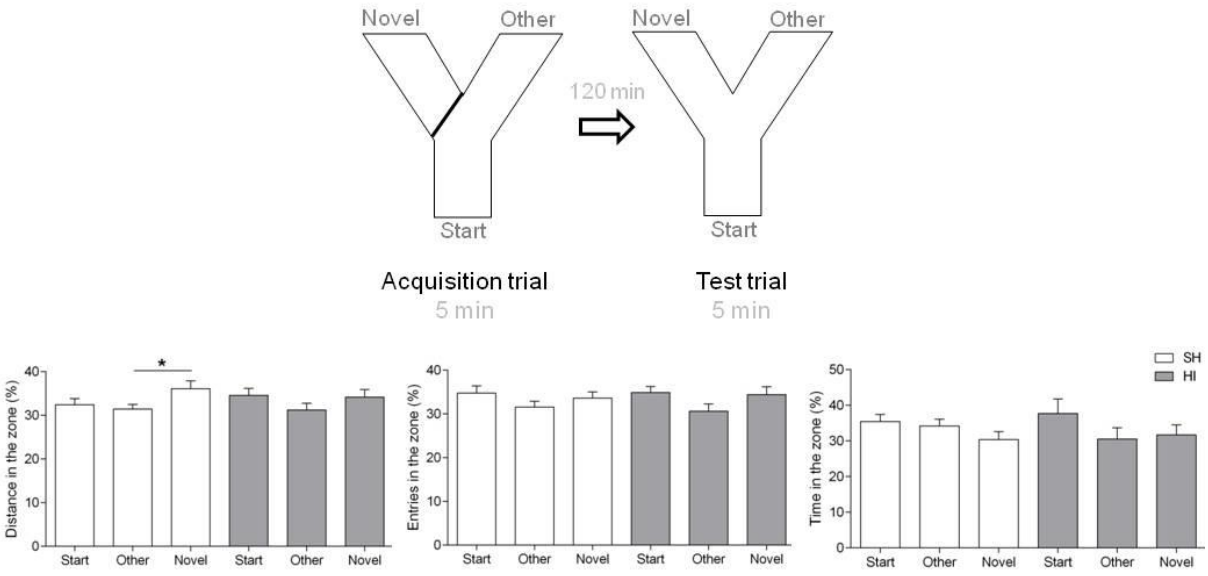


Figure 3 – Evaluation of learning and memory in Y-maze modified test. The protocol included an acquisition trial, in which a barrier prevented the access to the *novel* arm. The rat was placed in *start* arm and could explore only two regions (*start* and *other*) for 5 minutes. After a 120 min-interval the barrier was removed, releasing the access to the *novel* arm. The analysis of the percentage of distance travelled in the zones revealed a significant difference between data related to *novel* arm in comparison with *other* arm. HI group, however, did not exhibit this difference. There were no differences in relation to the percentages of entries and time spent in each zone. Data expressed as means±SEM; *p<0,05; n= 13-14/group.

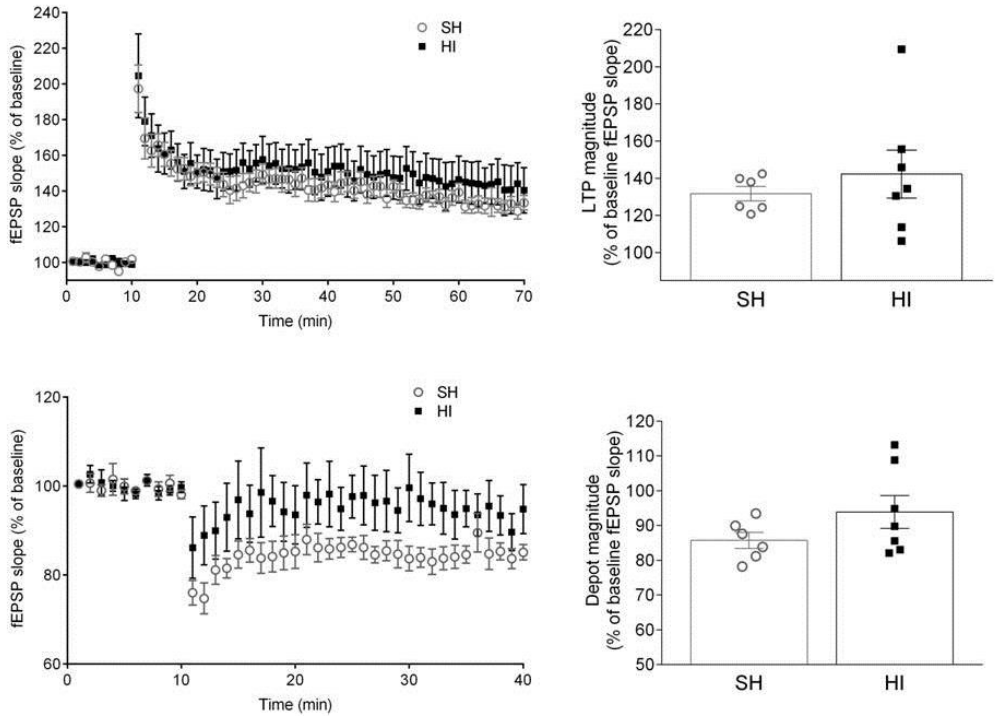


Figure 4 – Extracellular recordings in hippocampal slices. HI insult did not alter synaptic plasticity, measured as LTP and depotentiation. SH and HI presented similar amplitudes in both measurements. Data expressed as means±SEM; n=6-7/group.

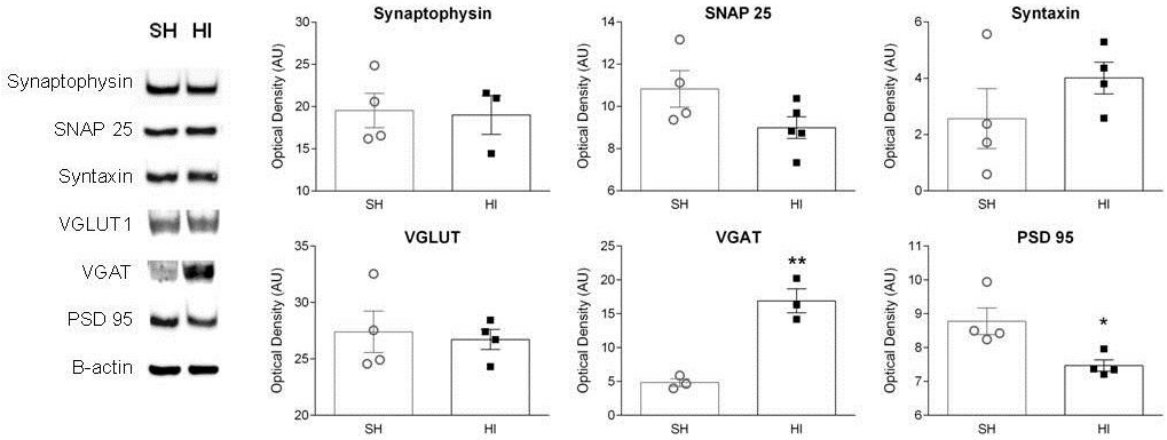


Figure 5 – Analysis of synaptic markers in hippocampal synaptosomes of SH and HI animals. We did not find differences between the groups in relation to the content of synaptophysin, SNAP 25, syntaxin and VGLUT1. The insult affected the levels of VGAT, which are increased in HI group, and PSD 95, significantly reduced in HI. Data expressed as means±SEM; *p<0,05; **p<0,01; n=3-5/group.

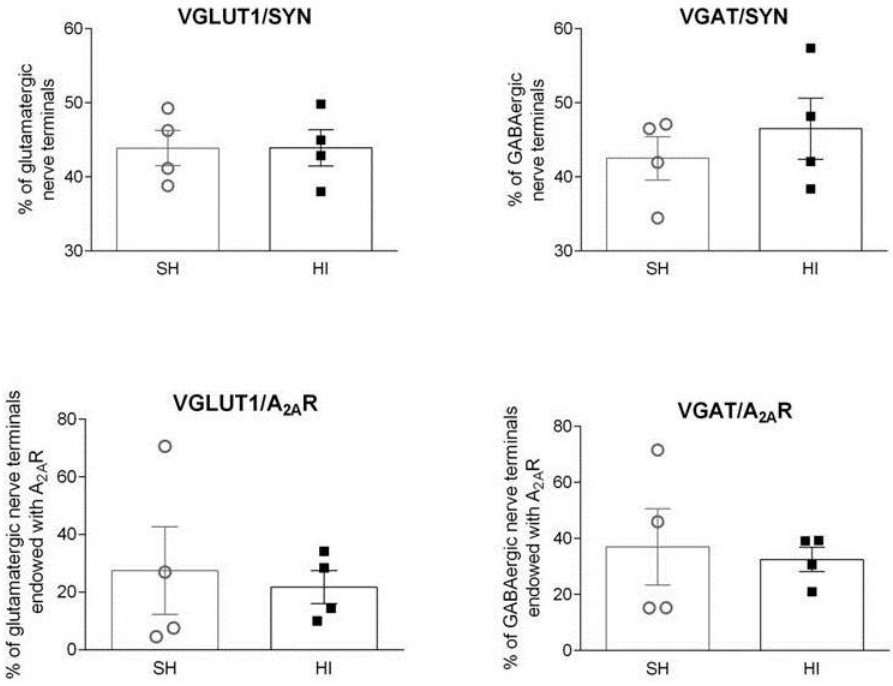


Figure 6 – Quantification of the percentage of glutamatergic or GABAergic synaptic terminals (VGLUT1/SYN and VGAT/SYN, respectively) and quantification of glutamatergic or GABAergic terminals endowed with $A_{2A}R$ immunoreactivity (VGLUT1/ $A_{2A}R$ and VGAT/$A_{2A}R$, respectively). No significant differences were found between the groups. Data expressed as means±SEM; n=4/group.

Master and PhD students from the group. From the left: Patricia Santos, Ana Elisa Speck, Marta Rodrigues (ISN-CAEN fellow), Sara Reis, Anna Pliassova and Marlene Pereira.

Purines Team. From the left: Daniel Moreira, Inês Amaral, Marta Rodrigues (ISN-CAEN fellow), Dr. Rodrigo Cunha, Dr. João Pedro Lopes, Francisco Queiroz, Dr. Paula Canas, Dr. Paula Agostinho, Dr. Angelo Tomé, Dr. Ana Patricia Simões and Dr. Henrique Silva.