Hyperactivity Disorder (ADHD) of mixed type through nonlinear EEG synchronization measures.

Methods: Signals: digitised monopolar EEGs (sampling rate: 256 Hz) from an ADHA group (12 males, 6-10 years) and a control (CONT) group (11 males, 6-10 years). Recording electrodes: Fp1/C3/T3/O1 (reference A2) and Fp2/C4/T4/O2 (reference A1). Pairwise interdependence between electrodes was assessed through a nonlinear index (L) for the assessment of generalized synchronization between the amplitudes of the signals. Also, properly generated surrogate data were used to test the significance of the interdependences. Intra cortical connectivity for the left and right hemisphere was obtained averaging all possible intrahemispheric pairs L values. Interhemispheric connectivity was computed averaging the homotopic hemisphere electrodes pairs L values. Conditions considered were: basal eyes-open and basal eyes-closed. ANOVA test for repeated measures and post-hot Bonferroni test was used for between-group comparisons.

Results: Intrahemispheric interdependences did not shown between-groups differences in any experimental conditions considered. Nevertheless, interhemispheric interdependence was greater ($P < 0.001$) for the ADHA group in both experimental conditions considered without significant differences between them.

Conclusions: ADHD children of mixed type show increased generalized nonlinear interhemispheric synchronization. Nonlinear EEG interhemispheric synchronization measures might be considered as clinical diagnostic help for ADHD children

583

LACTOFERRIN SUPPLEMENTATION PROTECTS BRAIN DEVELOPMENT IN RAT PUPS EXPOSED TO PRENATAL DEXAMETHASONE

P. Larvaron¹, Y. Van de Looij^{1,2}, E. Somm¹, B. Wang³, R. Gruetter^{2,4}, P. Huppi¹, **S. Sizonenko**¹

¹Department of Pediatrics, University of Geneva, Geneva, ²Laboratory for Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, ³Nutrition an Health, Nestlé Research Center, Vers-chez-les-Blancs, ⁴Departments of Radiology, University of Geneva and of Lausanne, Geneva-Lausanne, Switzerland

Background: In rats, in utero exposure to dexamethasone mimics gestational stress and

showed reduction in brain weight and myelination. At postnatal day 7 (P7), in vivo 1H-MRS showed alterations of cerebral metabolism in cortex and hippocampus, suggesting impairment of brain structure, energy metabolism and neurotransmitter. Lactoferrin (Lf) an iron-binding glycoprotein secreted in milk with an antioxidant activity is transferred into the the brain after oral administration.

Aims: We hypothesized that Lf could influence brain development and prevent cerebral damages following prenatal exposure to glucocorticoids.

Methods: Rats were treated with DEX (100 µg/kg/day) during the third week of gestation with access to standard diet (Ctrl-DEX), or Lactoferrin-enriched diet (1 g/kg/day) during gestation and lactation (Lf-DEX). Sham rats not exposed to DEX fed with standard diet (Ctrl-Veh). In vivo 1H-MRSpectroscopy of hippocampus and cortex were acquired at P7 and compared using a Kruskal-Wallis test.

Results: Ctrl-DEX group showed decreased of NAA-NAAG in the hippocampus compared to Ctrl-Veh group ($2.55 \pm 0.26 \text{ mM}$ vs $3.37 \pm 0.10 \text{ mM}$, $p < 0.05$). A decrease of Glu-Gln was observed in both the hippocampus ($3.74 \pm 0.85 \text{ mM}$ vs $5.32 \pm 0.66 \text{ mM}$, $p < 0.05$) and the cortex ($2.95 \pm 0.66 \text{ mM}$ vs $5.62 \pm 0.78 \text{ mM}$, $p < 0.05$). No differences have been found between Lf-DEX group and both Ctrl-Veh and Ctrl-DEX groups.

Conclusions: Variation of NAA-NAAG and Glu-Gln, considered as markers of neuronal integrity and function, confirmed the altered brain development after Dex exposure and revealed the potential protective effect of the lactoferrin in a rat model of prenatal exposure to adverse conditions.

584

EFFECT OF RESCUE AND PROPHYLAXIS WITH L-ARGININE ON APOPTOTIC CELL DEATH IN NEONATAL RATS

C. Turkyilmaz¹, Z. Turkyilmaz², E. Onal¹, Y. Atalay¹, F. Soylemezoglu³, B. Celasun⁴

¹Pediatrics, ²Pediatric Surgery, Gazi University Medical Faculty, ³Pathology, Hacettepe University Faculty of Medicine, ⁴Pathology, Baskent University Medical Faculty, Ankara, Turkey

Hypoxic ischemic brain damage presents significant mortality and morbidity rates in newborns. Various animal models of hypoxia-ischemia have made important contribution to the trial of pathogenesis

and new treatment modalities. Seven day old rat hypoxia-ischemia model can be used to evaluate the effect of different agents. The complex role of nitric oxide and its antagonists in neonatal hypoxic ischemia has been widely known. However effects of L-arginine, an NO precursor, on apoptotic cell death in neonatal hypoxia-ischemia animal models have not been studied before. The aim of this study is to investigate the effects of the pre and post treatment of L-arginine on apoptotic cell death in hypoxia-ischemia neonatal rat model. Seven-day-old rats were administered L-arginine intraperitoneally before and after hypoxic ischemia inflicted through right carotid artery ligation and the exposed to 8 % oxygen. Apoptotic cell death was shown by Tunnel method after three-day recovery. Right hippocampal and striatal apoptotic cell death percentage in L-arginine post-treated group were significantly lower than those of vehicle groups, but no change was found in cortex. L-arginine pre-treatment was found to have no effect on apoptotic cell death in the three brain regions.

As a result, nitric oxide may play an important dual role in apoptotic cell death in neonatal hypoxic-ischemic brain injury. Pre-treatment of L-arginine, an NO precursor has not been proven to reduce apoptotic cell death, while early treatment after hypoxic ischemia has antiapoptotic effect on neurons in neonatal rat model.

585

CELL DEATH AND AUTOPHAGY AFTER SEVERE HYPOXIC-ISCHEMIC ENCEPHALOPATHY IN HUMAN NEONATES

M.-P. Pittet¹, V. Ginet², J.P. Puyal², M.C. Osterheld³, P.G. Clarke², A.C. Truttmann¹

¹*Department of Pediatrics and Pediatric Surgery; Division of Neonatology, University Hospital Center and University of Lausanne,* ²*Department of Biology and Cellular Morphology,* ³*Institute of Pathology, University of Lausanne, Lausanne, Switzerland*

Perinatal asphyxia remains one of the challenging conditions in neonatology, with high prevalence, high mortality and neurological morbidity and no pharmacological neuroprotective strategy. Three main types of neuronal cell death have been described: apoptosis, necrosis and more recently, autophagic cell death, which is a physiological process of degradation, but excessive autophagy may be involved in cell death. Recent studies from

others as well as from our own group showed that inhibition of autophagy is neuroprotective in neonatal models of cerebral ischemia in animals. We showed in a model of perinatal asphyxia in rats an increased neuronal autophagic flux linked to cell death. Following our observations in animals, our objectives for the present study were:

- 1) to describe all the cell death features encountered in brain tissue samples from severely asphyxiated human neonates
- 2) to determine the presence of autophagy histologically and using immunohistochemistry
- 3) to determine the distribution and regional pattern of the different cell death types.

8 newborns at term who died in the Division of Neonatology from 2004-2008 were selected retrospectively and anonymously on the criteria: asphyctic event, Sarnat III, death and accorded autopsy. In histological sections from the frontal cortex, hippocampus, basal ganglia and thalamus, necrosis, apoptosis, but also autophagy were present, with a distinct distribution pattern. The different markers of autophagosomes, lysosomes and apoptosis were tested by immunohistochemistry, showing an increase of autophagic flux in areas affected by asphyxia. These observations lead to a new understanding in the mechanisms of neuronal cell death following perinatal asphyxia.

586

DIFFERENTIAL REGIONAL DYNAMICS OF UNCONJUGATED BILIRUBIN ENTRY AND CLEARANCE IN THE BRAIN OF GUNN RATS

S. Gazzin¹, J. Zelenka², R. Koníčková², L. Zdrahalova², P.J. Giraudi¹, A. Raseni³, A.L. Berengeno¹, L. Vitek², C. Tiribelli¹

¹*Italian Liver Foundation, Basovizza - Trieste, Italy,* ²*1st Faculty of Medicine, Charles University in Prague, Prague, Czech Republic,* ³*Burlo Garofolo, Trieste, Italy*

Background: “Kernicterus” historically describes the unconjugated bilirubin (UCB) accumulation in selective brain regions, leading to neurological damage in hyperbilirubinemic newborn babies and in Crigler-Najjar type I patients. Hyperbilirubinemic (jj) Gunn rats develop neurological deficits that parallels the human pathology and a similar pattern of regional brain UCB distribution under displacing agents treatment.