Effects of fetal Nicotine, Dexamethasone exposure and caloric restriction on the neurochemical profile of the postnatal rat.

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Introduction

The fetal brain is susceptible to insults during pregnancy that can adversely impact brain development resulting in impaired brain function. Exposure to nicotine (NIC), dexamethasone (DEX) or caloric restriction (CR) during pregnancy is affecting the endocrine system. The aim of the project is to evaluate potential differential effect of DEX, CR and NIC in the developing rat brain using proton spectroscopy (^{1}H-MRS).

Materials and Method

Female Sprague-Dawley dams (Charles River Laboratories, France) were used to do programming, three groups, on top of a control one, with the following prenatal interventions were examined, with n=8 in each four groups: 1) maternal CR of 30% (during the 3 weeks of gestation), this condition will be used a reference condition for intra-uterine growth retardation 2) prenatal glucocorticoid treatment with DEX (100 µg/kg/day during the last week of gestation) 3) NIC exposure (3mg/kg/day during the last 18 days of gestation). Pup rats were scanned at postnatal day 7 (P7) (n=4) and 21 (P21) (n=4) and were continuously anesthetized under a flow of 1.2 to 1.8% of isuflorane in oxygen.

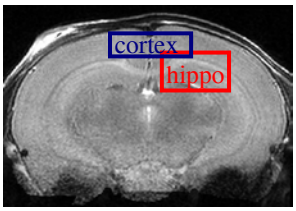

Figure 1: T2-weighted image showing the voxel position for localized spectroscopy

All experiments were performed on an actively-shielded 9.4T 31 cm bore magnet (Varian/Magnex Scientific). An actively shielded 12 cm inner diameter high-performance gradient with a rise time of 120 µs and a maximum gradient strength of 400 mT/m was used. Localized spectroscopy was performed with an ultra-short echo time STEAM with asymmetric RF pulses for slice selection as previously published [1] (TE/TM/TR = 2/20/4000 ms). Signal from the outer volume was suppressed by four blocks of 1.2 ms hyperbolic secant slice selective pulses, water signal was suppressed by the VAPOR sequence [1]. A 17 mm diameter two-loop quadrature coil was used both for RF excitation and signal reception. Field homogeneity was adjusted by the FASTMAP protocol [2,3]. The measured data were corrected for B_0 drift and eddy-current. The data were processed with LCModel [4] and a water spectra was used to get the absolute concentration. The regions of interest were hippocampus and cortex, the voxel size was adapted to brain size range from 18 to 32 µl (c.f. Figure 1).

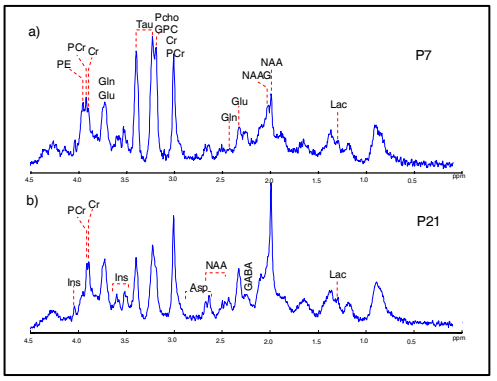

Figure 2: In-vivo spectra acquired on control rats in the hippocampus region at two ages, a) P7 and b) P21. Each spectra required 20 mn of acquisition.

Results

Preliminary results indicated a neurochemical profile in control rats (not shown) that was in excellent agreement with previous studies [5]. Analysis with one-way ANOVA test revealed significant differences in metabolite concentrations in all three groups compared to the control including but not restricted to glutamate (Glu), Inositol (Ins), posphorylethanolamine (PE), taurine (Tau), N-acetyl aspartate (NAA), glutamate to glutamine ratio (Glu/Gln) and phosphocreatine to creatine ratio (PCr/Cr). In addition, changes in ascorbic acid concentration (Asc), which can be measured at 9.4 T [6], were detected.

CR and DEX showed similar concentration differences compared to the control group such as Asc, Glu, Ins, Tau (c.f. Figure 3 a), b) c) and e)) which were not present in the NIC, which may reflect similarities in their mechanism of action (disrupting the steroid receptor).

In contrast, the NIC group showed a significantly higher Glu/Gln ratio at P7 compared to the controls in the cortex and hippocampus (c.f. Figure 3 f)).

All three prenatal treatment groups at P21 indicated a decrease in the concentration of total NAA compared to controls in the hippocampus and a trend showing an increase in PCr/Cr ratio.

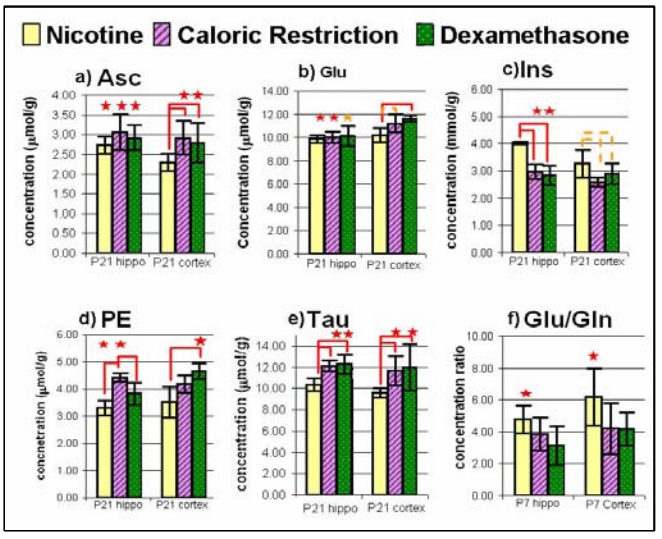

Figure 3: Absolute concentration of a) vitamin C (Asc) b) glutamate (Glu) c) inositol (Ins) d) posphorylethanolamine e) taurine (Tau) at P21 and f) glutamate to glutamine ratio at P7 in the hippocampus and cortex
— p<0.05 - - p<0.08 and ★ p<0.05 relative to the control

Discussion and Conclusions

The changes in neurochemical profile in cortex and hippocampus suggest a measurable, yet different impact of the three adverse prenatal conditions examined. The changes in the putative neuronal marker, NAA, which has been implicated in myelin synthesis, could imply a delay in myelination. The altered PCr/Cr ratio reflects the phosphorylation potential and thus an altered energetic status of the brain. Finally the increased Glu/Gln ratio in NIC group suggests an effect on glutamatergic action.

In conclusion, the results suggest that nicotine, dexamethasone, and caloric restriction in the prenatal period likely result in specific alterations if neurochemical profile during postnatal development.

Acknowledgements

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