NMR-BASED METABOLOMICS APPROACH TO EXPLORE BRAIN METABOLIC CHANGES INDUCED BY PRENATAL EXPOSURE TO AUTISM-INDUCING CHEMICALS AS A FUNCTION OF AGE AND SEX

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IX SIMPOSIO de Investigación EN CIENCIAS EXPERIMENTALES

Introduction

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders characterized by deficits in social interaction, social and verbal communication along with the presence of repetitive, restricted and stereotyped patterns of interests and/or behaviors. Its origin is largely unknown, but current theories pose that autism can be caused by environmental factors, like the exposure to some chemicals. **Valproic acid (VPA)**, an antiepileptic drug, and **chlorpyrifos (CPF)**, an organophosphorus pesticide, are **linked to greater occurrence of ASD in humans**. Identification of **biomarkers** can be a challenge considering the complexity and diversity of **molecular pathways** implicated in most neurological disorders such as ASD, so **metabolomics** works in this way. This technique assures the characterization of an individual metabolic phenotype, that permits the detection of rapid biochemical pathway alterations and unravels multiple biomarker panels. In this study, we aimed to explore **brain metabolic changes** in a rat model of ASD induced by **prenatal exposure to VPA and CPF in rats** at different postnatal ages in the metabolome of different tissues of the brain (**hippocampus and cerebellum**). The biochemistry in these two anatomical regions is expected to be disturbed to different extents by the administration of these xenobiotics, and also depending on the age and sex. The metabolomic analysis was achieved by an analytical platform based on a liquid **high-resolution NMR spectrometer** and a high-sensitive cryoprobe, which is the method of choice for efficient quantification of tissue metabolites.

Procedure

1

3 GROUPS:

CPF group: one subcutaneous injection of 1mg/kg dissolved in DMSO each day

VPA group: 400 mg/kg dissolved in 0.9% saline for a concentration of 250 mg/mL and three daily subcutaneous injections only with saline

Control group (CNT): one daily subcutaneous injection of 1mL/kg of dimethyl sulfoxide (DMSO) for four days

THREE AGES:
A: Neonatal (day 1)
B: Adolescence (day 47-48)
C: Adults (day 202-206)

10 pups per dam (5 females and 5 males)

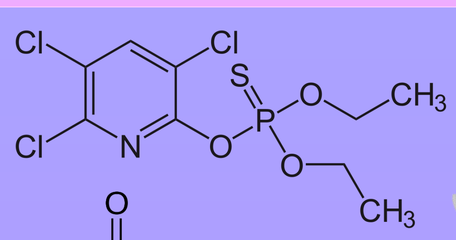
2

Frozen brain tissue samples were freeze-dried for 24 h and mortared to a powder

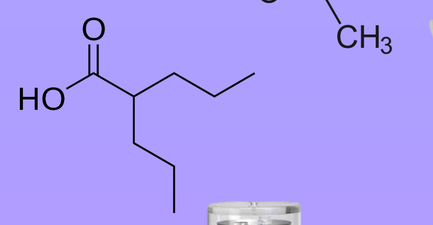
3

12 mg sample + 0.8 mL of a mixture of 50:50 (v/v) CH_3OH-d_4 and D_2O KH_2PO_4 buffer (pH 6.0) containing TSP (0.01% w/w) and NaN_3 (90 µM).

CPF



VPA



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Samples were sonicated for 20 min, mixed for 10 min (600 rpm) and centrifuged (5 min, 13500 rpm).

5

500 µL of the supernatants were transferred to 5 mm NMR tubes and measured in a Bruker Avance III 600 spectrophotometer operating at a ^1H frequency of 600.13 MHz using a 5 mm QCI quadruple resonance pulse field gradient cryoprobe.

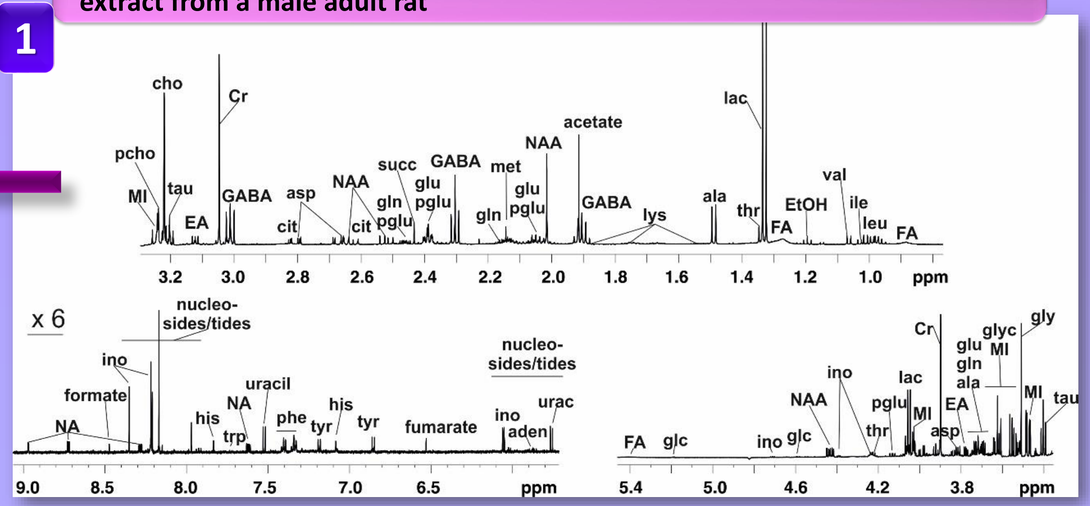
6

Multivariate Data Analysis was performed using the SIMCA-P software (v. 14.0, Umetrics); scaling was done to Unit Variance for PCA and PLS models and to Pareto for OPLS models.

Results and discussion

1

Subregions of a ^1H NMR spectrum (600 MHz) of the left cerebellum tissue extract from a male adult rat



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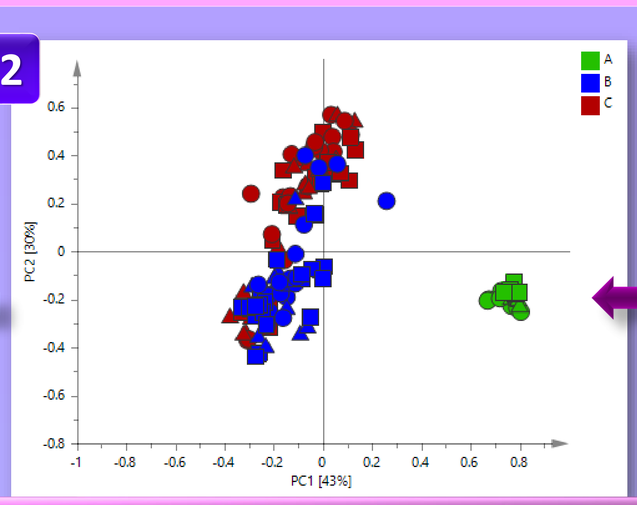
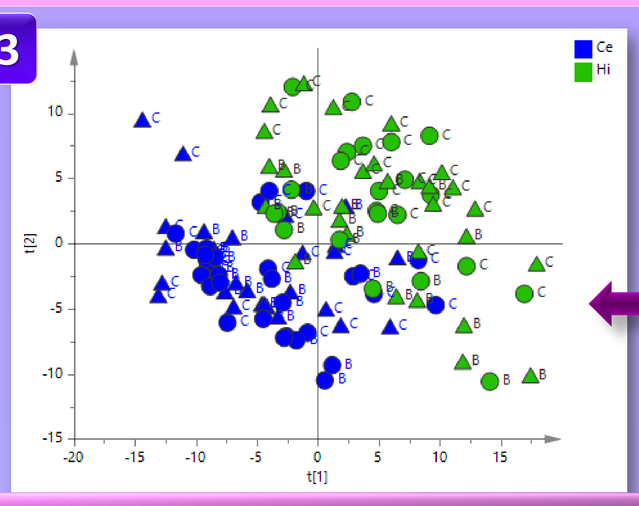


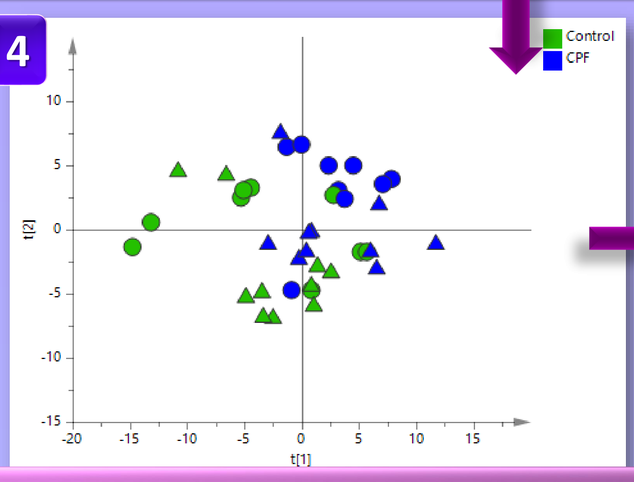
PCA scores plot shows that neonatal (A) brains discriminated from teenager (B) and adults (C) brains.

3



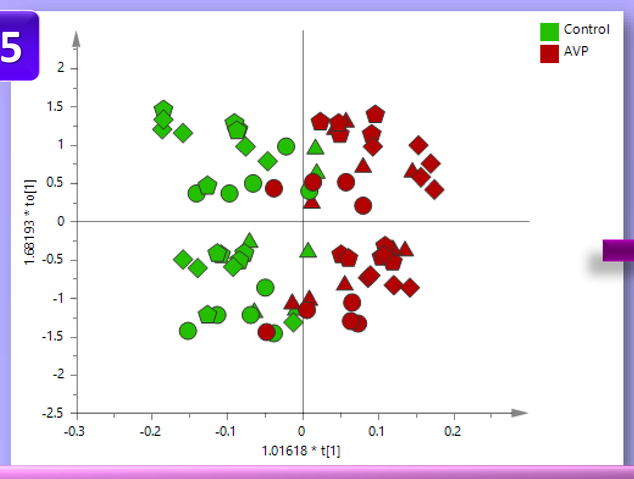
PLS-DA scores plot shows the discrimination between extracts of different parts of B and C brains: hippocampus (green scores) and cerebellum (blue scores).

4



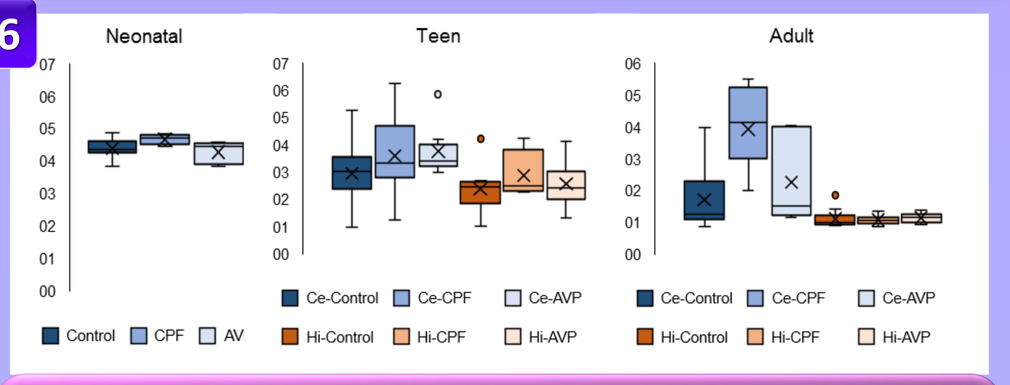
PLS-DA scores plots reveals discrimination between cerebellum tissues from B and C brains from control and CPF groups.

5



OPLS-DA scores plot shows the discrimination between tissues of hippocampus and cerebellum from B and C brains from control and VPA groups.

6



Box plots representations of the relative changes in Glx/GABA ratio. Glx (Glu/Gln) levels increased and GABA concentrations decreased across the two brain regions in all age groups, after administration of VPA and/or CPF comparing to control. Impaired glutamate/GABA-glutamine cycle have been linked to ASD related phenotypes and behavior.

Conclusions

- VPA and CPF prenatal exposure may evoke both specific and common metabolic responses associated to an increased risk of ASD occurrence in different tissues of the brain, depending on the aging process, but no correlation was found with sex.
- Concerning the exposure to VPA and CPF, changes in some metabolites were common in all group ages and brain tissues: NAA generally increased while acetate and choline generally decreased with VPA and/or CPF exposure.
- Reduced GABA along with differences in glutamate and Gln concentrations might indicate a cortical excitation/inhibition imbalance, which may contribute to the development of ASDs.
- These observations provided broad insight for systemic effects of the exposure to important ASD-inducing chemicals on brain metabolism.

Acknowledgments

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