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# Gestational exposure to bisphenol A induces region-specific changes in brain metabolomic fingerprints in sheep

Davy Guignard [a], Cécile Canlet [a,b], Marie Tremblay-Franco [a,b], Elodie Chaillou [d], Roselyne Gautier [a,b], Véronique Gayrard [a], Nicole Picard-Hagen [a], Henri Schroeder [c], Fabien Jourdan [a], Daniel Zalko [a], Catherine Viguié [a,1,*], Nicolas J. Cabaton [a,1]

[a] Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, Toulouse, France
[b] Metatoul-AXIOM Platform, National Infrastructure for Metabolomics and Fluxomics: MetaboHUB, Toxalim, INRAE, Toulouse, France
[c] Université de Lorraine, INSERM U1256, NGERE, Nutrition Génétique et Exposition aux Risques Environnementaux, 54000 Nancy, France
[d] CNRS, IFCE, INRAE, Université de Tours, PRC, Nouzilly, France

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ABSTRACT

*Abstract:* Fetal brain development depends on maternofetal thyroid function. In rodents and sheep, perinatal BPA exposure is associated with maternal and/or fetal thyroid disruption and alterations in central nervous system development as demonstrated by metabolic modulations in the encephala of mice. We hypothesized that a gestational exposure to a low dose of BPA affects maternofetal thyroid function and fetal brain development in a region-specific manner. Pregnant ewes, a relevant model for human thyroid and brain development, were exposed to BPA (5 μg/kg bw/d, *sc*). The thyroid status of ewes during gestation and term fetuses at delivery was monitored. Fetal brain development was assessed by metabolic fingerprints at birth in 10 areas followed by metabolic network-based analysis. BPA treatment was associated with a significant time-dependent decrease in maternal TT4 serum concentrations. For 8 fetal brain regions, statistical models allowed discriminating BPA-treated from control lambs. Metabolic network computational analysis revealed that prenatal exposure to BPA modulated several metabolic pathways, in particular excitatory and inhibitory amino-acid, cholinergic, energy and lipid homeostasis pathways. These pathways might contribute to BPA-related neurobehavioral and cognitive disorders. Discrimination was particularly clear for the dorsal hippocampus, the cerebellar vermis, the dorsal hypothalamus, the caudate nucleus and the lateral part of the frontal cortex. Compared with previous results in rodents, the use of a larger animal model allowed to examine specific brain areas, and generate evidence of the distinct region-specific effects of fetal BPA exposure on the brain metabolome. These modifications occur concomitantly to subtle maternal thyroid function alteration. The functional link between such moderate thyroid changes and fetal brain metabolomic fingerprints remains to be determined as well as the potential implication of other modes of action triggered by BPA such as estrogenic ones. Our results pave the ways for new scientific strategies aiming at linking environmental endocrine disruption and altered neurodevelopment.

## 1. Introduction

Over the past decades, the incidence of neurodevelopmental behavioral, cognitive, emotional disorders and IQ loss have increased dramatically. The societal economical and affective costs of such disorders is estimated to be very high (Gaylord et al., 2020). Human exposure to endocrine disrupting chemicals (EDC), in particular those targeting thyroid function during fetal development, is suspected to contribute to this increase (Bellanger et al., 2015; Braun et al., 2006; Caporale et al., 2022; Grohs et al., 2019; Mhaouty-Kodja et al., 2018; O'Shaughnessy et al., 2021). According to the World Health Organization, a cause effect relationship between an endocrine mode of action and an adverse effect is mandatory for a substance to be classified as an Endocrine Disrupting Chemical (EDC) (https://apps.who.int/iris/bitstream/handle/10665/78102/WHO_HSE_PHE_IHE_2013.1_eng.pdf).

This is a critical issue as EDC are meant to be regulated severely. To

* Corresponding author.
*E-mail address:* catherine.viguie@inrae.fr (C. Viguié).
[1] N. Cabaton and C. Viguié equally contributed to this work, as D. Guignard's PhD mentors.

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fulfill this criterion a comprehensive understanding of the chemical mode of action is required. Scientific approaches allowing large scale screening of biological networks impacted by chemical exposure might constitute very performing tools to address such issue. Some biomonitoring surveys in humans suggest that bisphenol A (BPA) exposure is associated with thyroid disruption (Andrianou et al., 2016; Park et al., 2017) including in neonates (Chevrier et al., 2013; Derakhshan et al., 2021) and/or neurobehavioral abnormalities (Ejaredar et al., 2017; Grohs et al., 2019; Lin et al., 2017; Mustieles et al., 2020). These data are consistent with results obtained in different animal models, demonstrating that BPA affects thyroid function, especially when exposure occurs during the developmental period (Fernandez et al., 2018; Gorini et al., 2020; Guignard et al., 2017). Many effects of early exposure to BPA on the developing brain and/or neurocognitive-behavioral developments have been reported in rodents (Cabaton et al., 2013; Elsworth et al., 2013; Mathisen et al., 2013; Mhaouty-Kodja et al., 2018; Xu et al., 2019; Zalko et al., 2016) and non-human primates (Elsworth et al., 2013; Leranth et al., 2008). The interplay between thyroid disruption and brain development is a complex process involving a variety of possible modes of actions, modulation of metabolic pathways, and even anatomical changes. An integrative biology approach is required to unravel this high level of complexity. A key strategy to address the effect of thyroid disruption on brain development is to use global approaches with no a priori hypotheses, relying on untargeted metabolomics. A pioneering study in this field demonstrated that perinatal exposure to a low-dose of BPA produced differences in metabolic fingerprints at the level of whole brain extracts from 3-week old F1 mice. Metabolites discriminating animals perinatally exposed to BPA from control animals were in a large part related to key amino acids of excitatory/inhibitory pathways pivotal for neural development and plasticity. BPA exposure disrupted neural pathways through modulation of transaminase activity in the brain of rodents (Cabaton et al., 2013; Zalko et al., 2016).

Although critical insights have been made by using metabolomics approaches in rodent models (Cabaton et al., 2013; Constantinou et al., 2011; Maga-Nteve et al., 2017; Rosique et al., 2019; Zalko et al., 2016), investigating the potential link between these “omics” modulations and a potential functional impact requires taking into account the functional and structural complexity of the encephalon. One strategy is to examine several thyroid-dependent brain areas at the same time. This implies working on large brains so that multiple structures may be dissected rapidly (Menassol et al., 2011; Wood et al., 2003). From this standpoint, the sheep is a very interesting model for which detailed data on encephalon anatomy are readably available. In addition, the sheep has long been recognized as a reference model for physiology of gestation and *in utero* development (Barry and Anthony, 2008). Our laboratory showed that the pregnant ewe is well suited to predict the effects of fetal exposure to BPA and its main metabolite BPA-Glucuronide (BPA-G) (Gauderat et al., 2017). The sheep is also a very relevant model to evaluate thyroid physiology, allowing researchers to overcome many of the limitations of rodent models concerning their relevance toward regulation of thyroid function in humans (Viguié et al., 2020). Furthermore, the synchronized development of the thyroid and the central nervous system in sheep is very similar to that in humans (Fisher, 1991).

In this context, we conducted a study in sheep to explore the effect of gestational exposure to a chronic low dose of BPA (5 μg/kg bw/d subcutaneously (*sc*) from gestational day (GD) 28 to few days before the expected delivery) on materno-fetal thyroid function and metabolomic profiles of specific fetal brain regions. The BPA dose is close to the tolerable daily intake (TDI) of 4 μg/kg bw/d) defined by the European Food Safety Agency for the oral route at the time of the experiment. We developed an investigational strategy relying on an integrated approach combining exploration of *in vivo* endocrine physiological endpoints and metabolomics carried out on a wide range of brain regions. Our specific goal was to use metabolomic phenotyping to identify thyroid- and/or toxicant-dependent cerebral biomarkers in selected fetal brain areas, in order to decipher, using computational modeling, the biochemical pathways impacted by gestational exposure to BPA.

## 2. Materials and methods

All materials used for the preparation of solutions, samplings, and samples processing and analysis were either glass or BPA-free plastic.

### 2.1. Chemicals

BPA 99% purity (Sigma-Aldrich, Saint-Quentin Fallavier, France) was dissolved in corn oil / absolute ethanol (6/1: v/v) to obtain a concentration of 200 μg/mL. A new solution was prepared monthly. The solutions were stored in amber glass bottles at + 4 °C. Deuterium oxide ($D_2O$) and sodium 3-trimethylsilyl-2,2,3,3-tetradeuteriopropionate (TMSP) were obtained from Eurisotop (Saint-Aubin, France).

### 2.2. Animal husbandry and treatments

All animal procedures were carried out in accordance with the accepted standards of humane animal care under agreement #311155515 for animal experimentation from the French Ministry of Agriculture and validated by the local ethical committee (TOXCOM 0123/CV) and the French Ministry for Higher Education and Scientific Research (APAFIS#4649). Adult pregnant Lacaune ewes (1–4 years old) were housed in the sheep research facility of the National Veterinary School of Toulouse. Ewes were synchronized for their estrous cycle and were artificially inseminated with semen from a single ram to minimize the impact of the paternal genetic background. Animals were kept under natural photoperiodic and temperature conditions, with free access to water and hay ad libitum and vegetable pellets (Brebilac® RAGT, Rodez, France); a vitamin and iodine-enriched mineral supplementation (Alimal gestante® Alliance Elevage, Souvigny, France) was provided to cover the specific requirements of gestation. The quantity of pellets was adjusted regularly to meet pregnancy requirements and changes in body weight.

Ewes were allocated randomly to two groups (n = 13 per group) balanced for TT4 serum concentration, bodyweight and age. They received BPA (5 μg/kg) or vehicle daily *sc* administrations from Gestational Day (GD) 28 to GD 137–138. The ewes were weighted twice a month and the administered volume was regularly adjusted to the most recently recorded body weight. The *sc* route was chosen to control the individual dose while maintaining the sheep in collective pens and feeders with respect to the gregarious behavior of these animals.

On GD 138-139 and at least 24 h after the last BPA/vehicle injection, ewes were euthanized by pentobarbital overdose (60 mg/kg of Dolethal® Vetoquinol, Lure, France, delivered intravenously) and fetuses were quickly delivered by cesarean section and euthanized (60 mg/kg of Dolethal®, IV). There were 26 (15 males and 11 females) and 24 (13 males and 11 females) fetuses delivered from vehicle-treated and BPA-treated ewes, respectively. When an ewe bore more than two fetuses, only the two first born were kept for the study.

### 2.3. Monitoring internal concentrations of BPA and BPA-G

#### 2.3.1. Sampling

Fetal blood, cord blood and amniotic fluid were collected immediately after fetal delivery. Blood samples were centrifuged for 20 min at 3000 g and 4 °C, and sera were decanted and stored in polypropylene tubes at −20 °C until assay.

#### 2.3.2. BPA and BPA-G measurements

Concentrations of BPA and its main metabolite BPA-G were measured in maternal and fetal jugular, cord blood, and amniotic fluid, using a previously described UPLC/MS/MS method (Lacroix et al., 2011). Limits of quantification (LOQ) were validated at 1 and 5 ng/mL

for BPA and BPA-G, respectively.

#### 2.3.3. Pharmacokinetic analyses

Given the known BPA and BPA-G pharmacokinetic parameters in the ewe and our assay LOQ, BPA and BPA-G concentrations in maternal serum were likely to be non-quantifiable. Thus, we used a pharmacokinetic model based on a tri-exponential equation to predict the time courses of maternal serum BPA and BPA-G concentrations after repeated *sc* administrations. The model was run with a set of BPA pharmacokinetic (PK) parameters obtained in 4 ewes during the courses of another experiment (Guignard et al., 2017).

### 2.4. Characterization of maternal and fetal thyroid status

#### 2.4.1. Sampling

Maternal blood samples were collected at GD 28 (before the first BPA administration), GD 29, GD 36, GD 43, GD 54, GD 68, GD 82, GD 96, GD 110, and GD 124 to monitor the time course of serum thyroid hormones concentrations throughout pregnancy.

#### 2.4.2. Thyroid hormone assays

Total T4 (TT4) concentrations were determined on maternal plasma samples collected throughout pregnancy using radioimmunoassay (RIA) kits (DE4533®, Demeditec, Kiel-Wellsee, Germany). TT4, TT3 and reverse T3 (rT3) concentrations in maternal and fetal plasma collected at delivery were measured with a UPLC/MS/MS method. The mean intra-assay and inter-assay coefficients of variation (CV) of 3 quality control pools were $\leq$ 15%, for all assays. For TT4, the LOQ was validated at 10 and 0.1 ng/mL for the RIA and the chromatographic method, respectively. For TT3 and rT3, the LOQ was validated at 0.1 ng/mL for the UPLC/MS method.

#### 2.4.3. Statistical analyses

The effect of treatment on the time course of maternal TT4 concentrations during the treatment period was analyzed by using a two-way ANOVA with time, treatment and their interaction as fixed factors and the animals nested in the treatment as the random effect factor. Thyroid hormones concentrations measured in samples collected from the fetuses at the end of the experiment were analyzed with a two-way ANOVA with sex and treatment, and their interaction as fixed effect factors. Analyses were performed with the R software R® (v3.2.3).

### 2.5. Proton nuclear magnetic resonance ($^1$H NMR) metabolomics

#### 2.5.1. Brain dissection and sampling

Whole fetal brains were removed and dissected immediately after fetal delivery. We focused on brain regions in which metabolism is altered in thyroid disorders, particularly in humans, namely the frontal lobe, the hippocampus, and areas that are highly dependent on thyroid hormones during development (medial and lateral parts of the frontal lobe (mFL and lFL, respectively), the caudate nucleus (Cd), the ventral and dorsal parts of the hypothalamus (vHy and dHy, respectively), the amygdala (A), the dorsal and ventral parts of the hippocampus (DH and VH, respectively), the cerebellar medial lobe including the vermis (CbV), and the cerebellar hemispheres (Cb). These regions were collected by using external landmarks (Fig. S1) as described in histological sheep brain atlas (Johnson, J.I. et al., n.d.). Samples were snap frozen in liquid nitrogen and stored at −80 °C until further analysis.

#### 2.5.2. Sample preparation for $^1$H NMR spectroscopy

Frozen brain samples were pulverized in liquid nitrogen (Retsch Mixer Mill MM 400), with the right and left parts of the same region pooled together. Approximately 100 mg of powdered frozen tissue was homogenized with a tissue lyser (FastPrep 24, MP Biomedicals, Santa Ana, CA, USA) in 400 μL of methanol and 85 μL of distilled water. Next, 400 μL of dichloromethane and 200 μL distilled water were added before centrifugation at 2870 g for 15 min at 4 °C. Aqueous phases were collected and lyophilized. Lyophilisates were then reconstituted in 700 μL of 0.25 M sodium potassium phosphate buffer (pH = 7) containing 1 mM TMSP, as a chemical reference at 0 ppm.

#### 2.5.3. $^1$H NMR spectroscopy

$^1$H NMR spectroscopy was run as described previously (Tremblay-Franco et al., 2015). Briefly, all $^1$H NMR spectra were obtained at 300 K using a Bruker Avance III HD 600 MHz NMR spectrometer (operating at 600.13 MHz for $^1$H resonance frequency, Bruker Biospin, Rheinstetten, Germany) equipped with a 5 mm TXI cryogenic probe. The Carr-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence with pre-saturation, with a total spin echo delay (2nτ) of 240 ms was used to attenuate broad signals from macromolecules and to suppress signals from water molecules. A total of 128 transients were collected in 32,000 data points by using a spectral width of 20 ppm, a relaxation delay of 2 s, and an acquisition time of 1.36 s. Samples were analyzed randomly. A quality control (QC) sample corresponding to a pool of all samples was analyzed 26 times during the sequence. The free induction decay (FID) obtained was multiplied by 0.3 Hz of exponential line broadening before Fourier transformation. All spectra were manually phased and baseline corrected and referenced to TMSP (chemical shift at 0 ppm) by using Bruker TopSpin 3.2 software (Bruker). Spectral assignment was based on matching one-dimensional (1D) data to reference spectra in a home-made reference database, as well as with other databases (http://www.brmb.wisc.edu and http://www.hmdb.ca), and reports in the literature.

#### 2.5.4. Data pre-treatment and multivariate analysis

Using the software AMIX® (version 3.9, Bruker), the spectral region $\delta$ 10–0.5 ppm was segmented into buckets 0.01 ppm wide. Spectral regions corresponding to the signal of water and methanol were suppressed. To account for differences in the sample amount, each integrated region was normalized to the total spectral area.

A principal component analysis (PCA) was applied to each brain region individually to evaluate the impact of the sex factor. None of the structure showed a sex effect, finding confirmed by a partial least squares discriminant analysis (PLS-DA) that failed to identify robust models discriminating male from female lambs (*i.e.,* no significant latent variable in the model). There was no significant discrimination between males and females in any of the structures. Thus, the subsequent analysis did not take sex into consideration. The multivariate statistical analyses used to identify the discriminant variables to differentiate treatments groups have already been described (Cabaton et al., 2013). Briefly, PCA followed by PLS-DA associated with an orthogonal signal correction filtering was performed to identify the most contributive variables for separation of the treatment groups. The percentage of the explained variance ($R^2Y$ parameter), predictive ability and robustness (permutation test) of the statistical models were assessed. A robust model should be characterized by $R^2 > 50\%$ and $Q^2 > 0.4$; the robustness of our models was post-validated by using permutation tests involving 200 iterations. Discriminant variables were determined by using the variable importance in the projection (VIP) value, a global measure of the influence of each variable on the PLS components. Variables with VIP > 1 were considered discriminant. A Wilcoxon test was then performed on variables selected from the PLS-DA model to determine the variables that were significantly different between treatment groups. False discovery rate (FDR) correction was used to account for multiple testing (Benjamini and Hochberg, 1995). NMR variables with $p < 0.05$ were considered significantly different. SIMCA-P software (V12; Umetrics AB, Umea, Sweden) was used to perform the multivariate analyses and R software (http://www.r-project.org) was used to perform the Wilcoxon test.

The data of two fetuses (one from each treatment group) were suppressed from the dataset because they were systematic outliers of statistical models. No explanation in terms of thyroid status and/or BPA

exposure could be found.

#### 2.5.5. Identification of modulated metabolic pathways

Metabolic network-based analysis of metabolomics datasets enables a holistic overview of mechanisms; it goes beyond pathway-based analysis by integrating all possible metabolic reactions into a single system. A global ovine metabolic network was built based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database of *Ovis aries* (1800 metabolic reactions and 1521 metabolites, MetExplore biosource 2937 (Kanehisa et al., 2017). A network-based algorithm was then applied to retrieve the metabolic reactions related to the treatment effects. Briefly, for each brain region, discriminating metabolic biomarkers were mapped in the ovine metabolic network. Then, reactions sequences (metabolic paths) connecting these significant metabolites were computed by using the lightest path algorithm (Frainay and Jourdan, 2017). The resulting pathways were merged into a single subnetwork containing few tenths of reactions specific for each metabolic profile. The MetExplore web server (https://metexplore.toulouse.inrae.fr) was used to detect these modulated subnetworks (Chazalviel et al., 2018; Cottret et al., 2010; Faust and van Helden, 2012; Frainay and Jourdan, 2017) and to overlay the metabolic pathways linking the biomarkers. The objective was to identify potential mechanisms and neural consequences of prenatal exposure to a low dose of BPA in specific fetal brain regions. Functional links between different modulated pathways were examined and could be established between different pathways, as long as at least one metabolite common to the different pathways was identified. Functional networks were built for the two structures to provide the best pictures of modulated pathways, including all the identified discriminant metabolites for that structure.

## 3. Results

### 3.1. Monitoring internal concentrations of BPA and BPA-G

Maternal serum concentrations of BPA, BPA-G and BPA-Sulfate (BPA-S) were, as expected, below the LOQ of the assay (<1 ng/mL) for all animals from the vehicle groups. In the treated group, BPA remained below the assay LOQ for 7 out of the 13 treated ewes. For the six ewes in which it was detected, the measured concentrations (1–5 ng/ml) were close to the assay LOQ. The measured BPA-G and BPA-S concentrations remained below the LOQ as were the values predicted by the model (Fig. 1).

BPA and BPA-G concentrations were not quantifiable in the different fetal compartments in control fetuses. BPA concentrations were quantifiable in amniotic fluid for 16 out of the 21 fetuses (1.2–3.7 ng/mL) from BPA-treated mothers. BPA was also quantifiable in fetal cord blood in 7 fetuses (2.1–9.5 ng/mL). By contrast, BPA-G concentrations were always quantifiable in amniotic fluid (23.8 ± 4.8 ng/mL), fetal serum (24.5 ± 2.3 ng/mL) and cord serum (22.8 ± 3.1 ng/mL). All fetal compartments were exposed similarly to BPA-G.

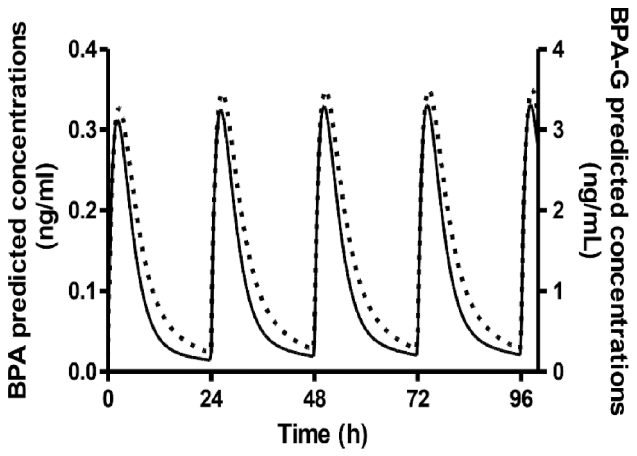


**Fig. 1.** Simulation of BPA (continuous line) and BPA-G (discontinuous line) serum concentrations time course for repeated subcutaneous administrations of BPA 5 µg/kg bw/day in pregnant ewes. The maternal serum BPA and BPA-G concentrations were predicted by using BPA PK parameters measured in pregnant ewes in another experiment (Guignard et al. 2017) and were simulated using a tri-exponential equation corresponding to extravascular administration.

### 3.2. Maternal and fetal thyroid status

Fig. 2 shows the time course of mean (±SEM) individual ratio of TT4 at a given timepoint to initial TT4 concentrations in pregnant ewes. BPA treatment was associated with a significant time-dependent decrease in maternal TT4 concentration ratios ($p = 0.04$) toward mid pregnancy. However a return to similar TT4 levels between BPA-treated and control ewes at term was noticed.

At the end of the exposure period (4 months), rT3 concentrations tended to be higher ($p = 0.06$) in the serum of BPA-exposed ewes, compared with controls ewes (Fig. 3). Free and total T3 concentrations were non-quantifiable in fetuses on GD 138–139, whatever the maternal treatment. Fetal free and total T4 concentrations did not differ significantly between groups.

### 3.3. $^{1}H$ NMR metabolomics

The different brain areas displayed similar metabolites, although changes in the relative intensities of some resonances were observed. The $^{1}H$ NMR spectra of the aqueous brain extract (pool of all samples) are presented in Fig. S1. Thirty-six metabolites were identified and Table S1 lists the assigned compounds, with $^{1}H$ chemical shift and coupling patterns.

A valid PLS-DA model was generated for control lambs only, run within the same sequence. This showed that different structures could be discriminated according to their specific metabolomic signatures (Fig. S2).

Because the first PCA score plots did not indicate a sex effect, data from male and female fetuses were pooled for subsequent PLS-DA of specific brain structures. The PLS-DA results demonstrated a marked discrimination between the two treatment groups for all structures except the Cb and VH (no valid model for these two) (Fig. 4). Figs. 4–6 need to be printed in color.

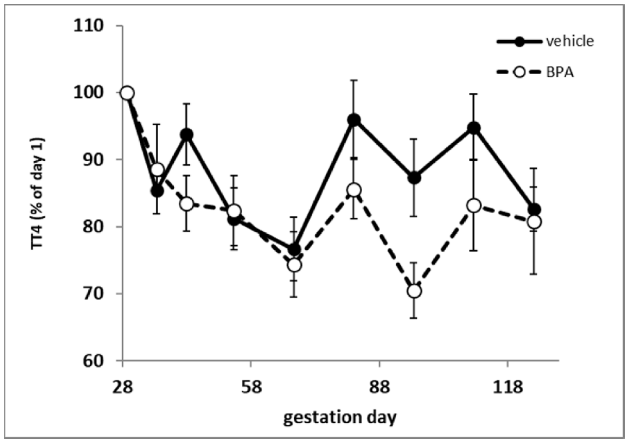


**Fig. 2.** Time course of mean (±SEM) individual ratio of TT4 at a given timepoint to initial TT4 concentrations in pregnant ewes treated with vehicle or BPA (5 µg/kg bw/day; *sc*) from GD 28 to GD 137–138. The time course of mean TT4 concentrations differed significantly between the two groups (Time × Treatment interaction: $p < 0.05$).

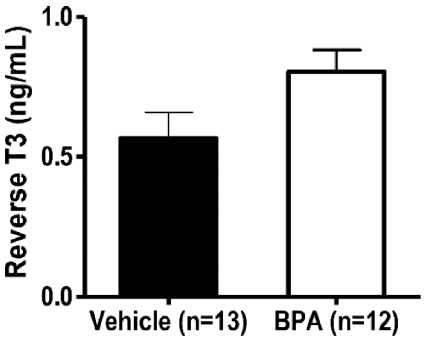


**Fig. 3.** Mean (±SEM) reverse-T3 (rT3) serum concentration in ewes on GD 137–138 ($p = 0.06$). Pregnant ewes were treated with BPA (5 μg/kg bw/d sc) from GD 28 to GD 137–138. rT3 was assayed in sample collected just before delivery.

Several metabolites contributed to inter-group discrimination, from which 15 could be identified by comparing $^{1}H$ chemical shift and coupling patterns of brain extracts NMR spectra with NMR spectra of reference compounds (Table 1). The pattern of modified metabolites differed according to the brain regions, with more discriminant variables for the lFL, DH, dHy and CbV (Table 1). Of note, glutamine was systematically increased in the 8 discriminated regions, namely lFL and mFL, Cd, DH, A, dHy and vHy, and CbV (Table1).

Discriminant metabolites from each brain region were mapped into the ovine metabolic network by using the MetExplore webserver, in order to identify the metabolic sub-networks modulated by BPA exposure. Using this specific metabolic network, dHy, the lFL and the CbV showed the most prominent modulations. Network analysis allowed us to investigate the biochemical processes modulated in these brain regions by *in utero* gestational exposure to BPA. The results obtained for the dHy highlight potential modulations in GABA, glutamate and Krebs' cycle metabolic pathways (Fig. 5).

Functional network prediction also revealed interplay between different pathways, as shown for the Cd and CbV (Fig. 6A and 6B, respectively). In both structures, metabolites involved in energy/oxidative metabolism and amino acid pathways appeared to be modulated. Moreover, cholinergic pathways were well represented in the CbV. In the Cd, common nodes between amino acid and lipid metabolism pathway markers on the one hand, and energy oxidative metabolism on the other hand. Meanwhile, there was no molecular link between cholinergic and energy/oxidative metabolism pathways in the CbV.

## 4. Discussion

Several neuronal and metabolic pathways have been identified as potential targets of developmental exposure to BPA in rodents (Kunz et al., 2011; Nakamura et al., 2010; Tonini et al., 2020; Yao et al., 2020). Most of these studies focused on only one specific structure or the whole brain. We hypothesized that a multifocal and large-scale chemical screening approach in multiple brain areas provides a more comprehensive understanding of the BPA effects on brain development. In this study, we found in sheep that gestational exposure to a low dose of BPA significantly impacted the metabolomic profiles of specific fetal brain regions. These effects were mostly related to neural amino-acid pathways and energy homeostasis, and most changes were region-specific. Moreover, there were subtle modifications in maternal but not fetal thyroid function.

We used *sc* administration as a way to control individual dosing. Previous studies conducted in the same model in our laboratory showed that *sc* administration leads to a 3-fold higher BPA maximum concentration (Cmax) compared with BPA delivered in the diet (Guignard et al., 2017). Despite similar Cmax, due to the difference in the time courses of BPA, the relative bioavailability of BPA by diet versus *sc* routes was only

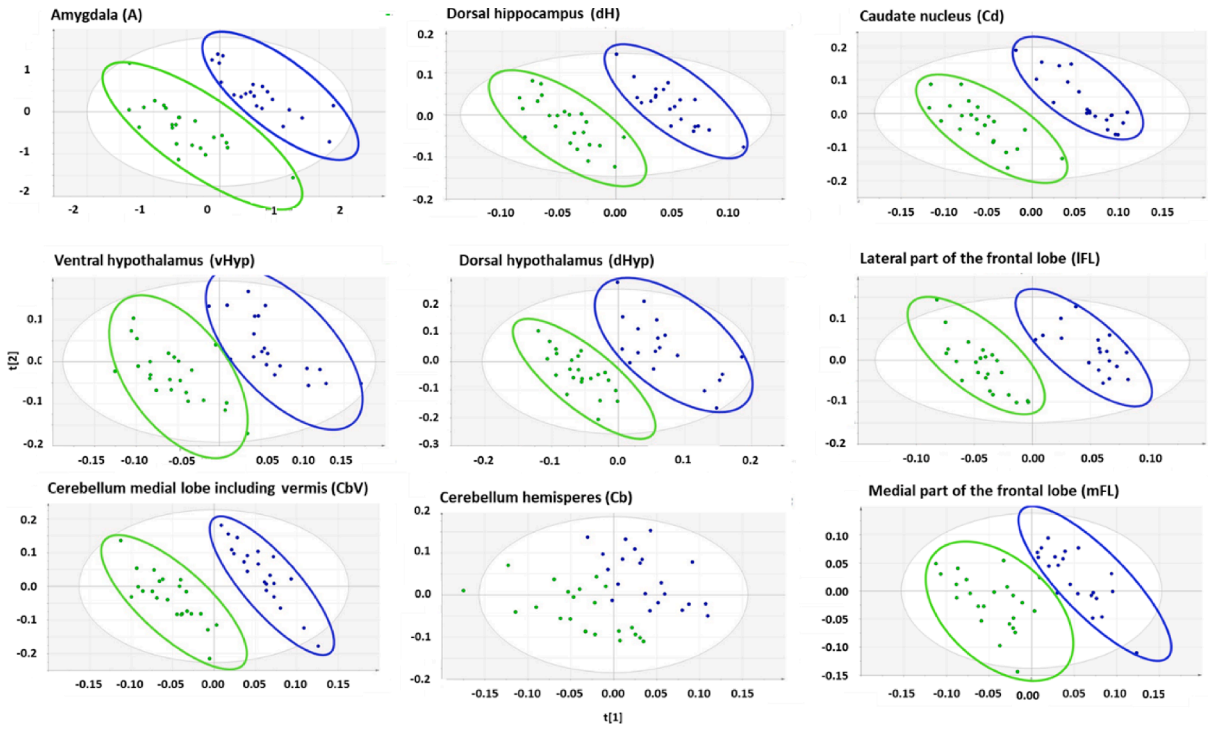


**Fig. 4.** Two-dimensional PLS-DA score plot of integrated 1H NMR spectra of brain structures: medial part of the frontal lobe (mFL) (vehicle, n = 23; BPA, n = 21; 1 latent component; R2Y = 0.984; Q2 = 0.801); caudate nuclei (Cd) (vehicle, n = 22; BPA, n = 21; 3 latent components; R2Y = 0.973; Q2 = 0.896); amygdala (A) (vehicle, n = 23; BPA, n = 21; 3 latent components; R2Y = 0.974; Q2 = 0.846); ventral part of the hypothalamus (vHyp) (vehicle, n = 23; BPA, n = 21; 2 latent components; R2Y = 0.911; Q 2 = 0.642); medial lobe of the cerebellum including the vermis (CbV) (vehicle, n = 22; BPA, n = 21; 4 latent components; R2Y = 0.992; Q2 = 0.932) and cerebellum hemispheres (Cb) (vehicle, n = 23; BPA, n = 21).

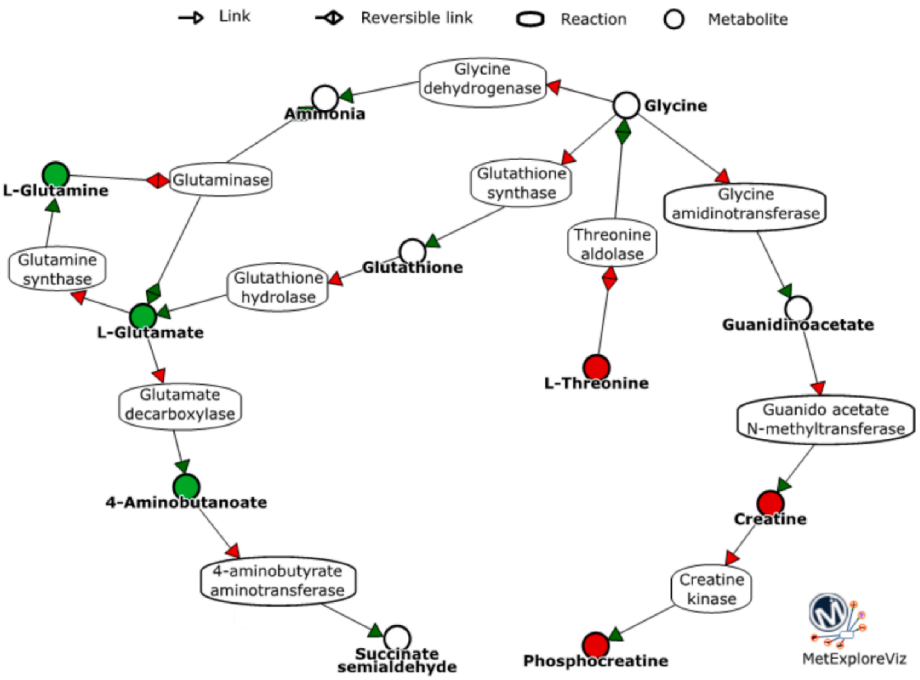


**Fig. 5.** Subnetwork extracted from the global ovine metabolic network after mapping of the metabolites that discriminate treatment groups in the dorsal hypothalamus. The green and red circles indicate metabolites that are increased and decreased, respectively (obtained from the webserver Metexplore®; www.metexplore.toulouse.inra.fr).

3%. Thus, despite a similar Cmax range, the ewes were likely subjected to higher internal BPA exposure than what would have occurred with BPA administered in the diet. Of note, maternal predicted BPA and BPA-G concentrations in ewes are relevant to the internal exposure levels in human, based on serum mean concentrations reported in biomonitoring surveys (cord blood within the range of 0.1–1 ng/mL) (Gerona et al., 2013).

In this context, we demonstrated that gestational exposure to BPA moderately altered the temporal trajectory of thyroid hormone status in ewes beginning in mid-gestation. This effect was no longer expressed at term. No BPA-induced changes in serum fetal TH at the end of gestation could be evidenced either We had previously shown similar alterations in the time course of maternal TT4 during gestations in the same model (albeit with different amplitudes (Guignard et al., 2017; Viguié et al., 2013). By contrast, we had only observed the effects on fetal TT4 at the end of gestation in one experiment at high dose of BPA (Viguié et al., 2013). We have discussed the possible explanations for such a discrepancy elsewhere (Guignard et al., 2017).

$^{1}$H NMR metabolomic unambiguously demonstrated the impact of gestational exposure to BPA on several fetal brain regions. For 8 out of the 10 examined brain structures, PLS-DA of NMR data provided a very strong discrimination of BPA-treated compared with vehicle-treated animals, with very high R2Y and Q2 scores, reflecting extremely valid and robust models. For two structures, no discriminating model could be found, attesting to the specificity of our approach. Interestingly, the discriminative metabolic profiles differed among the brain regions, highlighting the regional effects of BPA. In human, there were structure-dependent modifications of brain NMR metabolic profiles in hypothyroid infants living in iodine-deficient areas (Akinci et al., 2006). The large number of structures for which gestational exposure to BPA altered the metabolomic profile could be one explanation for the wide variety of neural, behavior, and cognitive adverse effects of developmental exposure to BPA (Mhaouty-Kodja et al., 2018; Rebolledo-Solleiro, 2021).

Most of the metabolic modulations highlighted in our study are emblematic of dialogs between neural amino acid pathways, and brain energy homeostasis related to the Krebs cycle (input and output). Our results suggest that oxidative energy metabolism in most of the fetal brain regions may be altered in BPA-exposure. Consistently with this hypothesis, in the rat, BPA exposure from GD 11 to PND 21 was associated with a decrease in glucose metabolism until postnatal day (PND) 90 in the prefrontal cortex and hippocampus offspring (Xu et al., 2019). Interestingly, those effects were observed concomitantly with modest thyroid disruption in the dams by the end of gestation (a 16%-18% decrease for both free T4 and TT4) and at PND 21 in offspring (a 10% decrease in free T4 and TT4). The effects of gestational exposure on glucose metabolism in our sheep model are consistent with the observed modifications of aspartate and acetate. Aspartate is involved in the tricarboxylic acid cycle due to its permanent balance with oxaloacetate. In addition, the decrease in creatine and phosphocreatine in the dHy and in the mFL could be markers of disrupted energy metabolism. Creatine protects axons from energy depletion in normal conditions (Shen and Goldberg, 2012). Its conversion into phosphocreatine and ATP by creatine kinase is a critical step that mediates its effect on energy homeostasis.

The glial cell-neuron network provides anatomical support for regulation of energy homeostasis and amino acid pathways. It is commonly solicited during physiological brain activation (Cherix et al., 2021; Hertz and Chen, 2017) or under pathological conditions (Xu et al., 2020). In the current study, glutamine, the glial precursor of glutamate and GABA, was upregulated in 8 of the 10 brain regions studied. This could reflect its key role at the interface between glial and neuronal functions as a byproduct of the Krebs cycle produced in astrocytes, and as a direct precursor of both glutamate and GABA (Hertz and Rothman, 2016). However, given the ubiquitous distribution of glutamine, one could speculate about the biological significance of this global upregulation of glutamine in the encephalon. Presynaptic glutamate replenishment results from an activity-dependent transfer of glutamine from astrocytes to presynapses. It is thus very likely that any changes in glutamine in the brain would somehow alter synaptic activity (Cheung et al., 2022). To the best of our knowledge, there is not data allowing to estimate to what extent the change of the glutamine/glutamate cycle of the magnitude identified in our study can impact neuronal function. Nevertheless, our results should prompt the scientific community to investigate how glutamine/glutamate cycle might contribute to BPA-

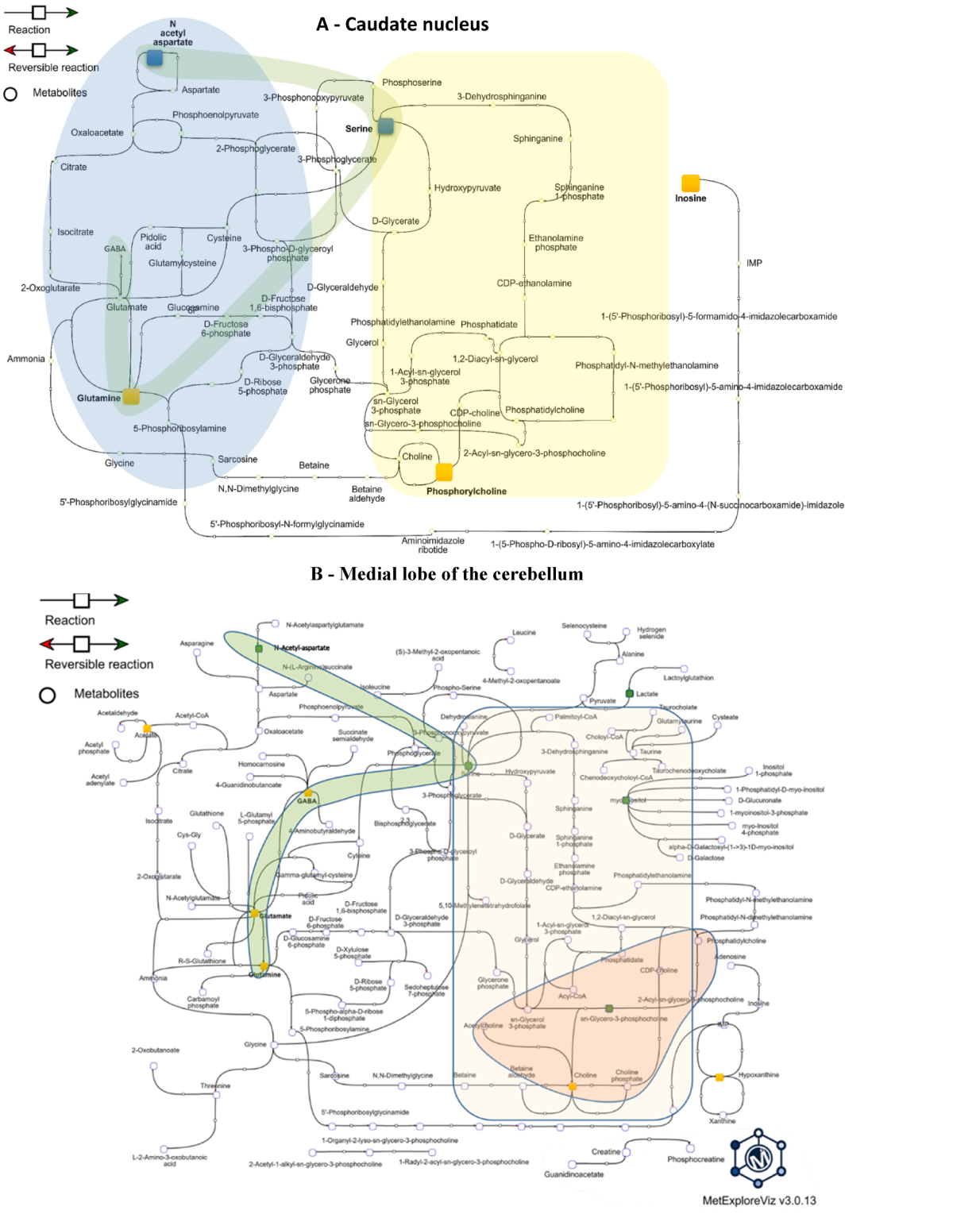


**Fig. 6.** Functional metabolic networks showing potential pathways modulated by gestational exposure BPA (5 µg/kg bw/d) in the caudate nucleus (A) and medial lobe of the cerebellum (B) in ovine term fetuses. The color-filled dots represent identified discriminative metabolites. The yellow area encompasses lipid biosynthesis pathways, the green area includes amino acid neuromediator pathways, the blue area is related to energy homeostasis and, the brown one cholinergic-related pathways.

induced neural alterations. Overall, our results show that when aspartate is a discriminant variable, it is decreased by BPA exposure (mFL, lFL, and DH), while when glutamate (A and dHy) and GABA (dHy and CbV) are discriminant variables, they are upregulated by BPA. Research in rodents also indicates that perinatal or prenatal exposure to BPA modulates neural amino acid pathways. Data from perinatally exposed mice have demonstrated an increase in glutamine in the whole brain alongside increased aspartate and decreased glutamate and/or GABA in BPA-exposed animals (Cabaton et al., 2013). The glutamate/aspartate ratio decreased in the hippocampus of rats perinatally exposed to BPA

**Table 1**
Modulation of endogenous metabolites induced by gestational exposure to BPA (5 µg/kg bw/d) in different brain areas of term fetuses. Fold changes (FC) correspond to the ratio of the mean NMR areas (exposed samples / control samples). p values were calculated with a nonparametric Wilcoxon test and corrected based on the false discovery rate.

| Metabolites | mFL | | iFL | | Cd | | DH | | A | | dHy | | vHy | | CbV | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FC | p | FC | p | FC | p | FC | p | FC | p | FC | p | FC | p | FC | p |
| Acetate | | | | | | | | | | | 1.11 | 0.022 | | | 1.16 | 0.015 |
| Alanine | | | 1.10 | 0.00025 | | | | | | | | | | | | |
| Aspartate | 0.94 | 0.040 | 0.92 | 0.021 | | | 0.92 | 0.0126 | | | | | | | | |
| Choline | 0.92 | 0.036 | 0.94 | 0.0307 | | | | | | | | | | | 1.13 | 0.03 |
| Creatine/Phosphocreatine | 0.95 | 0.0062 | | | | | | | | | 0.94 | 0.021 | | | | |
| GABA | | | | | | | | | | | | | | | 1.05 | 0.015 |
| Glutamate | | | | | | | | | 1.04 | 0.012 | 1.4 | 0.032 | | | | |
| Glutamine | 1.12 | 0.0009 | 1.11 | 0.00025 | 1.06 | 0.043 | 1.08 | 0.033 | 1.09 | 0.005 | 1.05 | 0.029 | 1.08 | 0.026 | 1.06 | 0.034 |
| Glycerophosphocholine | | | 0.92 | 0.041 | | | 0.94 | 0.025 | | | 0.90 | 0.033 | 0.94 | 0.0121 | 0.95 | 0.032 |
| Hypoxanthine | | | | | | | | | | | | | | | 1.36 | 0.0023 |
| Inosine | 0.82 | 0.002 | | | | | 1.19 | 0.00053 | | | | | | | | |
| Isoleucine | | | | | | | | | 0.85 | 0.005 | | | | | | |
| Leucine | | | | | | | | | 0.88 | 0.004 | | | | | | |
| Lactate | | | | | | | | | | | 0.90 | 0.00033 | 0.95 | 0.042 | 0.90 | 0.047 |
| Myo-inositol | 0.94 | 0.0048 | 0.94 | 0.021 | | | 0.96 | 0.0095 | | | 0.91 | 0.047 | 0.94 | 0.026 | 0.91 | 0.021 |
| N-acetylaspartate | | | | | 0.93 | 0.0074 | 0.95 | 0.039 | | | | | | | 0.91 | 0.015 |
| Phosphorylcholine | | | | | 1.08 | 0.0005 | 1.08 | 0.0019 | | | | | | | | |
| Propyleneglycol | | | 0.85 | 0.0019 | 0.85 | 0.00017 | 0.96 | 0.00033 | | | | | | | | |
| Taurine | | | 1.05 | 0.021 | 0.93 | 0.034 | 0.94 | 0.014 | | | | | 1.13 | 0.0027 | | |

Abbreviations: mFL: median part of frontal cortex – lFL: lateral part of frontal cortex – Cd: caudate nuclei – DH: dorsal hippocampus – A: amygdala – vHy: ventral part of the hypothalamus – dHy: dorsal part of the hypothalamus – CbV: cerebellar medial lobe.

(Kunz et al., 2011). Furthermore, in mice, perinatal exposure to BPA modulated GABA and glutamate concentrations (measured by LC-MS) in 9 cerebral regions including the hypothalamus, the amygdala and the hippocampus (Ogi et al., 2015). Taken together, these studies suggest a developmental effect of BPA on neural amino acids pathways involved in neural ontogenesis and plasticity as well as brain energy homeostasis.

Researchers have shown that gestational exposure to BPA disrupts thyroid homeostasis in dams and/or neonates of several species. Data on developmental hypothyroidism provide several lines of evidence that the neural amino-acids pathways are very sensitive to thyroid dysregulation. For example, similarly to what we observed in this study, the GABA concentration increased in the cerebellum of rat pups born from females maintained in a hypothyroid status from GD 1 to lactational day 21 (Ahmed et al., 2010). Researchers have also reported that the effect of thyroid disruption on neural amino acid pathways varies according to the target structure, the period of exposure and other parameters (Menezes et al., 2019; Sawano et al., 2013). These findings underscore the importance of examining different structures simultaneously, keeping in mind that the severity of the effect may vary not only with the structure but also with the dose.

Choline-related metabolites were also impacted by gestational BPA exposure in several fetal brain regions. Similarly, to glutamine, choline is a central node of the network linking neuro-mediators and metabolic pathways. In both parts of the frontal lobe, gestational exposure to BPA was associated with a decrease in choline. As both a precursor and a metabolite of acetylcholine, modification to the choline content might indicate a disruption in cholinergic neural pathways in both cortical areas and in the medial lobe of the cerebellum. In rats, perinatal exposure to BPA decreased acetylcholine and acetylcholine esterase in the prefrontal cortex and hippocampus (Xu et al., 2019). In sheep, the cholinergic system is functional in the near-term fetus (Shi et al., 2008). In addition, nitric oxide synthase—which produces nitric oxide, a second messenger of both the glutamatergic and cholinergic systems—varies depending on the brain region and gestational age in fetal sheep (Northington et al., 1997). Surprisingly, BPA did not affect cholinergic metabolites in the Cd, even though this neural pathway is highly represented in the caudate network. Metabolomics provides a "picture" of the metabolic state at a given time point. Therefore, the fact that we did not observe an effect on a given structure for a neural pathway, even though this pathway is predominant in the structure such as cholinergic pathway in the Cd, does not signify that gestational exposure to BPA might not have given rise to alterations of neural pathways in these structures at a different developmental age.

Choline, glycerophosphorylcholine, and phosphorylcholine are also involved in the biosynthesis of key lipids of the central nervous system such as sphingomyelins. The decrease in choline and/or glycerophosphorylcholine levels in several regions could be an indicator of altered membrane turnover and/or phospholipid incorporation in the membranes. The effects of BPA on lipid precursors that are critical for harmonious brain development highlight the need to couple metabolomic and lipidomic investigations when evaluating the impact of toxicants and/or thyroid disruptors on brain development.

Our results suggest BPA-induced remodeling of the metabolic network linking amino acid, and cholinergic pathways with energy and lipid metabolism in different cerebral areas of fetal lambs. The intricacy of these metabolic pathways is illustrated by the subnetwork analysis presented for two structures in Fig. 6.

Virtually all key processes of neurodevelopment have been described as potential targets of BPA (Mhaouty-Kodja et al., 2018) and many of these processes are dependent upon the pathways altered in our study (Welch and Mulligan, 2022). These processes and pathways are modulated by a variety of endocrine mediators including thyroid, estrogen, androgen, adrenal (Gilbert et al., 2007; Iacobas et al., 2013; Pozzi et al., 2020; Westerholz et al., 2010), as well as a more global physiological status of energy metabolism, oxidative stress (Brekke et al., 2015), and the immune system (Boulanger, 2009; Zhang et al., 2022). It thus

remains to be seen if the subtle modifications of the timeline of maternal TT4 we observed contribute to the BPA-induced modulations of metabolomic profiles in different brain areas. While suggestive of a potential thyroid mode of action for BPA, it is not possible at this stage to determine to what degree the evidenced metabolomic modulations may contribute to long-lasting alterations of behavioral and cognitive functions reported for BPA. Additional studies allowing to increase the panel of identified discriminant metabolites using for example high resolution MS thus improving the resolution of the network analysis would allow to refine the molecular mechanism at stake to mediate neurodevelopmental alteration due to *in utero* exposure to BPA. Moving toward implementation of multiomic approaches should enhance the diversity of molecular levels interrogated. Progress in both of these areas will significantly contribute to an integrated, concomitant fine analysis of molecular, neuronal, anatomical, and functional networks and improve predictions of potential neurobehavioral/cognitive consequences stemming from toxicant exposure.

*CRediT authorship contribution statement*

**Davy Guignard:** Investigation, Formal analysis, Writing – review & editing. **Cécile Canlet:** Investigation, Formal analysis, Writing – review & editing. **Marie Tremblay-Franco:** Formal analysis, Writing – review & editing. **Elodie Chaillou:** Writing – review & editing. **Roselyne Gautier:** Methodology. **Véronique Gayrard:** Investigation, Writing – review & editing. **Nicole Picard-Hagen:** Investigation, Writing – review & editing. **Henri Schroeder:** Writing – review & editing. **Fabien Jourdan:** Data curation. **Daniel Zalko:** Supervision, Funding acquisition, Writing – review & editing. **Catherine Viguié:** Conceptualization, Supervision, Writing – review & editing. **Nicolas J. Cabaton:** Conceptualization, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107336.

## References

Ahmed, O.M., Abd El-Tawab, S.M., Ahmed, R.G., 2010. Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: I. The development of the thyroid hormones–neurotransmitters and adenosinergic system interactions. Int. J. Dev. Neurosci. 28, 437–454. https://doi.org/10.1016/j.ijdevneu.2010.06.007.

Akinci, A., Sarac, K., Güngör, S., Mungan, I., Aydin, O., 2006. Brain MR spectroscopy findings in neonates with hypothyroidism born to mothers living in iodine-deficient areas. Am J Neuroradiol 27, 2083–2087.

Andrianou, X.D., Gängler, S., Piciu, A., Charisiadis, P., Zira, C., Aristidou, K., Piciu, D., Hauser, R., Makris, K.C., Carvalho, D.P.d., 2016. Human exposures to Bisphenol A, Bisphenol F and Chlorinated Bisphenol A derivatives and thyroid function. PloS One 11 (10). https://doi.org/10.1371/journal.pone.0155237.

Barry, J.S., Anthony, R.V., 2008. The pregnant sheep as a model for human pregnancy. Theriogenology 69, 55–67. https://doi.org/10.1016/j.theriogenology.2007.09.021.

Bellanger, M., Demeneix, B., Grandjean, P., Zoeller, R.T., Trasande, L., 2015. Neurobehavioral deficits, diseases, and associated costs of exposure to endocrine-disrupting chemicals in the European Union. J. Clin. Endocrinol. Metab. 100, 1256–1266. https://doi.org/10.1210/jc.2014-4323.

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Boulanger, L.M., 2009. Immune Proteins in Brain Development and Synaptic Plasticity. Neuron 64, 93–109. https://doi.org/10.1016/j.neuron.2009.09.001.

Braun, J.M., Kahn, R.S., Froehlich, T., Auinger, P., Lanphear, B.P., 2006. Exposures to Environmental Toxicants and Attention Deficit Hyperactivity Disorder in U.S. Children. Environ. Health Perspect. 114, 1904–1909. https://doi.org/10.1289/ehp.9478.

Brekke, E., Morken, T.S., Sonnewald, U., 2015. Glucose metabolism and astrocyte–neuron interactions in the neonatal brain. Neurochem. Int. 82, 33–41. https://doi.org/10.1016/j.neuint.2015.02.002.

Cabaton, N.J., Canlet, C., Wadia, P.R., Tremblay-Franco, M., Gautier, R., Molina, J., Sonnenschein, C., Cravedi, J.-P., Rubin, B.S., Soto, A.M., Zalko, D., 2013. Effects of low doses of Bisphenol A on the metabolome of perinatally exposed CD-1 mice. Environ Health Perspect 121 (5), 586–593.

Caporale, N., Leemans, M., Birgersson, L., Germain, P.-L., Cheroni, C., Borbély, G., Engdahl, E., Lindh, C., Bressan, R.B., Cavallo, F., Chorev, N.E., D'Agostino, G.A., Pollard, S.M., Rigoli, M.T., Tenderini, E., Tobon, A.L., Trattaro, S., Troglio, F., Zanella, M., Bergman, Å., Damdimopoulou, P., Jönsson, M., Kiess, W., Kitraki, E., Kiviranta, H., Nånberg, E., Öberg, M., Rantakokko, P., Rudén, C., Söder, O., Bornehag, C.-G., Demeneix, B., Fini, J.-B., Gennings, C., Rüegg, J., Sturve, J., Testa, G., 2022. From cohorts to molecules: Adverse impacts of endocrine disrupting mixtures. Science 375, eabe8244. https://doi.org/10.1126/science.abe8244.

Chazalviel, M., Frainay, C., Poupin, N., Vinson, F., Merlet, B., Gloaguen, Y., Cottret, L., Jourdan, F., 2018. MetExploreViz: web component for interactive metabolic network visualization. Bioinformatics 34, 312–313. https://doi.org/10.1093/bioinformatics/btx588.

Cherix, A., Donati, G., Lizarbe, B., Lanz, B., Poitry-Yamate, C., Lei, H., Gruetter, R., 2021. Excitatory/inhibitory neuronal metabolic balance in mouse hippocampus upon infusion of [U-13C6]glucose. J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab. 41, 282–297. https://doi.org/10.1177/0271678X20910535.

Cheung, G., Bataveljic, D., Visser, J., Kumar, N., Moulard, J., Dallérac, G., Mozheiko, D., Rollenhagen, A., Ezan, P., Mongin, C., Chever, O., Bemelmans, A.-P., Lübke, J., Leray, I., Rouach, N., 2022. Physiological synaptic activity and recognition memory require astroglial glutamine. Nat. Commun. 13, 753. https://doi.org/10.1038/s41467-022-28331-7.

Chevrier, J., Gunier, R.B., Bradman, A., Holland, N.T., Calafat, A.M., Eskenazi, B., Harley, K.G., 2013. Maternal urinary bisphenol A during pregnancy and maternal and neonatal thyroid function in the CHAMACOS study. Environ. Health Perspect. 121, 138–144. https://doi.org/10.1289/ehp.1205092.

Constantinou, C., Chrysanthopoulos, P.K., Margarity, M., Klapa, M.I., 2011. GC-MS metabolomic analysis reveals significant alterations in cerebellar metabolic physiology in a mouse model of adult onset hypothyroidism. J Proteome Res 10, 869–879. https://doi.org/10.1021/pr100699m.

Cottret, L., Wildridge, D., Vinson, F., Barrett, M.P., Charles, H., Sagot, M.-F., Jourdan, F., 2010. MetExplore: a web server to link metabolomic experiments and genome-scale metabolic networks. Nucleic Acids Res. 38, W132–W137. https://doi.org/10.1093/nar/gkq312.

Derakhshan, A., Philips, E.M., Ghassabian, A., Santos, S., Asimakopoulos, A.G., Kannan, K., Kortenkamp, A., Jaddoe, V.W.V., Trasande, L., Peeters, R.P., Korevaar, T.I.M., 2021. Association of urinary bisphenols during pregnancy with maternal, cord blood and childhood thyroid function. Environ. Int. 146, 106160 https://doi.org/10.1016/j.envint.2020.106160.

Ejaredar, M., Lee, Y., Roberts, D.J., Sauve, R., Dewey, D., 2017. Bisphenol A exposure and children's behavior: A systematic review. J. Expo. Sci. Environ. Epidemiol. 27, 175–183. https://doi.org/10.1038/jes.2016.8.

Elsworth, J.D., Jentsch, J.D., VandeVoort, C.A., Roth, R.H., Jr, D.E.R., Leranth, C., 2013. Prenatal exposure to bisphenol A impacts midbrain dopamine neurons and hippocampal spine synapses in non-human primates. NeuroToxicology 35, 113–120. https://doi.org/10.1016/j.neuro.2013.01.001.

Faust, K., van Helden, J., 2012. Predicting Metabolic Pathways by Sub-network Extraction. Bact. Mol. Netw. Methods Protoc., Methods in Molecular Biology. https://doi.org/10.1007/978-1-61779-361-5_7.

Fernandez, M.O., Bourguignon, N.S., Arocena, P., Rosa, M., Libertun, C., Lux-Lantos, V., 2018. Neonatal exposure to bisphenol A alters the hypothalamic-pituitary-thyroid

axis in female rats. Toxicol. Lett. 285, 81–86. https://doi.org/10.1016/j.toxlet.2017.12.029.
Fisher, D.A., 1991. Thyroid system ontogeny in the sheep: a model for precocial mammalian species. Adv Exp Med Biol 299, 11–26.
Frainay, C., Jourdan, F., 2017. Computational methods to identify metabolic sub-networks based on metabolomic profiles. Brief. Bioinform. 18 (1), 43–56.
Gauderat, G., Picard-Hagen, N., Toutain, P.-L., Servien, R., Viguié, C., Puel, S., Lacroix, M.Z., Corbel, T., Bousquet-Melou, A., Gayrard, V., 2017. Prediction of human prenatal exposure to bisphenol A and bisphenol A glucuronide from an ovine semi-physiological toxicokinetic model. Sci. Rep. 7, 15330. https://doi.org/10.1038/s41598-017-15646-5.
Gaylord, A., Osborne, G., Ghassabian, A., Malits, J., Attina, T., Trasande, L., 2020. Trends in neurodevelopmental disability burden due to early life chemical exposure in the USA from 2001 to 2016: A population-based disease burden and cost analysis. Molecular and Cellular Endocrinology 502, 110666.
Gerona, R.R., Woodruff, T.J., Dickenson, C.A., Pan, J., Schwartz, J.M., Sen, S., Friesen, M.W., Fujimoto, V.Y., Hunt, P.A., 2013. Bisphenol-A (BPA), BPA glucuronide, and BPA sulfate in midgestation umbilical cord serum in a northern and central California population. Environ. Sci. Technol. 47, 12477–12485. https://doi.org/10.1021/es402764d.
Gilbert, M.E., Sui, L., Walker, M.J., Anderson, W., Thomas, S., Smoller, S.N., Schon, J.P., Phani, S., Goodman, J.H., 2007. Thyroid Hormone Insufficiency during Brain Development Reduces Parvalbumin Immunoreactivity and Inhibitory Function in the Hippocampus. Endocrinology 148, 92–102. https://doi.org/10.1210/en.2006-0164.
Gorini, F., Bustaffa, E., Coi, A., Iervasi, G., Bianchi, F., 2020. Bisphenols as environmental triggers of thyroid dysfunction: clues and evidence. Int. J. Environ. Res. Public. Health 17, 2654. https://doi.org/10.3390/ijerph17082654.
Grohs, M.N., Reynolds, J.E., Liu, J., Martin, J.W., Pollock, T., Lebel, C., Dewey, D., 2019. Prenatal maternal and childhood bisphenol A exposure and brain structure and behavior of young children. Environ. Health Glob. Access Sci. Source 18 (1). https://doi.org/10.1186/s12940-019-0528-9.
Guignard, D., Gayrard, V., Lacroix, M.Z., Puel, S., Picard-Hagen, N., Viguié, C., 2017. Evidence for bisphenol A-induced disruption of maternal thyroid homeostasis in the pregnant ewe at low level representative of human exposure. Chemosphere 182, 458–467. https://doi.org/10.1016/j.chemosphere.2017.05.028.
Hertz, L., Chen, Y., 2017. Integration between glycolysis and glutamate-glutamine cycle flux may explain preferential glycolytic increase during brain activation, requiring glutamate. Front. Integr. Neurosci. https://doi.org/10.3389/fnint.2017.00018.
Hertz, L., Rothman, D.L., 2016. Glucose, lactate, β-hydroxybutyrate, acetate, GABA, and succinate as substrates for synthesis of glutamate and GABA in the glutamine–glutamate/GABA cycle, in: Schousboe, A., Sonnewald, U. (Eds.), The Glutamate/GABA-Glutamine Cycle, Advances in Neurobiology. Springer International Publishing, Cham, pp. 9–42. https://doi.org/10.1007/978-3-319-45096-4_2.
Iacobas, D.A., Iacobas, S., Chachua, T., Goletiani, C., Sidyelyeva, G., Velíšková, J., Velíšek, L., 2013. Prenatal Corticosteroids Modify Glutamatergic and GABAergic Synapse Genomic Fabric: Insights from a Novel Animal Model of Infantile Spasms. J. Neuroendocrinol. 25, 964–979. https://doi.org/10.1111/jne.12061.
Johnson, J.I., Sudheimer, Keith D., Davis, Kristina K., Kerndt, Garrett M., Winn, Brian M, n.d. The sheep brain atlas.
Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., Morishima, K., 2017. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 45, D353–D361. https://doi.org/10.1093/nar/gkw1092.
Kunz, N., Camm, E.J., Somm, E., Lodygensky, G., Darbre, S., Aubert, M.L., Hueppi, P.S., Sizonenko, S.V., Gruetter, R., 2011. Developmental and metabolic brain alterations in rats exposed to bisphenol A during gestation and lactation. Int. J. Dev. Neurosci. 29, 37–43. https://doi.org/10.1016/j.ijdevneu.2010.09.009.
Lacroix, M.Z., Puel, S., Collet, S.H., Corbel, T., Picard-Hagen, N., Toutain, P.L., Viguié, C., Gayrard, V., 2011. Simultaneous quantification of bisphenol A and its glucuronide metabolite (BPA-G) in plasma and urine: applicability to toxicokinetic investigations. Talanta 85, 2053–2059. https://doi.org/10.1016/j.talanta.2011.07.040.
Leranth, C., Hajszan, T., Szigeti-Buck, K., Bober, J., MacLusky, N.J., 2008. Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates. Proc. Natl. Acad. Sci. 105, 14187–14191. https://doi.org/10.1073/pnas.0806139105.
Lin, C.-C., Chien, C.-J., Tsai, M.-S., Hsieh, C.-J., Hsieh, W.-S., Chen, P.-C., 2017. Prenatal phenolic compounds exposure and neurobehavioral development at 2 and 7years of age. Sci. Total Environ. 605–606, 801–810. https://doi.org/10.1016/j.scitotenv.2017.06.160.
Maga-Nteve, C., Vasilopoulou, C.G., Constantinou, C., Margarity, M., Klapa, M.I., 2017. Sex-comparative study of mouse cerebellum physiology under adult-onset hypothyroidism: The significance of GC-MS metabolomic data normalization in meta-analysis. J. Chromatogr. B-Anal. Technol. Biomed. Life Sci. 1041, 158–166. https://doi.org/10.1016/j.jchromb.2016.12.016.
Mathisen, G.H., Yazdani, M., Rakkestad, K.E., Aden, P.K., Bodin, J., Samuelsen, M., Nygaard, U.C., Goverud, I.L., Gaarder, M., Løberg, E.M., Bølling, A.K., Becher, R., Paulsen, R.E., 2013. Prenatal exposure to bisphenol A interferes with the development of cerebellar granule neurons in mice and chicken. Int. J. Dev. Neurosci. Off. J. Int. Soc. Dev. Neurosci. 31, 762–769. https://doi.org/10.1016/j.ijdevneu.2013.09.009.
Menassol, J.-B., Tautou, C., Collet, A., Chesneau, D., Lomet, D., Dupont, J., Malpaux, B., Scaramuzzi, R.J., 2011. The effect of an intracerebroventricular injection of metformin or AICAR on the plasma concentrations of melatonin in the ewe: potential involvement of AMPK? BMC Neurosci. 12, 76. https://doi.org/10.1186/1471-2202-12-76.
Menezes, E.C., Santos, P.R., Goes, T.C., Barboza Carvalho, V.C., Teixeira-Silva, F., Stevens, H.E., Badaue-Passos Junior, D.Jr., 2019. Effects of a rat model of gestational hypothyroidism on forebrain dopaminergic, GABAergic, and serotonergic systems and related behaviors. Behav. BRAIN Res. https://doi.org/10.1016/j.bbr.2019.03.027.
Mhaouty-Kodja, S., Belzunces, L.P., Canivenc, M.-C., Schroeder, H., Chevrier, C., Pasquier, E., 2018. Impairment of learning and memory performances induced by BPA: evidences from the literature of a MoA mediated through an ED. Mol. Cell. Endocrinol. 475, 54–73.
Mustieles, V., D'Cruz, S.C., Couderq, S., Rodríguez-Carrillo, A., Fini, J.-B., Hofer, T., Steffensen, I.-L., Dirven, H., Barouki, R., Olea, N., Fernández, M.F., David, A., 2020. Bisphenol A and its analogues: A comprehensive review to identify and prioritize effect biomarkers for human biomonitoring. Environ. Int. 144, 105811 https://doi.org/10.1016/j.envint.2020.105811.
Nakamura, K., Itoh, K., Yoshimoto, K., Sugimoto, T., Fushiki, S., 2010. Prenatal and lactational exposure to low-doses of bisphenol A alters brain monoamine concentration in adult mice. Neurosci. Lett. 484 (1), 66–70.
Northington, F.J., Tobin, J.R., Harris, A.P., Traystman, R.J., Koehler, R.C., 1997. Developmental and regional differences in nitric oxide synthase activity and blood flow in the sheep brain. J. Cereb. Blood Flow Metab Off. J. Int. Soc. Cereb. Blood Flow Metab. 17, 109–115. https://doi.org/10.1097/00004647-199701000-00014.
Ogi, H., Itoh, K., Ikegaya, H., Fushiki, S., 2015. Alterations of neurotransmitter norepinephrine and gamma-aminobutyric acid correlate with murine behavioral perturbations related to bisphenol A exposure. Brain Dev. 37, 739–746. https://doi.org/10.1016/j.braindev.2014.12.008.
O'Shaughnessy, K.L., Fischer, F., Zenclussen, A.C., 2021. Perinatal exposure to endocrine disrupting chemicals and neurodevelopment: How articles of daily use influence the development of our children. Best Pract. Res. Clin. Endocrinol. Metab. 35 (5) https://doi.org/10.1016/j.beem.2021.101568.
Park, C., Choi, W., Hwang, M., Lee, Y., Kim, S., Yu, S., Lee, I., Paek, D., Choi, K., 2017. Associations between urinary phthalate metabolites and bisphenol A levels, and serum thyroid hormones among the Korean adult population - Korean National Environmental Health Survey (KoNEHS) 2012–2014. Sci. Total Environ. 584–585, 950–957. https://doi.org/10.1016/j.scitotenv.2017.01.144.
Pozzi, D., Rasile, M., Corradini, I., Matteoli, M., 2020. Environmental regulation of the chloride transporter KCC2: switching inflammation off to switch the GABA on? Transl. Psychiatry 10, 349. https://doi.org/10.1038/s41398-020-01027-6.
Rebolledo-Solleiro, D., 2021. Impact of BPA on behavior, neurodevelopment and neurodegeneration. Front. Biosci. 26, 363–400. https://doi.org/10.2741/4898.
Rosique, C., Lebsir, D., Lestaevel, P., Benatia, S., Guigon, P., Caire-Maurisier, F., Benderitter, M., Bennouna, D., Souidi, M., Martin, J.-C., 2019. Assessment of the effects of repeated doses of potassium iodide intake during pregnancy on male and female rat offspring using metabolomics and lipidomics. J. Toxicol. Environ. Health A 82, 603–615. https://doi.org/10.1080/15287394.2019.1625474.
Sawano, E., Takahashi, M., Negishi, T., Tashiro, T., 2013. Thyroid hormone-dependent development of the GABAergic pre- and post-synaptic components in the rat hippocampus. Int. J. Dev. Neurosci. 31, 751–761. https://doi.org/10.1016/j.ijdevneu.2013.09.007.
Shen, H., Goldberg, M.P., 2012. Creatine pretreatment protects cortical axons from energy depletion in vitro. Neurobiol. Dis. 47, 184–193. https://doi.org/10.1016/j.nbd.2012.03.037.
Shi, L., Mao, C., Zeng, F., Zhang, Y., Xu, Z., 2008. Central cholinergic signal-mediated neuroendocrine regulation of vasopressin and oxytocin in ovine fetuses. BMC Dev. Biol. 8, 95. https://doi.org/10.1186/1471-213X-8-95.
Tonini, C., Segatto, M., Gagliardi, S., Bertoli, S., Leone, A., Barberio, L., Mandala, M., Pallottini, V., 2020. Maternal dietary exposure to low-dose bisphenol A affects metabolic and signaling pathways in the brain of rat fetuses. Nutrients 12, 1448. https://doi.org/10.3390/nu12051448.
Tremblay-Franco, M., Cabaton, N.J., Canlet, C., Gautier, R., Schaeberle, C.M., Jourdan, F., Sonnenschein, C., Vinson, F., Soto, A.M., Zalko, D., Gibert, Y., 2015. Dynamic metabolic disruption in rats perinatally exposed to low doses of Bisphenol-A. PLOS ONE 10 (10). https://doi.org/10.1371/journal.pone.0141698.
Viguié, C., Chaillou, E., Gayrard, V., Picard-Hagen, N., Fowler, P.A., 2020. Toward a better understanding of the effects of endocrine disrupting compounds on health: Human-relevant case studies from sheep models. Mol. Cell. Endocrinol. 505, 110711 https://doi.org/10.1016/j.mce.2020.110711.
Viguié, C., Collet, S.H., Gayrard, V., Picard-Hagen, N., Puel, S., Roques, B.B., Toutain, P.-L., Lacroix, M.Z., 2013. Maternal and fetal exposure to bisphenol a is associated with alterations of thyroid function in pregnant ewes and their newborn lambs. Endocrinology 154, 521–528. https://doi.org/10.1210/en.2012-1401.
Welch, C., Mulligan, K., 2022. Does bisphenol A confer risk of neurodevelopmental disorders? What we have learned from developmental neurotoxicity studies in animal models. Int. J. Mol. Sci. 23, 2894. https://doi.org/10.3390/ijms23052894.
Westerholz, S., de Lima, A.D., Voigt, T., 2010. Regulation of early spontaneous network activity and GABAergic neurons development by thyroid hormone. Neuroscience 168, 573–589. https://doi.org/10.1016/j.neuroscience.2010.03.039.
Wood, C.E., Giroux, D., Gridley, K., 2003. Fetal brain regional responses to cerebral hypoperfusion: modulation by estrogen. Brain Res. 993, 84–89. https://doi.org/10.1016/j.brainres.2003.09.001.
Xu, S., Liu, Y., Pu, J., Gui, S., Zhong, X., Tian, L., Song, X., Qi, X., Wang, H., Xie, P., 2020. Chronic stress in a rat model of depression disturbs the glutamine–glutamate–GABA cycle in the striatum, hippocampus, and cerebellum. Neuropsychiatr. Dis. Treat. 16, 557–570. https://doi.org/10.2147/NDT.S245282.
Xu, X., Fan, S., Guo, Y., Tan, R., Zhang, J., Zhang, W., Pan, B.-X., Kato, N., 2019. The effects of perinatal bisphenol A exposure on thyroid hormone homeostasis and

glucose metabolism in the prefrontal cortex and hippocampus of rats. Brain Behav. 9 (3) https://doi.org/10.1002/brb3.1225.

Yao, J., Wang, J., Wu, L., Lu, H., Wang, Z., Yu, P., Xiao, H., Gao, R., Yu, J., 2020. Perinatal exposure to bisphenol A causes a disturbance of neurotransmitter metabolic pathways in female mouse offspring: A focus on the tryptophan and dopamine pathways. CHEMOSPHERE 254. https://doi.org/10.1016/j.chemosphere.2020.126715.

Zalko, D., Soto, A.M., Canlet, C., Tremblay-Franco, M., Jourdan, F., Cabaton, N.J., 2016. Bisphenol A exposure disrupts neurotransmitters through modulation of transaminase activity in the brain of rodents. Endocrinology 157, 1736–1739. https://doi.org/10.1210/en.2016-1207.

Zhang, H., Yang, R., Shi, W., Zhou, X., Sun, S., 2022. The association between bisphenol A exposure and oxidative damage in rats/mice: a systematic review and meta-analysis. Environ. Pollut. Barking Essex 1987 (292), 118444. https://doi.org/10.1016/j.envpol.2021.118444.