



Bisphenol A in utero induced glutamate and D-serine metabolic dysregulation in the hippocampus of rats and primary cultured astrocytes

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ABSTRACT

Bisphenol A (BPA) is a widely used synthetic compound that could cause neurobehavioral abnormalities in mammals. Previous studies have suggested that NMDAR may be a potential target of BPA-induced neurotoxicity. However, the impact of exposure to BPA on glutamate (Glu) and D-serine (D-ser) metabolism (key regulators of NMDAR activation) has not been clarified yet. Pregnant SD rats were exposed to 0, 0.05, 0.5, 5 and 50 mg/kg/day BPA via oral gavage from gestational day (GD) 5 to GD 19, and primary cultured astrocytes (AS) from neonatal rats were exposed to 5, 10 and 20 $\mu\text{mol/L}$ BPA, respectively. Neurochemical experiments were conducted to investigate dysfunctions in Glu and D-ser metabolism both in the hippocampus of rats' offspring at different developmental stages and in AS. Results showed that BPA exposure in utero induced Glu accumulation and inhibited GS, GLS1, and GDH expression and activity at the gene, protein, and enzymatic levels in the hippocampus of rats' offspring during different developmental stages (GD 20, PND 21, and PND 56). However, BPA exposure in utero increased D-ser levels at GD 20 but decreased them from PND 21 onward. Additionally, BPA exposure in utero inhibited SR, asc-1, and ASCT2 expression, while promoting ASCT1 expression during these stages. Besides, BPA exposure in utero up-regulated DAO expression at GD 20 but down-regulated it from PND 21 onward. Moreover, BPA exposure inhibited the expression and activity of GS (except for its expression), GLS1, and GDH, while suppressing SR and DAO expression but increasing ASCT2 expression without altering ASCT21 expression in AS. These findings will provide new insights into the pathogenesis of BPA-induced neurotoxicity through Glu and D-ser metabolic pathways.

1. Introduction

Bisphenol A (BPA) remains one of the most extensively produced industrial chemicals globally, primarily serving as a plasticizer in numerous consumer products including: plastic bottles, infant toys, food packaging materials, thermal papers and so on (Tarafdar et al., 2022; Wang et al., 2023a; Ghahremani et al., 2024). Numerous studies have demonstrated BPA's potential to exert toxic effects on both humans and animals, as well as environmental contamination (Rahman et al., 2021; Costa and Cairrao, 2024; Mukherjee et al., 2024). Epidemiological data revealed detectable BPA in > 90 % of human urine specimens (Jiang et al., 2024), near 3.2 ng/ml serum levels in residents living near e-waste

facilities (Song et al., 2019), and close to 0.91 ng/g levels in postmortem brain specimens (Geens et al., 2012). BPA readily crosses the blood-brain barrier (BBB) (Hu et al., 2017; Liu et al., 2023), rendering the central nervous system (CNS) particularly vulnerable. Consequently, BPA-induced neurological impairments, including synaptic abnormalities and memory dysfunction, have emerged as major research focuses (Meng et al., 2023; Wang et al., 2023b). Furthermore, prenatal urinary BPA concentration were associated with some aspects of Children's neurodevelopment and behavior (Jiang et al., 2020; Braun et al., 2017) and animal studies have also confirmed that in utero exposure to BPA could cause abnormal neural development in offspring (López-Moreno et al., 2025; Yu et al., 2020). Growing recognition of

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BPA's adverse effects has prompted its phase-out in numerous countries and replacement with structural analogs such as bisphenol S (BPS), bisphenol F (BPF), and bisphenol AP (BPAP) (Xiao et al., 2018; Liu and Martin, 2019). They retain the biphenolic structure, change the functional group and used as BPA alternatives in plasticizers. However, emerging evidence indicates these BPA alternatives may similarly induce various adverse effects, particularly neurotoxicity, in both humans and animals (Yuan et al., 2019; Wu et al., 2023). The substantial existing data on BPA provided a foundation for elucidating its neurotoxic mechanisms, while also offering valuable insights for assessing the toxicity of other bisphenol analogs.

The N-methyl-D-aspartate receptor (NMDAR) plays a pivotal role in synaptic plasticity and learning and memory ability. Accumulating evidence from our and other studies demonstrated that BPA exposure alters NMDAR subunit (NR1, NR2A, and NR2B) expressions (Xu et al., 2010, 2011; Yu et al., 2020). These findings collectively suggested NMDAR as a key molecular target in BPA-induced neurodevelopmental abnormalities. Proper NMDAR function requires both adequate subunit expressions and agonist binding for channel activation. Data have shown that NMDAR activation requires simultaneous binding of glutamate (Glu) and a co-agonist, with D-serine (D-ser) being the predominant endogenous co-agonist (Wolosker and Radziszewsky, 2013; Bodner et al., 2020). As a result, this study represents the first investigation of BPA's neurotoxic effects on D-ser metabolism and provides the first systematic analysis of the mechanisms underlying BPA-induced Glu metabolic dysregulation in rat offspring during early development stages following in utero exposure.

Glu, among the most prevalent amino acids in the mammalian brain, is predominantly released from presynaptic neurons and activates postsynaptic Glu receptors, including NMDAR, thereby mediating rapid excitatory neurotransmission and modulating synaptic plasticity. However, excessive Glu release may over-activate postsynaptic Glu receptors, triggering calcium influx, intracellular Ca^{2+} overload, sustained neuronal excitation, and ultimately irreversible neurotoxicity (Molz et al., 2008). The Glu-Gln cycle is essential for maintaining synaptic Glu homeostasis, and requires coordinated astrocyte-neuron interactions (Andersen et al., 2021). Synaptic Glu is primarily taken up by astrocytes (AS) via Glu transporters (Schreiner et al., 2014), converted to Gln by glutamine synthetase (GS) (Bak et al., 2006), and subsequently released for neuronal uptake, where glutaminase (GLS) reconverts Gln to Glu, thus completing the cycle (Márquez et al., 2016). Additionally, glutamate dehydrogenase (GDH) in AS contributes to Glu clearance, working in concert with GS and GLS to maintain Glu homeostasis (Kugler and Schleyer, 2004).

Recently, D-ser has attracted considerable scientific interest due to its critical role as an NMDAR co-agonist. Abnormal D-ser levels have been associated with various neurological disorders, including Alzheimer's disease, schizophrenia, and anxiety disorders (Cho et al., 2016; Ishiwata et al., 2018; Wolosker and Balu, 2020). Therefore, maintaining D-ser homeostasis is essential for normal brain function. Although the complete mechanism of hippocampal D-ser metabolism remains unclear, Wolosker's "serine shuttle" hypothesis has gained widespread acceptance (Wolosker, 2011; Wolosker et al., 2016). This hypothesis posits that AS synthesize and release L-serine (L-ser), the essential precursor for D-ser production, then, L-ser release into the synaptic cleft, and absorbed by neuron, where neuronal serine racemase (SR) converts it to D-ser (Wolosker et al., 1999). D-ser catabolism occurs through two primary pathways: (1) oxidative deamination by D-amino acid oxidase (DAAO) (Kuo et al., 2022), and (2) SR-mediated α,β -elimination, yielding pyruvate and ammonia (Foltn et al., 2005). However, SR's L-ser-to-D-ser conversion rate substantially exceeds its α,β -elimination activity, confirming D-ser synthesis as its predominant function (Strisovsky et al., 2005). In addition, extracellular D-ser requires specialized transporters for cellular uptake, with its concentration regulated by Na^+ -dependent alanine-serine-cysteine-threonine transporter, such as ASCT1/ASCT2 (Slc1A4/5), and Na^+ -independent

alanine-serine-cysteine transporter, such as asc-1 (Slc7a10) (Fukasawa et al., 2000; Ribeiro et al., 2002; Rosenberg et al., 2013). ASCT1/ASCT2 are predominantly expressed in AS, whereas asc-1 is primarily neuronal, collectively constituting an essential regulatory system for hippocampal D-ser metabolism. In summary, hippocampal D-ser homeostasis depends on the coordinated function of SR, DAAO, and ASCT1/ASCT2/asc-1 transporter system.

As evidenced by these findings, proper Glu and D-ser metabolism are essential for NMDAR activation. AS are now recognized as central regulators of both Glu and D-ser metabolism, as demonstrated by accumulating evidence. Furthermore, AS interface with cerebral capillary endothelial cells, forming an integral component of BBB and maintaining cerebral extracellular homeostasis through selective substance exchange (Schiera et al., 2024). Given BPA's ability to cross the BBB, AS are likely among the first neural cells affected. We hypothesize that BPA exerts neurotoxicity primarily through AS-mediated dysregulation of Glu and D-ser metabolism. Existing studies report conflicting findings regarding BPA's effects on hippocampal Glu levels in rodents, with reports of downregulation (Cabaton et al., 2013), upregulation (Zhang et al., 2019) and no significant change (Meng et al., 2023). However, the precise metabolic sites, underlying mechanisms of BPA-induced Glu dysregulation, and D-ser metabolic alterations remain poorly understood. This study therefore investigates BPA's effects on Glu and D-ser metabolism using both in vivo (in utero stage) and in vitro (primary cultured AS) methods, aiming to identify initial targets and elucidate mechanisms underlying BPA-induced cognitive impairment.

2. Materials and methods

2.1. Materials

The main reagents, antibodies and kits used in this study were listed in Table S1.

2.2. Animals and treatment procedures

This research obtained the permission from the Ethics Committee of Shenyang Medical College (No.SYYXY2021030801). SD Rats were purchased from Liaoning Changsheng biotechnology (Certificate number: SCXK2020-0001, Liaoning, China), and the feeding and treatment were performed according to our previously published paper (Yu et al., 2020). Briefly, sixty pregnant rats were treated with corn oil, 0.05 (Tolerable daily intake, TDI), 0.5 (ten times higher than TDI), 5 (No observed adverse effect level, NOAEL) and 50 mg/kg/day (Lowest observed adverse effect level, LOAEL) of BPA previously set by U.S. EPA and WHO (U.S. EPA, 2010; WHO, 2009) from gestational day 5 (GD 5) to GD 19 through gavage, with twelve rats in each group after pregnancy confirmed. The brain and hippocampal tissues of fetal pups (half male and half female) at GD 20, postnatal day 21 (PND 21) and PND 56 (after Morris water maze test) were separated for the following tests.

2.3. Morris water maze

Six pups of each group (half male and half female) at PND 56 were selected for the Morris water maze test. Briefly, after pre-training, rats were performed successive five training days for place trials, and the escape latencies were noted and analyzed. Then, rats were conducted the probe for one day after place trials, and the time spent in the target quadrant with the search strategies were recorded and analyzed. The details described in Supplementary Materials.

2.4. AS cultures

Brains of neonatal (1–3 days) rats purchased from Liaoning Changsheng biotechnology (Certificate number: SCXK2020-0001, Liaoning, China) were used for primary AS cultures and the details were

performed according to the published paper (Wang et al., 2021) with the detailed information described in [Supplementary Materials](#).

2.5. Immunofluorescence staining

Briefly, cells attached on the glass chamber were fixed with 4 % paraformaldehyde for 20 min, and then incubated in 0.2 % Triton X-100 for 10 min. The cells were subsequently incubated with the blocking solution containing normal goat serum for 30 min, and with primary rabbit anti-GFAP antibody at 4 °C overnight followed by a incubation with goat anti-rabbit-Alexa Fluor 594 secondary antibody for 45 min at 37 °C. After counterstaining with DAPI, cells were observed and captured under a fluorescence microscope (Nikon ECLIPSE Ci-E) and a digital camera system (Nikon DS-Fi3).

2.6. Quantification of cell viability

AS were exposed to culture medium (as blank control group), 0.1 % DMSO (as solvent control group), 2.5, 5, 10, 20, 40, or 200 μ mol/L BPA for 12 h, 24 h, or 48 h. Cell viability was quantitatively evaluated and calculated by CCK-8 assay as the kit's instruction.

2.7. H&E staining

Three pups rats (two male pups and one female pup) from each group at PND 21 and PND 56 were deeply anesthetized and heart perfusion with 4 % paraformaldehyde. The brain tissues of rats were separated and fixed immediately with 4 % paraformaldehyde, then embedded in paraffin. Section (5 μ m) were collected, stained with hematoxylin and eosin (H&E) according to the kit's instruction and captured.

2.8. UHPLC-MS/MS

Total Glu, Gln, L-ser and D-ser levels in the hippocampus of rats (six pups in each group with half male and half female at each developmental point) were quantified by an UHPLC-MS/MS, and the detailed process was shown in [Supplementary Materials](#).

2.9. Real-time PCR

Six rats' hippocampal tissues (with half male and half female at each developmental point) and four AS (primary cultured 4 batches from different neonatal rats) in each group were used to detect the mRNA levels of *GS*, *GLS1*, *GDH*, *SR*, *DAAO*, *asc-1*, *ASCT1*, *ASCT2* and *GAPDH* (as internal control). The method of real-time PCR described in our published paper (Yu et al., 2020). The primer details of these genes were summarized in [Table S2](#).

2.10. Western blot

Six rats' hippocampal tissues (with half male and half female at each developmental point) and four AS (primary cultured 4 batches from different neonatal rats) in each group were used to detect the protein levels of *GS*, *GLS1*, *GDH*, *SR*, *DAAO*, *asc-1*, *ASCT1* and *ASCT2*. 50 μ g per lane for hippocampal sample and 30 μ g per lane for AS sample were performed western blot analysis as our published paper (Yu et al., 2020). The incubation ratios of primary antibodies were *GS* (1:1000), *GLS1* (1:1000), *GDH* (1:1000), *SR* (1:1000), *DAAO* (1:500), *asc-1* (1:1000), *ASCT1* (1:1000), *ASCT2* (1:1000), β -actin (1:2000) and secondary antibodies were (1:5000), respectively.

2.11. GS, GLS and GDH activities

Six rats' hippocampal tissues (with half male and half female at each developmental point) and four AS (primary cultured 4 batches from different neonatal rats) in each group were used for detecting *GS*, *GLS*,

GDH activities as the instruction of commercial kits.

2.12. Statistical analysis

All data were presented as mean $\pm$ standard deviation (SD) and assessed for normality and variance similarity using the Shapiro-Wilk test and the Levene's test, respectively. If data exhibited normal distribution and homogeneity of variance, the statistical differences among groups were assessed by One-way ANOVA with least significant difference (LSD) multiple comparisons performed to evaluate the differences among groups. However, the Kruskal-Wallis test was used for data with non-normal distribution or non-homogeneity of variance, followed by Dunn's multiple comparisons test. All the statistical analysis were performed using the SPSS software, version 22.0 (SPSS Inc., Illi-nois, USA), $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Exposure to BPA in utero caused abnormal general growth of rats after birth and induced learning and memory ability dysfunctions of PND 56 rats

The results demonstrated that in utero exposure to 5 and 50 mg/kg/day BPA induced significant weight loss of rats only at GD 20 ([Fig. 1A](#)). However, BPA exposure did not cause comparable body weight reduction at PND 21 or PND 56, nor did it affect hippocampal weights at any of the three developmental stages examined ([Fig. 1A-B](#)).

The Morris water maze test results demonstrated that in utero exposure to 5 and 50 mg/kg/day BPA significantly prolonged the escape latency of rats in the place trials starting from the fourth training day ([Fig. 1C](#)), accompanied by significantly reduced time spent in the target quadrant during the probe trial ([Fig. 1D-E](#)).

3.2. Exposure to BPA in utero caused abnormal morphological structure in hippocampus of PND 21 and 56 rats

Representative H&E staining images of rat hippocampal structures at PND 21 and 56 were presented in [Fig. 2](#). Microscopic examination revealed that hippocampal neurons in all three regions of the control group exhibited compact organization with typical morphology and tight arrangement. However, neuronal edema and disorganized pyramidal cell layers were evident in both CA3 and DG hippocampal regions mainly in 50 mg/kg/day group at PND 21 and 56. Neuronal morphological abnormalities were observed in CA1, CA3, and DG regions following 5 and 50 mg/kg/day BPA exposure (more obvious in 50 mg/kg/day group). Additionally, inflammatory cell infiltration was detected specifically in the DG regions in 5 mg/kg/day BPA groups at PND 21.

3.3. Exposure to BPA in utero eventuated the alternations of Glu, Gln, L-ser and D-ser levels in the hippocampus of rats

As shown in [Fig. 3A-B](#), hippocampal Glu and Gln levels were significantly elevated following in utero BPA exposure, except for Gln levels in the 0.05–5 mg/kg/day BPA groups at PND 21. Hippocampal L-ser levels were significantly increased in all BPA-exposed groups at GD 20 and in high-dose groups (5 and 50 mg/kg/day) at PND 21 ([Fig. 3C](#)). In contrast, L-ser levels were significantly reduced across all BPA-exposed groups at PND 56 ([Fig. 3C](#)). D-ser level alterations following BPA exposure exhibited patterns similar to L-ser changes ([Fig. 3D](#)). D-ser levels were significantly elevated in the 0.5–50 mg/kg/day BPA groups relative to controls at GD 20 ([Fig. 3D](#)). Conversely, BPA exposure significantly suppressed hippocampal D-ser levels at both PND 21 and 56 ([Fig. 3D](#)).

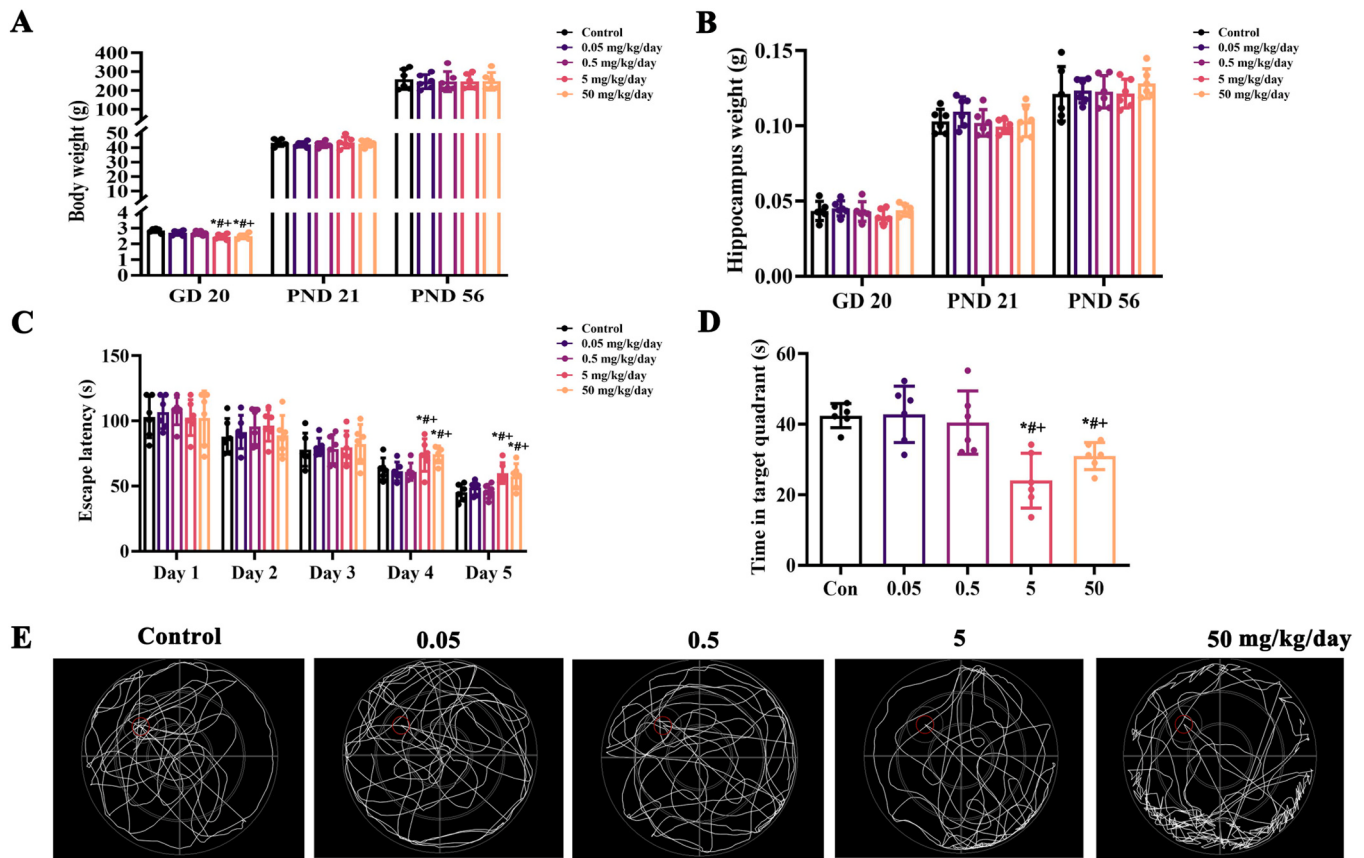


Fig. 1. Changes in the general growth condition and learning and memory ability of rats induced by exposure to BPA in utero. (A) Body weight. (B) Hippocampus weight. (C) Escape latency of rats in place trail. (D) Time spent in the target quadrant of rats in probe trail. (E) Typical track map of rats from each group in probe trail. (Mean $\pm$ SD, $n = 6$ per group; * indicated significance with control group; # indicated significance with 0.05 mg/kg/day BPA group; + indicated significance with 0.5 mg/kg/day BPA group; $P < 0.05$).

3.4. Exposure to BPA in utero led to Glu metabolism disorders in the hippocampus of rats at various growth periods

Analysis of hippocampal GS mRNA expression revealed significant reductions in the 50 mg/kg/day BPA group at GD 20 and PND 21, and all BPA groups at PND 56 (Fig. 4A). Similarly, Fig. 4D-E demonstrated that hippocampal GS protein levels were significantly decreased across all tested developmental stages following BPA exposure (0.05–50 mg/kg/day). Furthermore, GS enzymatic activity assays showed significant decreases in all BPA groups at GD 20, and the 50 mg/kg/day group at both PND 21 and 56 (Fig. 4F). Fig. 4B demonstrated that hippocampal GLS1 mRNA levels were significantly reduced across all developmental stages following in utero BPA exposure (0.05–50 mg/kg/day). Additionally, Figs. 4D and 4G showed that hippocampal GLS1 protein expressions were significantly decreased in the 50 mg/kg/day group at GD 20 and PND 21, and the 0.5–50 mg/kg/day groups at PND 56. Consistent with GLS1 protein changes, GLS1 enzymatic activities were significantly reduced in the 5 and 50 mg/kg/day groups at GD 20 and PND 21, and the 50 mg/kg/day group at PND 56 (Fig. 4H).

Fig. 4C showed that while in utero BPA exposure did not alter hippocampal GDH mRNA expressions at GD 20, significant reductions were observed at both PND 21 and 56 across all BPA doses (0.05–50 mg/kg/day). Moreover, although hippocampal GDH protein expressions remained unchanged at GD 20, significant decreases were detected in the 50 mg/kg/day group at PND 21, and all BPA groups at PND 56 (Fig. 4D and 4I). Additionally, GDH enzymatic activities were significantly reduced in the 5 mg/kg/day group at GD 20, and all 50 mg/kg/day groups across developmental stages (Fig. 4J).

3.5. Exposure to BPA in utero triggered D-ser metabolism confusion in the hippocampus of rats at various growth periods

Fig. 5A demonstrated that hippocampal SR mRNA expressions were significantly reduced in 50 mg/kg/day group at PND 21, and all BPA groups at PND 56. Similarly, SR protein levels showed significant decreases in the 0.5–50 mg/kg/day groups at GD 20 and PND 21, and all BPA groups at PND 56 (Fig. 5F-G).

Fig. 5B showed that hippocampal DAAO mRNA levels were significantly elevated at GD 20 following BPA exposure (0.5–50 mg/kg/day). Conversely, significant reductions in DAAO mRNA levels were observed at all BPA doses at PND 21, and 50 mg/kg/day at PND 56 (Fig. 5B). Fig. 5F and 5H demonstrated significant increase in hippocampal DAAO protein levels across all BPA groups compared with control at GD 20 (Fig. 5F and 5H). Notably, DAAO protein levels exhibited downward trends in 5 and 50 mg/kg/day group at both PND 21 and 56 (Fig. 5F and 5H).

Fig. 5 illustrated that hippocampal asc-1 mRNA levels were significantly reduced across all developmental stages following BPA exposure (0.05–50 mg/kg/day), except for the 0.05 mg/kg/day group at PND 56. Correspondingly, asc-1 protein expressions showed significant decreases in 5 and 50 mg/kg/day groups at GD 20, and all BPA groups at PND 21 and 56 relative to control groups (Fig. 5F and 5I).

Hippocampal ASCT1 mRNA expression exhibited significant upregulation at GD 20 and PND 21 following exposure to higher than 0.5 mg/kg/day BPA, and increased levels at PND 56 across all BPA doses (Fig. 5D). Concurrently, ASCT1 protein expressions were significantly elevated in the 50 mg/kg/day group at both GD 20 and PND 21 (Fig. 5F and 5J). Moreover, significant increases in ASCT1 protein levels were

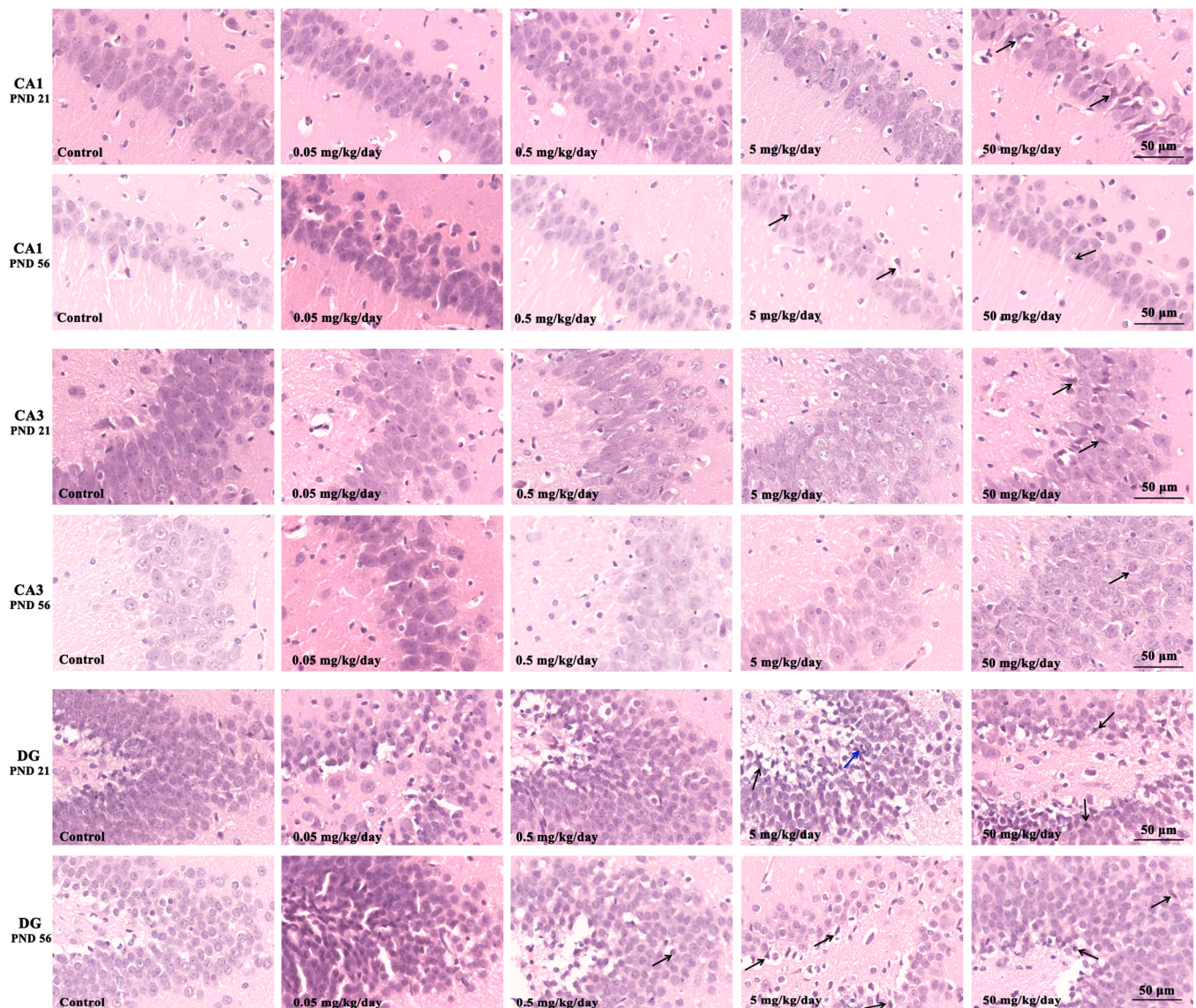


Fig. 2. Changes in the hippocampal morphological structure of PND 21 and 56 rats induced by exposure to BPA in utero. Hippocampal morphological structure in CA1, CA3 and DG regions of PND 21 and PND 56 rats were presented, respectively. (n = 3 pups per group; The black arrow denoted abnormal cell morphology, while the blue arrow marked inflammatory cell infiltration.).

observed at PND 56 across all BPA doses relative to controls (Fig. 5F and 5J).

Fig. 5E demonstrated that hippocampal ASCT2 mRNA expressions were significantly downregulated across all developmental stages following BPA exposure (0.05–50 mg/kg/day), with the exception of the 0.05 mg/kg/day group at PND 56. Furthermore, significant reductions in ASCT2 protein expressions were observed in the 0.5–50 mg/kg/day groups at GD 20, and the 50 mg/kg/day group at both PND 21 and 56 relative to controls (Fig. 5F and 5K).

3.6. Exposure to BPA eventuated cytotoxicity in primary cultured AS

Fig. S1A showed GFAP-immunofluorescence-positive AS exhibiting red cytoplasm (with DAPI-stained blue nuclei). Subsequent experiments were conducted using AS populations with > 95 % purity. To assess BPA-induced cytotoxicity, cell viability was measured using CCK-8 assays. Dose-dependent inhibitions of cell viability were observed at BPA concentrations > 20 μmol/L for 12–24 h, with significant reductions at > 5 μmol/L after 48 h exposure. Notably, 2.5 μmol/L BPA showed no significant effect on AS viability, whereas 40–200 μmol/L caused severe

astrocytic damage. Therefore, 5, 10 and 20 μmol/L BPA with 12 and 24 h exposure were selected for subsequent experiments (Fig. S1B). Additionally, 20 μmol/L BPA exposure for 24 h induced astrocytic morphological alterations including decreased cell density (Fig. S1C).

3.7. Exposure to BPA caused Glu metabolism disorders in primary cultured AS

Fig. 6A demonstrated that BPA exposure significantly upregulated GS mRNA expressions in all treatment groups at 12 h and in 20 μmol/L BPA group at 24 h. Additionally, GS protein expressions were significantly increased across all BPA-treated groups at both 12 h and 24 h (Fig. 6D–E). In contrast, GS enzymatic activities were significantly reduced in all BPA-treated groups at 12 h and in the high-dose (20 μmol/L) group at 24 h (Fig. 6F). Figs. 6B, 6D and 6G show that GLS1 mRNA and protein levels were significantly reduced in AS following 12 h and 24 h BPA treatment. Moreover, GLS1 enzymatic activities were significantly suppressed in 20 μmol/L BPA group at 12 h and in all treatment groups at 24 h (Fig. 6H). Figs. 6C, 6D and 6I illustrated that GDH mRNA and protein expressions were significantly downregulated in all BPA-

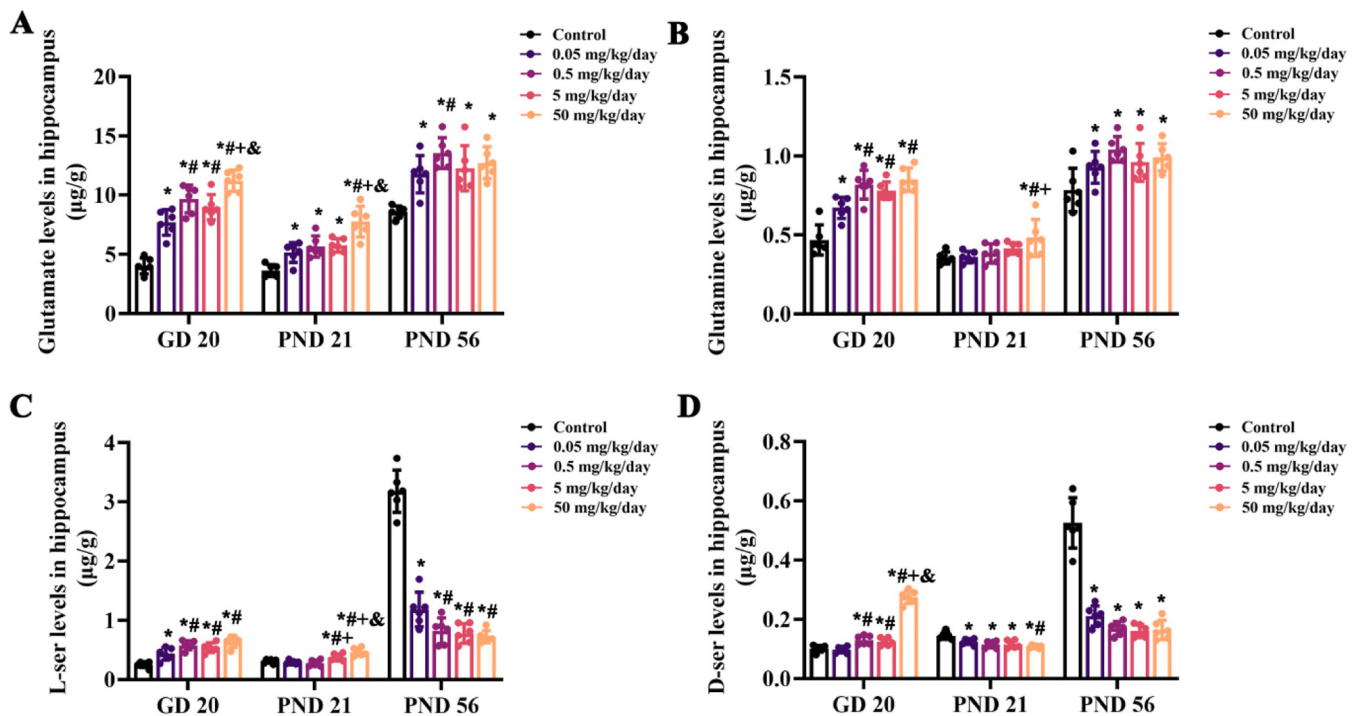


Fig. 3. Effects of exposure to BPA in utero on hippocampal Glu, Gln, L-ser and D-ser levels of rats at three growth stages. (A–D) Change in Glu, Gln, L-ser and D-ser levels based on UHPLC-MS/MS, separately. (Mean $\pm$ SD, $n = 6$ pups per group; * indicated significance with control group; # indicated significance with 0.05 mg/kg/day BPA group; + indicated significance with 0.5 mg/kg/day BPA group; & indicated significance with 5 mg/kg/day BPA group; $P < 0.05$).

treated groups at both 12 h and 24 h. Furthermore, GDH enzymatic activities in 20 $\mu\text{mol/L}$ BPA group were significantly reduced compared with controls at both 12 h and 24 h (Fig. 6J).

3.8. Exposure to BPA induced abnormal D-ser metabolism in primary cultured AS

The SR mRNA levels in AS were significantly reduced in 10 and 20 $\mu\text{mol/L}$ BPA groups at 12 h, as well as in all BPA groups at 24 h (Fig. 7A). Furthermore, the SR protein levels in AS showed significant suppression in all BPA groups after 12 h and 24 h of exposure (Fig. 7E–F). As shown in Fig. 7B, the DAAO mRNA levels in AS remained unchanged after 12 h of BPA exposure, whereas significant decreases were observed in all BPA groups at 24 h. Similarly, the DAAO protein levels in AS showed significant reductions in 20 $\mu\text{mol/L}$ BPA group at 12 h and in all BPA groups at 24 h (Figs. 7E and 7G). However, BPA exposure had no significant effects on ASCT1 expressions at either the mRNA or protein level after 12 h or 24 h under these experimental conditions (Figs. 7C, E, and 7H). In contrast to the unchanged ASCT-1 levels, ASCT-2 mRNA levels in AS exhibited a significant upward trend in all BPA groups, peaking in 5 $\mu\text{mol/L}$ BPA group at 12 h, and increased in a dose-dependent manner from the 10 $\mu\text{mol/L}$ BPA group at 24 h (Fig. 7D). Likewise, the ASCT-2 protein levels in AS were significantly elevated in all BPA groups compared with their respective controls at both 12 h and 24 h (Figs. 7E and 7I).

4. Discussion

Epidemiological data indicated that BPA was detectable in maternal serum, fetal serum, amniotic fluid, placental tissue, and breast milk (Lee et al., 2018; Jin et al., 2020), suggesting that pregnant women are readily exposed to BPA. Moreover, the embryonic stage, which represents the first critical period of neurodevelopment, is highly sensitive to BPA and may exert long-term negative effects on postnatal neurobehavior (Wang et al., 2014, 2016, 2020; Suresh et al., 2022). However,

few studies have focused on the neurotoxic effects of prenatal BPA exposure. Therefore, pregnant rats were exposed to varying doses of BPA via intragastric administration from GD 5 to GD 19, covering the period from implantation to pre-delivery, as described in our previous study (Yu et al., 2020). The Morris water maze test results showed that exposure to higher than 5 mg/kg/day BPA in utero could prolonged escape latency in place trial and shorten the time staying in the target area (probe trial), indicating that in utero exposure to BPA at doses exceeding 5 mg/kg/day may lead to learning and memory impairments in offspring, suggesting that early-life BPA exposure may exert long-term adverse effects on cognitive function. The hippocampus plays a critical role in higher cognitive functions, and is particularly vulnerable to BPA. Previous pharmacokinetic analyses confirmed hippocampal BPA concentrations of 0.181 $\mu\text{g/g}$ in female rats at 48 h after oral exposure to 100 mg/kg (Kim et al., 2004). Based on these findings, hippocampal tissue was chosen as the target for this in vivo study.

Glu can mediate NMDAR activation and support learning and memory processes, however, elevated Glu levels may induce excitotoxicity, resulting in neuronal damage. Therefore, maintaining stable Glu concentrations and normal metabolic homeostasis is crucial. Although several studies have investigated the effects of BPA exposure on Glu levels in CNS, their results remain inconsistent. Specifically, high-dose BPA exposure [125 mg/kg/day from PND 20 to PND 117 (Essawy et al., 2021); 5 mg/kg/day from PND 7 to PND 21 (Zhang et al., 2019)] was shown to elevate hippocampal Glu levels. Similarly, lower-dose BPA exposure (1 mg/ml via drinking water, from GD 6 to PND 20) also increased hippocampal Glu levels (Kunz et al., 2011). In contrast, similar dose of BPA administered exclusively during the juvenile stage reduced Glu levels (Hu et al., 2017). Furthermore, a lower BPA dose (0.5 $\mu\text{g/kg/day}$, PND 10–40) had no significant effect on Glu levels (Meng et al., 2023). Collectively, these studies suggested that high-dose BPA exposure elevates hippocampal Glu levels, whereas low-dose exposure either reduces or has no effect on them. Notably, the effects of BPA exposure on Glu levels are more pronounced during gestation than during postnatal stages. Critically, most previous studies focused on

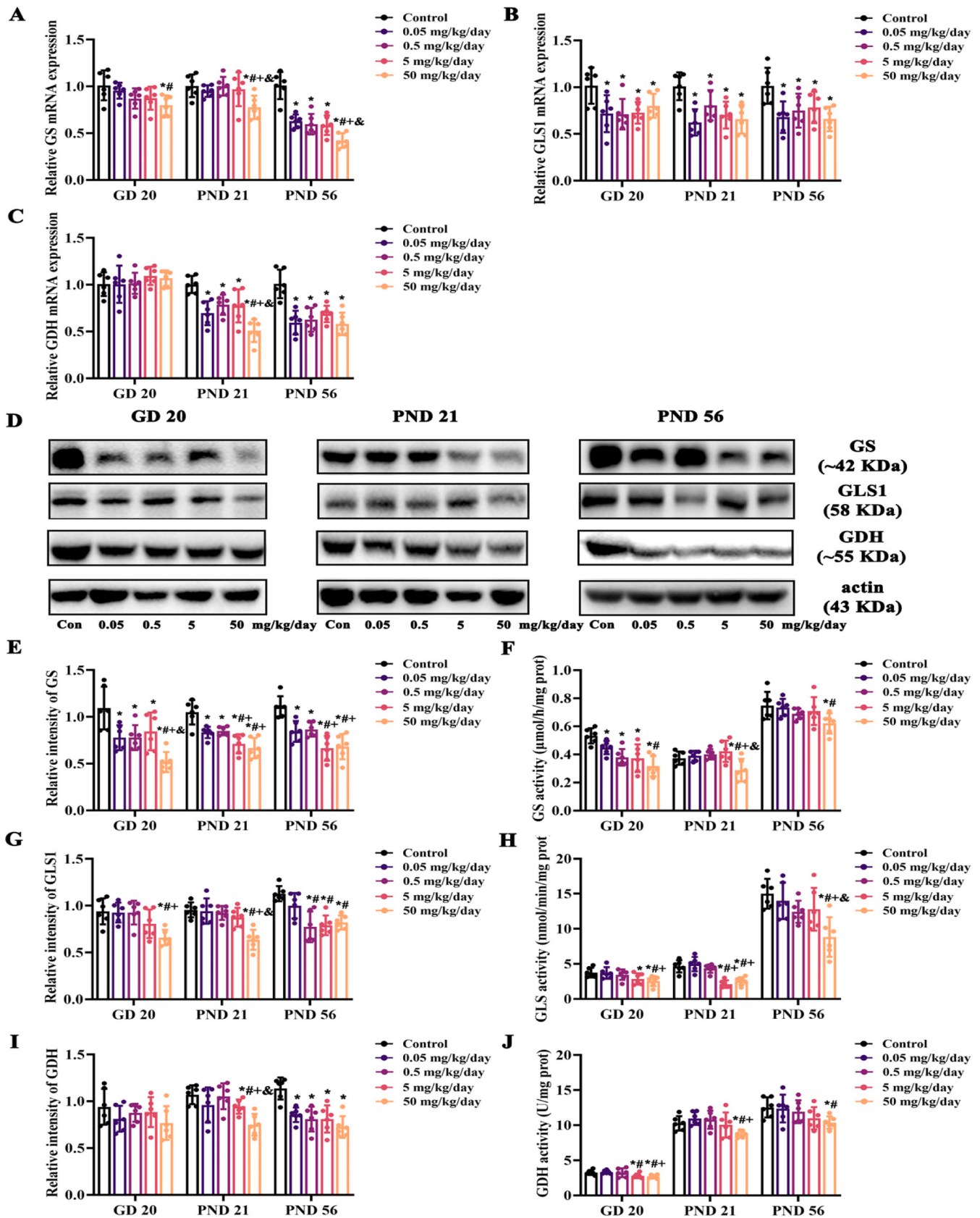


Fig. 4. Effects of exposure to BPA in utero on hippocampal Glu metabolism of rats at three growth stages. (A-C) Statistical analysis based on real-time PCR for GS, GLS1, GDH mRNA levels, respectively. (D) Representative blots of WB analysis for GS, GLS1 and GDH. (E) Statistical analysis based on WB for GS protein levels. (F) Analysis for GS activity. (G) Statistical analysis based on WB for GLS1. (H) Analysis for GLS activity. (I) Statistical analysis based on WB for GDH. (J) Analysis for GDH activity. (Mean $\pm$ SD, $n = 6$ pups per group; * indicated significance with control group; # indicated significance with 0.05 mg/kg/day BPA group; + indicated significance with 0.5 mg/kg/day BPA group; & indicated significance with 5 mg/kg/day BPA group; $P < 0.05$).

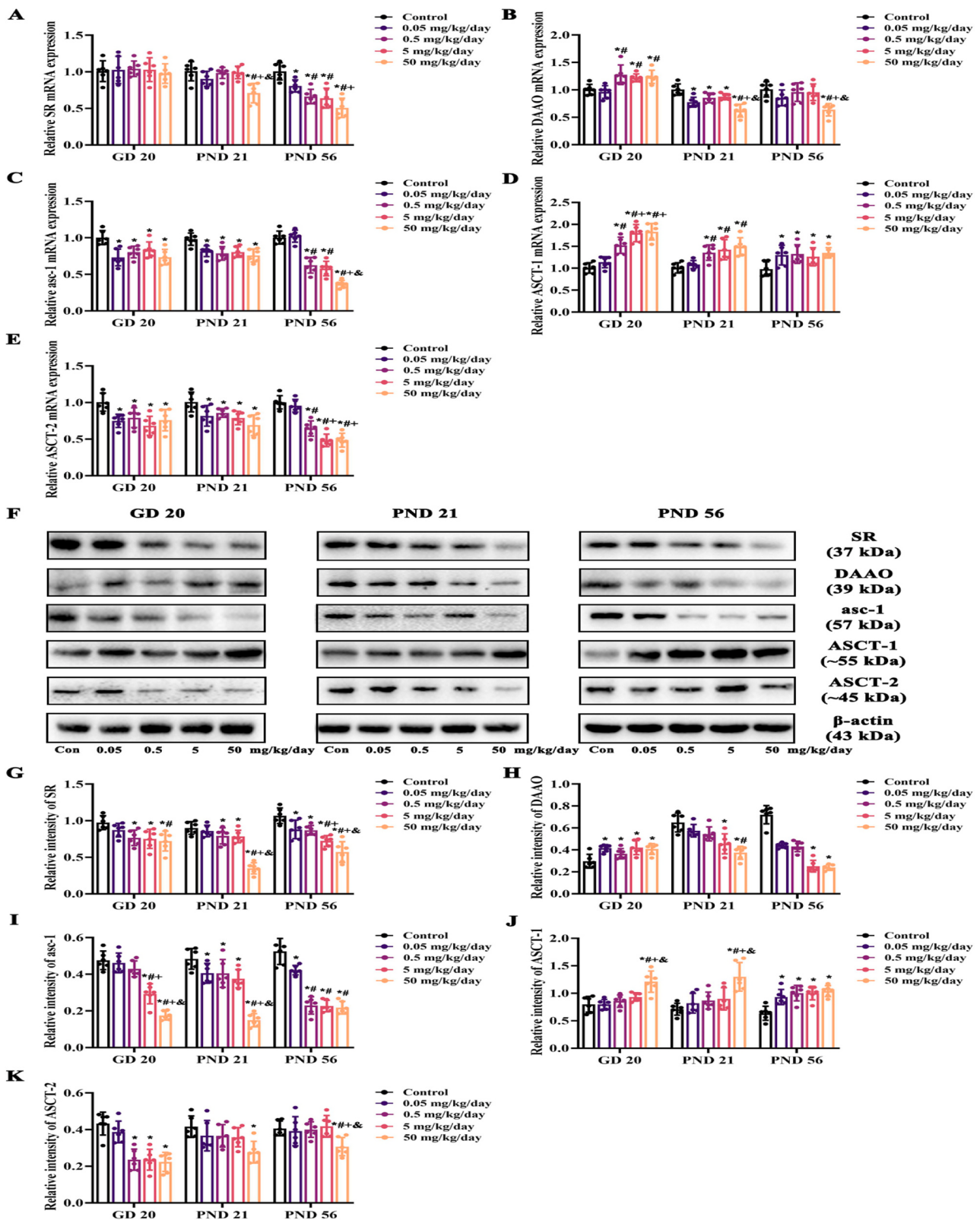


Fig. 5. Effects of exposure to BPA in utero on hippocampal D-ser metabolism of rats at three growth stages. (A-E) Statistical analysis based on real-time PCR for SR, DAAO, *asc-1*, ASCT-1 and ASCT-2 mRNA levels, respectively. (F) Representative blots of WB analysis for SR, DAAO, *asc-1*, ASCT-1 and ASCT-2. (G-K) Statistical analysis based on WB for SR, DAAO, *asc-1*, ASCT-1 and ASCT-2 protein levels, respectively. (Mean $\pm$ SD, $n = 6$ pups per group; * indicated significance with control group; # indicated significance with 0.05 mg/kg/day BPA group; + indicated significance with 0.5 mg/kg/day BPA group; & indicated significance with 5 mg/kg/day BPA group; $P < 0.05$; Statistical analysis of WB test for DAAO at PND 21 and PND 56, together with ASCT-2 at GD 20 and PND 21 were using Kruskal-Wallis test followed by Dunn's multiple comparisons test.).

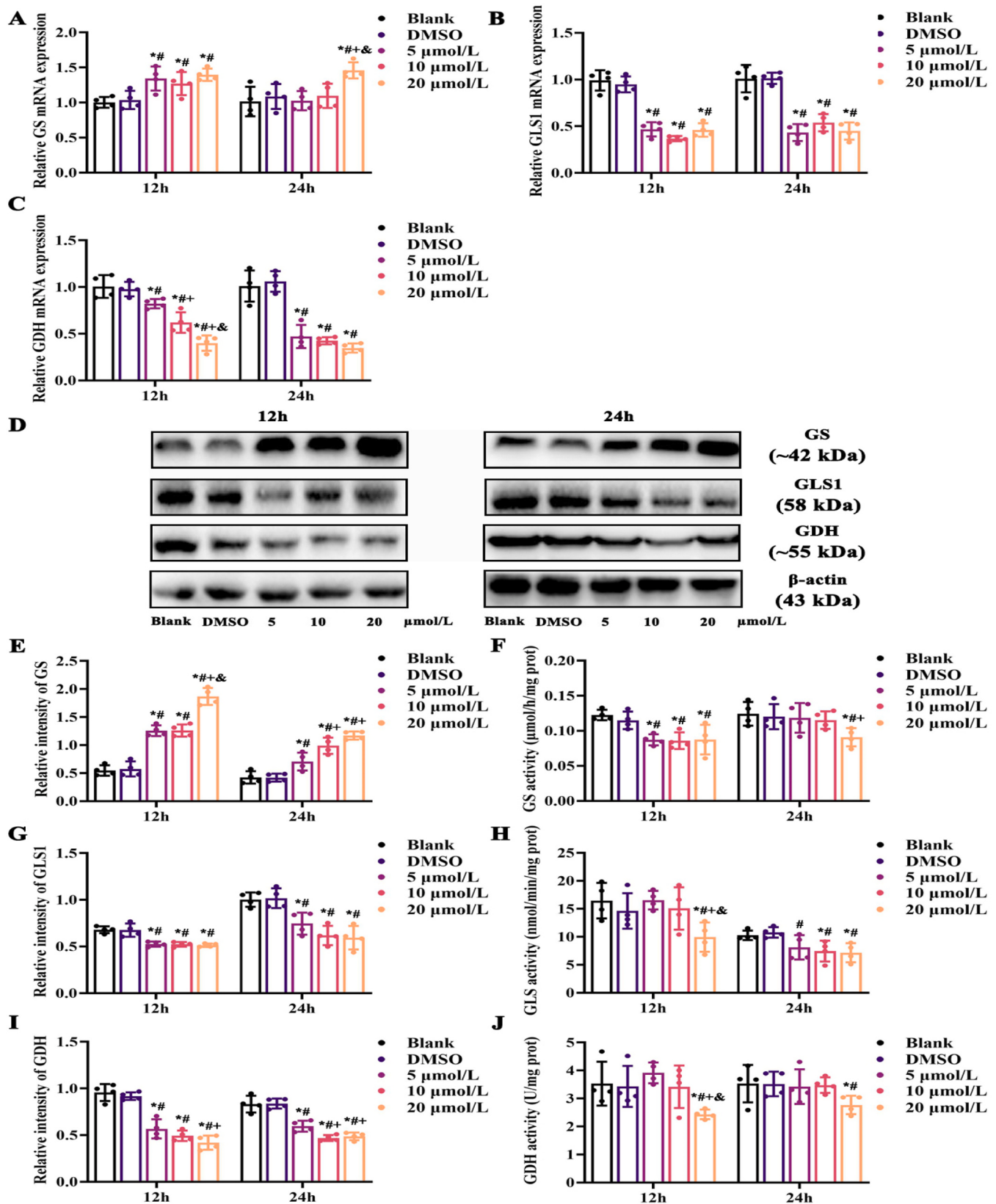


Fig. 6. Effects of exposure to BPA on Glu metabolism in primary cultured AS. (A-C) Statistical analysis based on real-time PCR for GS, GLS1 and GDH mRNA in AS, respectively. (D) Representative blots of WB analysis for GS, GLS1 and GDH in AS. (E) Statistical analysis based on WB for GS protein levels in AS. (F) Analysis for GS activity in AS. (G) Statistical analysis based on WB for GLS1 in AS. (H) Analysis for GLS activity in AS. (I) Statistical analysis based on WB for GDH in AS. (J) Analysis for GDH activity in AS. (Mean $\pm$ SD, $n = 4$. * indicated significance with blank control group; # indicated significance with DMSO group; + indicated significance with 5 $\mu\text{mol/L}$ BPA group; & indicated significance with 10 $\mu\text{mol/L}$ BPA group; $P < 0.05$).

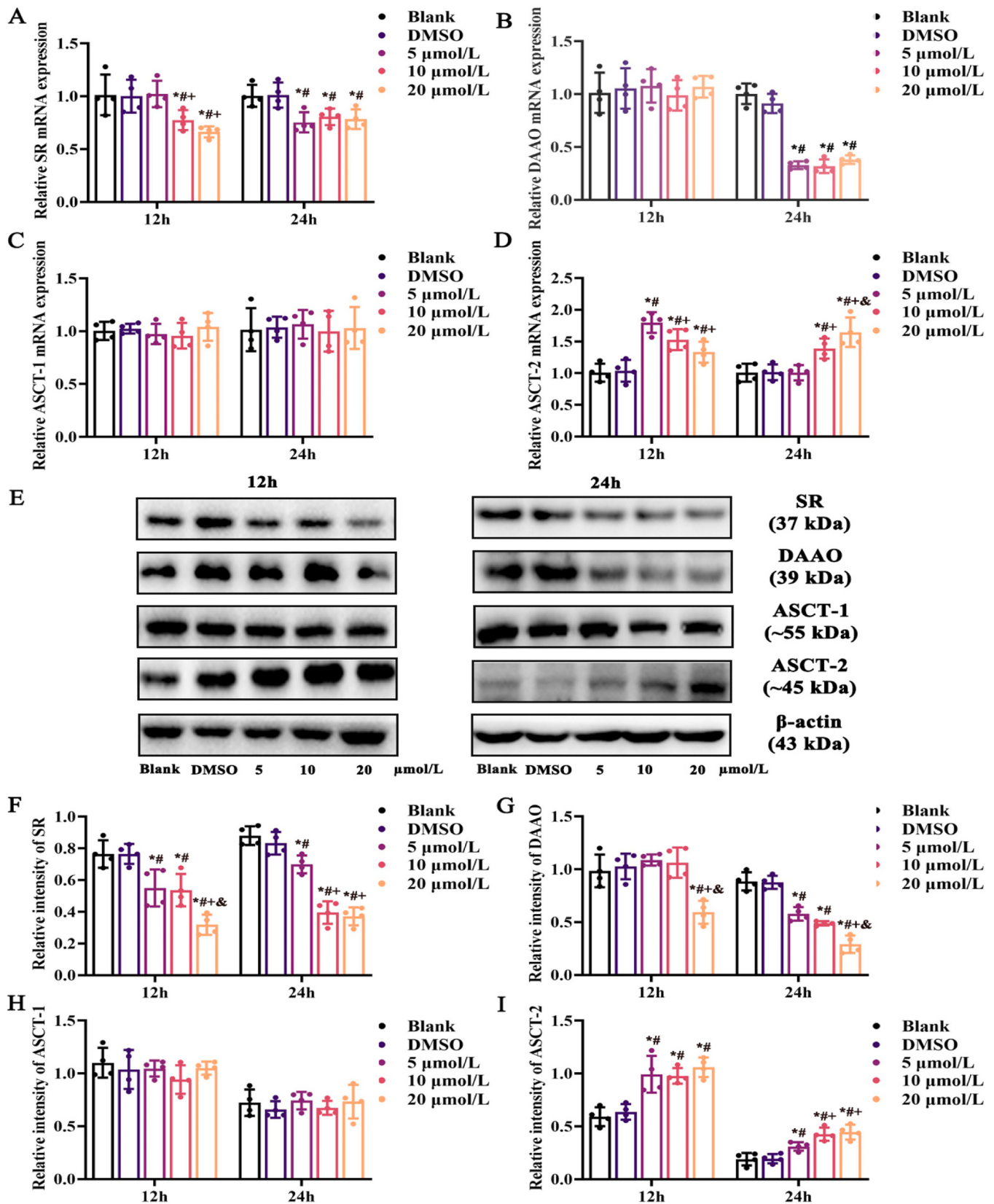


Fig. 7. Effects of exposure to BPA on D-ser metabolism in primary cultured AS. (A-D) Statistical analysis based on real-time PCR for SR, DAAO, ASCT-1 and ASCT-2 mRNA levels in AS, respectively. (E) Representative blots of WB analysis for SR, DAAO, ASCT-1 and ASCT-2. (F-I) Statistical analysis based on WB for SR, DAAO, ASCT-1 and ASCT-2 protein levels in AS, respectively. (Mean $\pm$ SD, $n = 4$. * indicated significance with blank control group; # indicated significance with DMSO group; + indicated significance with 5 $\mu\text{mol/L}$ BPA group; & indicated significance with 10 $\mu\text{mol/L}$ BPA group; $P < 0.05$).

postnatal or combined gestation-to-lactation BPA exposure, with limited investigation of exposure exclusively in utero (a critical period for nervous system development). To address this gap, we investigated the impact of gestational BPA exposure on hippocampal Glu levels. Our results demonstrated that in utero exposure to 0.05 mg/kg/day BPA significantly increased hippocampal Glu and Gln levels at early developmental stages, and the accumulation of Glu and Gln might cause NMDAR activation abnormality and excitotoxic effects in hippocampus, thereby participating in the later behavioral changes induced by BPA. Besides, as Glu and Gln levels altered starting from lower exposure dose and early stage, making them might be the molecular indicators for cognitive impairment caused by BPA. Glu homeostasis in the mammalian CNS is primarily maintained by the Glu-Gln cycle, which depends on the coordinated action of GS, GLS, and GDH. The Glu-Gln cycle will not work without AS; therefore, we investigated Glu metabolism in both the hippocampus of rats' offspring and primary AS. GS, a marker enzyme of AS, catalyzes the conversion of Glu to Gln, representing a key step in the Glu-Gln cycle. Our findings demonstrated that BPA exposure in utero significantly inhibited GS expression in the hippocampus of rats' offspring. Notably, the inhibitory effect on GS protein was more pronounced than on its gene expression: while GS protein levels were significantly reduced from GD 20 to PND 56 since exposure to 0.05 mg/kg/day BPA, significant reductions in GS gene expression occurred only at the higher dose (50 mg/kg/day) on GD 20 and PND 21. Similarly, GS activity was significantly inhibited after BPA exposure during early developmental stages, whereas this inhibitory trend attenuated postnatally and was observed only in the high-dose group (50 mg/kg/day). In contrast to the in vivo findings, 5 μ mol/L BPA up-regulated GS expression in AS at both mRNA and protein levels at 12 h, with the protein-level effects being more pronounced than the transcriptional effects at 24 h. All BPA treatment groups exhibited significant suppression of GS activity after 12 h exposure. This inhibitory effect was transient in low-dose groups, disappearing at 24 h, while persisting only in the high-dose group. Intriguingly, GS activity demonstrated a significant decrease in AS despite significant protein upregulation, a phenomenon whose underlying mechanism requires further investigation.

As a pivotal component of the Glu-Gln cycle, GLS catalyzes the deamidation of Gln to regenerate Glu, thereby completing the cycle. The expression of GLS in glial cells has been debated for years, as AS possess high-capacity Glu uptake systems that appeared to compensate for the apparent absence of GLS. However, recent studies have demonstrated GLS expression in hippocampal AS of both rats and humans, as well as in cultured AS, with confirmed catalytic activity. AS express four GLS transcript variants with marked expression differences, showing a striking 144:1 ratio of GLS1 to GLS2 isoforms (Cardona et al., 2015). Therefore, we hypothesized that GLS1 plays a more critical role in Glu metabolism and thus selected this isoform for analysis. Exposure to 0.05 mg/kg/day BPA significantly suppressed GLS1 gene expression in rats' offspring hippocampus from GD 20 through PND 56. However, GLS1 protein levels and activities were less responsive, requiring higher BPA doses for significant inhibition. AS GLS1 alterations were consistent with in vivo findings, demonstrating significant BPA-induced reductions in GLS1 mRNA, protein, and enzymatic activity.

Following uptake by AS, Glu undergoes two primary metabolic pathways: one is conversion by GS to Gln, and the other is metabolism via GDH to α -ketoglutarate, which subsequently enters the tricarboxylic acid cycle (Treberg et al., 2014). In this study, BPA exposure did not significantly alter hippocampal GDH mRNA or protein expressions at GD 20. However, 0.05 mg/kg/day BPA significantly suppressed GDH gene expression beginning at PND 21. Notably, the inhibitory effects on GDH protein were weaker than those on its transcript, with significant downregulation observed only after 50 mg/kg/day BPA at PND 21 or 0.05 mg/kg/day BPA at PND 56. Furthermore, high-dose BPA exposure significantly inhibited hippocampal GDH activity across all examined developmental stages. We propose that GDH repression may represent a

protective mechanism triggered by Glu homeostatic alterations during early neurodevelopment. Astrocytic GDH expressions were highly sensitive to BPA: even low doses significantly reduced GDH at both transcriptional and translational levels, additionally, higher concentrations (20 μ mol/L) also suppressed its enzymatic activity.

Comparative analysis of Glu metabolic regulators suggested GS may serve as an early biomarker for BPA-induced Glu dysregulation, as GS abnormalities were detectable simultaneously at mRNA, protein, and functional levels during earlier developmental phases. BPA-induced reductions in GS expression and activity may impair Gln synthesis from Glu, thereby increasing hippocampal Glu levels. Furthermore, suppressed GDH expression and activity would inhibit Glu catabolism, potentially exacerbating Glu accumulation. Collectively, these findings suggest that hippocampal Glu accumulation likely results from the combined inhibitory effects of BPA on both GS and GDH activity. Although GS downregulation would be expected to reduce Glu production, we observed a concomitant increase in both Gln and Glu levels. We hypothesize this phenomenon reflects BPA's potent inhibition of GLS1, which attenuates Gln hydrolysis, thereby exerting a more pronounced effect on Gln homeostasis than GS downregulation.

Given that D-ser is the primary endogenous co-agonist for NMDARs, we also investigated BPA-induced alterations in D-ser metabolism both in vivo and in vitro. In vivo, L-ser and D-ser level changes exhibited greater complexity than those of Glu, which showed that low-dose BPA exposure elevated hippocampal L-ser levels at GD 20 and PND 21, however, switch to down-regulate its levels at PND 56. D-ser dynamics followed a similar pattern, but with an earlier transition (PND 21): exposure to 0.5 mg/kg/day BPA increased D-ser levels at GD 20 but significantly decreased them from PND 21 onward. In addition, due to the important role of NMDAR in learning and memory process, as well as the irreplaceability of D-ser in NMDAR activation, we propose that abnormal D-ser levels might also participate in BPA induced learning and memory impairment in later life.

SR, the key biosynthetic enzyme for D-ser, catalyzes the racemization of L-ser to D-ser in the mammalian CNS. SR knockout (SR-KO) mice display hyperactivity and cognitive deficits accompanied by reduced brain D-ser levels, which are ameliorated by D-ser supplementation (Shindo et al., 2022). Similarly, Jami et al. demonstrated > 90 % reductions in CNS D-ser in SR-KO mice, which recapitulated key neurochemical and behavioral features of schizophrenia (Jami et al., 2021). These studies collectively established D-ser's critical role in neurobehavioral disorders and SR's essential function in maintaining D-ser homeostasis. Our in vivo and in vitro data demonstrated BPA dose- and time-dependently suppresses SR expression, with protein levels being more sensitive than mRNA, showing that significant reductions at lower doses during early development or shorter exposures (12 h) in vitro.

Nevertheless, D-ser homeostasis also depends on its degradation via DAAO-catalyzed deamination. DAAO inhibitors elevated brain D-ser levels, enhanced neuronal signaling, and ameliorated neuropsychiatric deficits including depression and cognitive impairment in rats (Nagy et al., 2021). We therefore examined DAAO expressions, finding that BPA exposure upregulated DAAO at GD 20 but downregulated it at PND 21 and PND 56 in vivo. In vitro, BPA similarly suppressed DAAO at both mRNA and protein levels in AS.

Emerging evidence suggested that two distinct transporter systems govern D-ser/L-ser exchange between AS and neurons: the asc-1 system and the ASCT1/ASCT2 family. The asc-1 transporter mediates high-affinity D-ser uptake and release in neurons (Rutter et al., 2007). In contrast, the mechanisms governing D-ser transport in AS remain controversial. Early studies prioritized ASCT2 over ASCT1 for the principal AS D-ser transporter, as evidenced by studies using primary AS cultures (Bröer et al., 1999; Shao et al., 2009) or C6 glioma cells model (Sikka et al., 2010). Besides, ASCT2 exhibits higher affinity for L-ser than D-ser (Lee et al., 2017) with a broader substrate promiscuity (Bröer and Bröer, 2017; Kaplan et al., 2018). However, recent research reported that ASCT1-KO mice, but not ASCT2-KO mice, showed altered

brain L-ser and D-ser levels, and they also assumed that ASCT1 may mediate L-ser export from AS through hetero-exchange with D-ser (Kaplan et al., 2018). Another study confirmed ASCT1's primary role in AS D-ser transport, though proposed it primarily mediates D-ser release rather than uptake (Tapanes et al., 2022). We herein also tested the expressions of these D-ser/L-ser transporters. Low-dose BPA significantly suppressed asc-1 at both mRNA and protein levels. Given asc-1's predominant neuronal expression, we did not assess its levels in AS. Unlike asc-1, BPA upregulated hippocampal ASCT1 expression, with more pronounced transcriptional than translational effects (evident at lower doses). However, BPA did not significantly alter ASCT1 expression in cultured AS. Notably, BPA downregulated hippocampal ASCT2 expressions, with greater mRNA than protein reduction at equivalent stages. In vitro, BPA paradoxically increased ASCT2 at both transcriptional and translational levels.

Considering the alterations in L-ser and D-ser contents in relation to SR and DAO expression, it can be hypothesized that D-ser levels were primarily dependent on L-ser changes. However, due to the persistently low expression of SR, the racemization of L-ser into D-ser was inhibited, potentially explaining the decrease in D-ser levels despite high L-ser concentrations at PND 21. Furthermore, we propose that the upregulation of DAO expression at GD 20 may be attributed to high D-ser levels, suggesting this could represent an adaptive response to reduce excessive D-ser. Both SR and DAO expressions were simultaneously inhibited from PND 21 onward. We hypothesize that BPA-induced inhibition of SR played a more significant regulatory role in controlling D-ser levels, as evidenced by the observed reduction in D-ser at this developmental stage. Moreover, impaired function of D-ser transporters (including asc-1, ASCT1 and ASCT2) may also contribute to abnormal L-ser and D-ser levels in the hippocampus. However, the precise mechanisms responsible for the differential effects of these transporters following BPA exposure remain unclear. This limitation of the current study will be addressed in future research.

5. Conclusions

In summary, exposure to BPA in utero was found to inhibit the GS, GLS1, and GDH at both transcriptional and functional levels, leading to Glu accumulation in the hippocampal region of rats' offspring across multiple developmental stages. Additionally, exposure to BPA also down-regulated SR, asc-1 and ASCT2 expressions, while up-regulated ASCT1 expressions, and modulated DAO expressions. These alterations might collectively disrupt D-ser metabolism in the rats' offspring hippocampus. Notably, this study revealed the vulnerability of Glu and D-ser metabolic pathways in primary cultured AS, highlighting BPA-induced neurotoxicity mediated through astrocytic dysfunction. The dysregulation of Glu and D-ser metabolism may contribute to the learning and memory impairments associated with BPA exposure. However, this study also has several limitations like the lack of interventional studies targeting for key regulators involved in BPA-induced Glu and D-ser metabolic dysregulation and the insufficient mechanistic exploration of how these metabolic alterations contribute to BPA-induced neurobehavioral changes. These critical questions will be systematically addressed in our future research.

CRedit authorship contribution statement

Yichen Zhao: Software, Data curation. **Mengwenhui Shi:** Software. **Panjie Wang:** Software, Data curation. **Mingyue Ma:** Writing – review & editing, Visualization. **Zhiwen Duan:** Supervision, Resources. **Xiucong Pei:** Supervision, Resources. **Xiao Li:** Software. **Xinran Li:** Writing – original draft, Software, Methodology. **Luxin Zhang:** Writing – original draft, Software, Methodology. **Haiyang Yu:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Haiyang Yu reports financial support was provided by National Natural Science Foundation of China. Haiyang Yu reports financial support was provided by Natural Science Foundation of Liaoning Province. Haiyang Yu reports financial support was provided by Basic Research Projects of Educational Department of Liaoning Province. If there are other authors, they 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. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2025.118651](https://doi.org/10.1016/j.ecoenv.2025.118651).

Data Availability

Data will be made available on request.

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