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Perinatal exposure to bisphenol A or S: Effects on anxiety-related behaviors and serotonergic system

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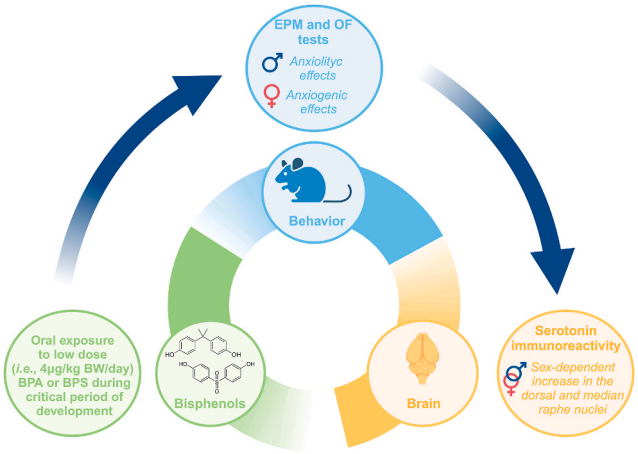
HIGHLIGHTS

- Exposure to low-dose bisphenol A or S during the perinatal period represents a risk to the developing organism.
- Anxiety-related behaviors are altered in bisphenol A or S-exposed mice.
- Serotonergic system within dorsal and median raphe nuclei is altered in bisphenol A or S exposed mice.
- Perinatal exposure to bisphenol A or S affected differentially the two sexes.
- New and more stringent regulations on the use of BPA and its analogue BPS are needed.

GRAPHICAL ABSTRACT



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ABSTRACT

Bisphenols, synthetic organic compounds used in the production of plastics, are an extremely abundant class of Endocrine Disrupting Chemicals, *i.e.,* exogenous chemicals or mixtures of chemicals that can interfere with any aspect of hormone action. Exposure to BPs can lead to a wide range of effects, and it is especially dangerous if it occurs during specific critical periods of life. Focusing on the perinatal exposure to BPA or its largely used substitute BPS, we investigated the effects on anxiety-related behaviors and the serotonergic system, which is highly involved in controlling these behaviors, in adult mice. We treated C57BL/6J dams orally with a dose of 4 µg/kg body weight/day (*i.e.*, EFSA TDI) of BPA or BPS dissolved in corn oil or with vehicle alone, at the onset of mating and continued treatment until the offspring were weaned. Adult offspring of both sexes performed the

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elevated plus maze and the open field tests. Then, we analyzed the serotonergic system in dorsal (DR) and median (MnR) raphe nuclei by immunohistochemical techniques. Behavioral tests highlighted alterations in BPA- and BPS-treated mice, suggesting different effects of the bisphenols exposure on anxiety-related behavior in males (anxiolytic) and females (anxiogenic). The analysis of the serotonergic system highlighted a sex dimorphism in the DR only, with control females showing higher values of serotonin immunoreactivity (5-HT-ir) than control males. BPA-treated males displayed a significant increase of 5-HT-ir in all analyzed nuclei, whereas BPS-treated males showed an increase in ventral DR only. In females, both bisphenols-treated groups showed a significant increase of 5-HT-ir in dorsal DR compared to the controls, and BPA-treated females also showed a significant increase in MnR.These results provide evidence that exposure during the early phases of life to BPA or BPS alters anxiety and the raphe serotonergic neurons in a sex-dependent manner.

1. Introduction

Growing literature considered the Endocrine Disrupting Chemicals (EDCs), *i.e.*, "exogenous chemicals, or mixtures of chemicals, that interfere with any aspect of hormone action", as substantial and costly public health problems due to their pervasiveness and their associations with chronic disease (Gore et al., 2015; Kahn et al., 2020).

More recently, there are growing concerns that EDCs alter brain development, neurochemistry, and behaviors (Bakoyiannis et al., 2021). Among EDCs, exposure to bisphenols (BPs), and in particular to bisphenol A (BPA), seems to impact the behavioral outcome, especially when the exposure occurs during critical periods of development, such as the pre- or perinatal one (Bakoyiannis et al., 2021; Rebolledo-Solleiro et al., 2021).

However, because of several health issues, the European Food Safety Authority (EFSA), after completing a full risk assessment in 2006 (EFSA, 2006), established a tolerable daily intake (TDI) for BPA of 50 μg/kg of body weight (BW)/day, which was reduced in 2015 from 50 to 4 μg (EFSA, 2015). Subsequently, the European Commission has imposed BPA removal from some consummatory goods, such as infant feeding bottles (European Commission, 2011) and other foodstuffs (Andersson et al., 2018). Considering BPA limitations, some substitutes have been proposed. Thanks to its increased stability (Kuruto-Niwa et al., 2005), one of the most commonly used replacements is bisphenol S (BPS), which shares structural similarities and endocrine-disrupting properties with BPA (Eladak et al., 2015; Gramec Skledar and Peterlin Masic, 2016; Naderi and Kwong, 2020; Rochester and Bolden, 2015). In fact, emerging evidence suggests that BPS is not a safe alternative to BPA. Despite these findings, the health risks of BPS remain poorly investigated, and no specific regulations are currently available (den Braver-Sewradj et al., 2020; Mustieles et al., 2020; Naderi and Kwong, 2020; Thoene et al., 2020).

Anxiety consists of several reactions (somatic, cognitive, emotional, and behavioral), which are mostly conserved among mammals, to survive or cope with threatening stimuli (Hohoff, 2009). Because correct activation of the anxiety state is needed to react properly to specific stimuli, anxiety enables an individual to adapt to environmental challenges (Gold, 2015; Hohoff, 2009). If anxiety responses are inappropriate, the ability to adapt to environmental conditions is compromised (Gold, 2015).

There is some evidence that exposure to BPA at any time of life alters anxiety responses in rodents (Bakoyiannis et al., 2021; Rebolledo--Solleiro et al., 2021), producing anxiogenic or anxiolytic effects, depending on the considered dose, period of exposure, sex, and experimental model (Rebolledo-Solleiro et al., 2021). Interestingly, perinatal exposure to BPA, which induces alteration in anxiety, has been associated with changes in hormone receptors' levels within the brain.

In particular, BPA anxiogenic effects, seen mainly in developmentally exposed females in rats (Gioiosa et al., 2013; Poimenova et al., 2010; Zhou et al., 2015), seem to be linked to increased levels of plasma corticosterone (Chang et al., 2016; Poimenova et al., 2010) and to decreased estrogen receptor α (ERα) (Chang et al., 2016), estrogen receptor β (ERβ), and melanocortin receptors (Patisaul et al., 2012) in different regions of the brain.

More recent works also investigated the effects of BPS on anxiety-related behaviors. It has been recently demonstrated that 10-week post-lactational exposure of male mice to BPS at a dose of 100 μg/kg/day mediated anxiogenic effects (Mornagui et al., 2019); juvenile exposure of male mice to either BPA or BPS (dose of 1 mg/kg BW/day or 100 μg/kg BW/day) also led to an increase in the anxiety state, along with hyperactivity of basolateral amygdala (BLA), which is strongly involved in fear and anxiety responses (Hu et al., 2022). Interestingly, only a few studies have investigated the effects of perinatal BPS exposure in rodents. At a dose of 0.2 mg/kg BW/day in mice and 10 μm/kg BW/day or 50 mg/kg BW/day in rats, BPS exposure is associated with an increase in anxiety-like behaviors, mainly in males (Kim et al., 2015).

The serotonergic system, within the dorsal (DR) and median (MnR) raphe nuclei, is particularly relevant for the control of anxiety behaviors (Ren et al., 2018; Zangrossi and Graeff, 2014). Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter synthesized from the amino acid tryptophan (Zhang et al., 2004). In the central nervous system (CNS), 5-HT is produced in small clusters of cells, defined as raphe nuclei, extending from the midbrain to the medulla oblongata and reaching several CNS areas (Hornung, 2010). Of the nine raphe nuclei (B1–B9), five (rostral) are in the midbrain and in the rostral pons, and four (caudal) are in the caudal pons and medulla (Hornung, 2010). From the rostral nuclei, especially DR and MnR, the projections reach different forebrain structures, but from the caudal nuclei, projections are primarily sent to the spinal cord (Ren et al., 2019).

Recent evidence supports the view of a dual role of 5-HT (anxiolytic and anxiogenic) in the control of anxiety-like behaviors (Gordon and Hen, 2004). Griebel has proposed that the dual effects, anxiolytic and anxiogenic, of 5-HT are due to the different involvement and distribution within brain areas of 5-HT receptors (Griebel, 1995). Daekin and Graeff proposed that 5-HT increases or decreases anxiety-related responses depending on the targeted brain areas (*i.e.*, enhancing it in the forebrain areas and reducing it in subcortical structures) (Deakin and Graeff, 1991). Gray and McNaughton hypothesize that 5-HT promotes fear response by activating the amygdala and the periaqueductal gray; but decreases anxiety by inhibiting the hippocampus (Davidson and Jarrard, 2004).

Notably, DR and MnR display high specialization in terms of functional connectivity (Ren et al., 2018). This is in line with the theory proposed by Deakin and Graeff, according to which two main pathways are involved in the control of anxiety behaviors (Deakin and Graeff, 1991). The first originates in the DR, goes through the medial forebrain bundle, and reaches the amygdala and the frontal cortex, facilitating avoidance behaviors (Deakin and Graeff, 1991). The second starts in the MnR and reaches the hippocampus, promoting resistance to chronic stress (Deakin and Graeff, 1991).

As BPA has been reported to alter anxiety-related behaviors (Bakoyiannis et al., 2021; Rebolledo-Solleiro et al., 2021), some studies have investigated its impact on the serotonergic system. In particular, it has been shown, in male rats, that a single intracranial injection of BPA (0.1–10 μg/kg BW) on postnatal day 2 led to an increase in 5-HT levels in the hippocampus, appreciable not only 5 days but also 28 days after the injection (Matsuda et al., 2010). Another study, performed in mice, highlighted that perinatal exposure to BPA (20 μg/kg BW/day) led to an

increase in 5-HT in different brain areas (caudate and putamen nuclei, dorsal raphe nucleus, thalamus, and substantia nigra) appreciable at 3, 10 and 15 postnatal weeks in both males and females (Nakamura et al., 2010). Furthermore, female mice perinatally exposed to a low-dose BPA (250 ng/kg/day) exhibited an increase in the 5-HT turnover in the hippocampus (Matsuda et al., 2013). Taken together, these results suggest that perinatal exposure to BPA may perturb 5-HT metabolism and signaling also in a long-term fashion.

Considering the increasing level of exposure to both BPs due to their persistence in the environment (D. Chen et al., 2016; Vasiljevic and Harner, 2021; Wu et al., 2018), and the lack of data regarding BPS, in this study, we aimed to evaluate the potential sexually dimorphic effects on anxiety-related behaviors (tested through the Elevated Plus Maze and the Open Field test) (Carola et al., 2002; Lezak et al., 2017) of adult male and female mice perinatally exposed to low-dose (4 μg/kg BW/day) of BPA or BPS. In parallel, we investigated the possible alterations of the serotonergic system in the DR and MnR, which is known to be involved in the control of different aspects of anxiety-related behaviors (Ren et al., 2018; Zangrossi and Graeff, 2014) and to be targeted at least by BPA exposure (Castro et al., 2015; Matsuda et al., 2010, 2013; Nakamura et al., 2010).

2. Methods

2.1. Animals

C57BL/6J mice (originally purchased from Envigo, S. Pietro al Natisone, Udine, Italy) from our colony at the Neuroscience Institute Cavalieri Ottolenghi were housed under standard conditions (room temperature 22 ± 2 °C; 12:12 light/dark cycle, lights on at 08:00 a.m.) with food (standard mouse chow 4RF21, Mucedola Srl, Settimo Milanese, Italy) and water *ad libitum*.

The animals were cared for and handled according to the European Union Council Directive of September 22, 2010 (2010/63/UE); all the procedures reported in the present study were approved by the Italian Ministry of Health (407/2018-PR) and the Ethical Committee of the University of Torino (Project n° 360384) and conforms to the ARRIVE guidelines (Kilkenny et al., 2010).

2.2. Treatments

We performed the oral treatment with bisphenols as described in Bonaldo et al. (2023). Briefly, we dissolved BPA (Sigma Aldrich, 239658, CAS 80-05-7) or BPS (Sigma Aldrich, 103039, CAS 80-09-1) in corn oil (Sigma-Aldrich, C8267) and administered them daily to pregnant dams (n = 4/group), from mating to offspring weaning (postnatal day 28, PND28). Bisphenol-treated dams received a daily dose of 4 μg/kg BW (*i.e.*, EFSA TDI for BPA) of BPA or BPS. Control dams received vehicle alone.

Weaned pups were housed in monosexual groups of 3–4 mice and monitored weekly until adulthood (PND90), when behavioral tests were performed.

Specifically, we obtained the following experimental groups from the weaned pups.

- Oil-treated males (Oil M, n = 10);
- Oil-treated female (Oil F, n = 11);
- BPA-treated male (BPA M, n = 11);
- BPA-treated females (BPA F, n = 9);
- BPS-treated males (BPS M, n = 11);
- BPS-treated females (BPS F, n = 11).

All the behavioral and immunohistochemical analyses described in the following sections were performed by blind investigators.

Table 1
Parameters analyzed for each mouse in the EPM recorded trials.

Parameter	Description
Cumulative Duration (CD)	The cumulative time (s) spent by the tester mouse in the center, in the open arms or in the closed ones.
Frequency of entrance	The number of times the tester mouse entered in the center, in the closed or open arms. The mouse was considered to have entered an arm if all four paws had left the center square.
Distance	The total distance traveled (cm) by the tester mice in the center, in each arm and in the total arena.
Latency to the first entry in open arms	The time passed (s) until the mouse first entered the open arms.
Mean velocity (v)	The mean velocity (cm/s) displayed by the tester mouse during the trial.
Protected head-dipping	The number of times the tester mouse scanned over the side of the center of the platform towards the floor.
Unprotected head-dipping	The number of times the tester mouse scanned over the side of the open arms of the platform towards the floor.
Head-dipping	Total number of times the tester mouse scanned over the side of the platform (center and open arms) towards the floor.

2.3. Behavioral tests

Around PND90, anxiety-related behaviors were evaluated by performing the Elevated Plus Maze (EPM) test and the Open Field (OF) test (n = 10 ± 1/group) (Carola et al., 2002; Kraeuter et al., 2019; Kulesskaya and Voikar, 2014; Lezak et al., 2017; Seibenhener and Wooten, 2015; Walf and Frye, 2007). Females were tested in the estrus phase, assessed by vaginal smear (McLean et al., 2012). On the day of the test, mice were placed in the room in which the test was performed at least 2 h before starting to allow for habituation to the room. Before starting and between each trial, the testing apparatuses were cleaned with 70% ethanol, and thoroughly dried to avoid exposure of mice to alcohol. The EPM is particularly sensitive to testing conditions (Albani et al., 2015; Shoji and Miyakawa, 2021), therefore it was the first test to be performed, followed by the OF, after at least 1 h (Carola et al., 2002; Schmitt and Hiemke, 1998). The tests were performed in the dark, using only a 25-W red light, which mice cannot see, to help the operator manipulate the animals (Palanza et al., 2002). Each test was recorded with an infrared camera placed above the apparatus in order to subsequently perform the

Table 2
Parameters analyzed for each mouse in the OF recorded trials.

Parameter	Description
Cumulative Duration (CD)	The cumulative time (s) spent by the tester mouse in the center or in the border of the arena
Frequency of entrance	The number of times the tester mouse entered in center or in the border. The mouse was considered to have entered the zone if all four paws had overtaken the borderline between the two.
Distance	The total distance traveled (cm) by the tester mice in the center, in the border and in the total arena.
Latency to the first entry in the center of arena	The time passed (s) until the mouse first entered the center of the arena.
Mean velocity (v)	The mean velocity (cm/s) displayed by the tester mouse during the trial.
Grooming	The number of times the tester mouse licked or scratched its fur, washed its face, or licked its genitalia.
Protected rearing	The number of times the tester mouse reared on its hind paws in the border of the arena.
Unprotected rearing	The number of times the tester mouse reared on its hind paws in the center of the arena.
Rearing	Total number of times the tester mouse reared on its hind paws in the center or in the border of the arena.

behavioral analysis through the Ethovision XT Software (Noldus Information Technology, Wageningen, The Netherlands).

2.3.1. Elevated plus maze (EPM)

The EPM test apparatus was a plus-cross-shaped platform comprising two open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm x 15 cm walls) originating from a central platform (5 cm × 5 cm) and raised 60 cm above the floor (Longo et al., 2014). At the beginning of the recorded session, the tester mouse was gently placed in the center of the platform. Then it was allowed to freely explore the apparatus for 10 min. After the recording, the parameters (Carola et al., 2002) described in Table 1 were analyzed for the first 5 min of the test using Ethovision XT Software (Noldus Information Technology, Wageningen, The Netherlands).

2.3.2. Open field (OF)

OF apparatus consisted of an unfamiliar arena (45 cm × 45 cm x 38 cm walls), which was divided into a central (20 × 20 cm) and a peripheral zone (Longo et al., 2014). At the beginning of the test, the mouse was placed in the corner of the apparatus and was allowed to explore the arena for 10 min. After the recording, the parameters (Carola et al., 2002) described in Table 2 were analyzed for the first 5 min of the test using Ethovision XT Software (Noldus Information Technology, Wageningen, The Netherlands).

2.4. Fixation and tissue sampling

Sacrifice and tissue sampling were performed as described in our previous works (Bonaldo et al., 2020; Bonaldo et al., 2022; Bonaldo et al., 2023). Briefly, at least ten days after performing the behavioral tests, mice were sacrificed by deep irreversible anesthesia (Zoletil 80 mg/kg/Rompum 10 mg/kg intraperitoneally injected) and transcardially perfused with 4% paraformaldehyde (PFA) solution. Females were sacrificed in the estrus phase, assessed by vaginal smear (McLean et al., 2012). Removed brains were post-fixed in 4% PFA and cryoprotected in order to be frozen and stored at −80 °C until sectioning (Bonaldo et al., 2020, 2022).

Brains (n = 4/group) were serially cut in the coronal plane at 30 μm thickness with a cryostat in four series, and sections were collected and stored in a cryoprotectant solution (Watson Jr. et al., 1986). The sectioning plane was oriented to match the drawings corresponding to the coronal sections of the mouse brain atlas (Paxinos and Franklin, 2001).

2.5. Serotonin immunohistochemistry

The presence of serotonin (5-HT) was detected by immunohistochemistry performed on free-floating sections from one series. After an overnight wash in 0.01 M phosphate buffer (PBS) pH 7.3, the sections were first incubated in citrate buffer (10 mM citric acid, 0.05% Tween, pH 6.0) that was previously heated to 95 °C for antigen retrieval. Next, the sections were washed in PBS containing 0.5% Triton X-100 for 30 min and then treated with a solution of PBS containing methanol/hydrogen peroxide for 20 min to inhibit endogenous peroxidase activity. Next, sections were incubated for 30 min in blocking solution containing normal horse serum (Vector Laboratories, Burlingame, CA, USA) and bovine serum albumin (Sigma-Aldrich, Milan, Italy) diluted in PBS and 0.5% Triton X-100. After blocking, the sections were incubated for two nights at +4 °C with anti-5-HT antibody (Immunostar,#20079, Goat, 1:2.500) diluted in the blocking solution. Next, sections were incubated in biotinylated horse anti-goat secondary antibody (Vector Laboratories, Burlingame, CA, USA) diluted in PBS, pH 7.3–7.4, containing 0.2% Triton X-100 was then employed at a dilution of 1:200 for 60 min at room temperature. The antigen-antibody reaction was revealed after a 60 min incubation with avidin–peroxidase complex (Vectastain ABC Kit Elite, Vector Laboratories, Burlingame, CA, USA). The peroxidase activity was visualized with a solution containing 0.400 mg/ml 3,3-diamino-benzidine (Sigma-Aldrich, Milan, Italy) and 0.004% hydrogen peroxide in 0.05 M Tris–HCl buffer at pH 7.6. Sections, mounted on chromallum-coated slides and air-dried, were cleared in xylene and coverslipped with New-Entellan mounting medium (Merck, Milano, Italy). This antibody was successfully used in previous studies (García-González et al., 2017; Yi Li et al., 2020), and its specificity was tested by the factory (https://www.biocompare.com/9776-Antibodies/2874940-5-HT-Serotonin-Goat-Antibody/#citations). As a further control, we omitted the primary antiserum or the biotinylated secondary one and replaced it with PBS. In both cases, positive cell bodies and fibers were totally absent.

2.6. Quantitative analysis

For quantitative analysis, selected standardized sections covering the Dorsal Raphe Nucleus (DR, Bregma −4.60 to −4.84 mm) and Median Raphe Nucleus (MnR, Bregma −4.36 to −4.48 mm) were chosen according to the mouse brain atlas (Paxinos and Franklin, 2001). A single section for each nucleus was acquired with a NIKON DS-U1 digital camera (Software of acquisition: NIS-Element AR 2.10) connected to a NIKON Eclipse 90i microscope (Nikon Italia S. p.S., Firenze, Italy). Images were digitized by using a 20x objective for nuclei acquisition. Digital images were processed and analyzed by ImageJ (version 2.10/1.53c; Wayne Rasband, NIH, Bethesda, MD, USA). Measurements were performed within predetermined fields (region of interest, ROI), boxes of fixed size and shape that are inserted inside each labeled nucleus (456.706 mm^2 for the DR; 238.818 mm^2 for the MnR). The DR was also divided into two subregions, the dorsal region (DRD, 375.348 mm^2) and the ventral region (DRV, 81.268 mm^2), following the specific distribution within the nucleus of the analyzed system (Paxinos and Franklin, 2001; Ren et al., 2018).

We evaluated the immunoreactivity of cell bodies, dendrites, and fibers in all the selected nuclei as fractional area (FA) covered by immunopositive material (Bonaldo et al., 2020, 2022; Viglietti-Panzica et al., 1994). Additionally, we counted the number of 5-HT-positive cells in the two analyzed nuclei.

2.7. Statistical analysis

Data were analyzed by two-way analysis of variance (ANOVA) (sex and treatment used as independent variables) with SPSS 27 statistic software (SPSS Inc., Chicago, USA). If the ANOVA was significant, the *post hoc* analysis was performed using Tuckey's HSD test. Differences were considered statistically significant for values of $p \leq 0.05$. Data are shown as mean ± SEM (mean standard error).

3. Results

3.1. Effects of perinatal exposure to BPA or BPS on anxiety-related behaviors

Results obtained from the analysis of the EPM and OF tests are summarized in Table 3 and Table 4, respectively. Here we highlight the most interesting results (Figs. 1 and 2).

3.1.1. Elevated plus maze (EPM)

The statistical analysis of the EPM highlighted some significant differences among the groups (summarized in Table 3). First, as expected, we observed that control males were more anxious compared to control females, as they spent less time in the open ($p = 0.003I$, Fig. 1B) and more time in the closed arms ($p = 0.012$, Fig. 1C), displayed an increased latency to the first entry in open arms ($p = 0.008$, *Fig. 1H)* and lower frequency of head-dipping behaviors ($p = 0.021$, Fig. 1I). Interestingly, the treatment with both BPs seemed to disrupt these sex-driven differences (Table 3 and Fig. 1).

Table 3
Results obtained from the analyzed parameters within the first 5 min of the EPM test. Data are reported as Mean ± SEM. Two-way ANOVA (sex and treatment used as independent variables) revealed a significant effect for $p \leq 0.05$, highlighted in bold.

Parameter	Oil		BPA		BPS		ANOVA	
	Males	Females	Males	Females	Males	Females	$F_{(5, 57)}$	p
Cumulative duration (s) in:								
Center	44.641 ± 4.819	82.66 ± 7.826	69.489 ± 3.63	84.891 ± 8.627	52.651 ± 3.252	57.28 ± 6.562	6.628	**<0.001**
Open arms	27.883 ± 4.144	68.966 ± 8.602	53.626 ± 8.771	39.276 ± 9.299	43.54 ± 5.782	41.145 ± 5.620	3.760	**0.005**
Closed arms	226.986 ± 8.499	148.516 ± 10.878	174.604 ± 10.286	175.994 ± 13.437	199.824 ± 8.059	199.842 ± 11.084	6.390	**<0.001**
Frequency of entrance in:								
Center	24.6 ± 1.31	32.727 ± 2.232	31.455 ± 2.006	29.75 ± 2.372	28 ± 2.284	27.455 ± 2.738	1.656	0.160
Open arms	8.9 ± 0.888	15.909 ± 1.786	16.909 ± 2.395	12.25 ± 1.287	12.636 ± 1.439	15.364 ± 1.835	2.973	**0.019**
Closed arms	14.5 ± 0.764	16.455 ± 1.021	16.455 ± 0.666	17.875 ± 1.623	16.636 ± 1.771	14.091 ± 1.131	1.123	0.359
Distance (cm) traveled in:								
Arena	46603.921 ± 3156.563	45885.65 ± 7783.543	77262.609 ± 2833.946	80483.599 ± 841.290	73579 ± 8679.094	75878.268 ± 5133.699	5.715	**<0.001**
Center	22599.16 ± 1673.799	21562 ± 291 ±3815.782	36798.473 ± 1267.868	37800.025 ± 4469.577	34287.418±	37853 ± 4582 ±414	4.058	**0.003**
Open arms	22014.96 ± 1688.617	21488.58 ± 4214.155	36475 ± 1956 ±229	38163.05 ± 4214.903	36293.245 ± 4337.169	31545.582 ± 2915.674	4.485	**0.002**
Closed arms	1989.801 ± 339.334	2834.779 ± 580.471	3988.336 ± 611.876	4520.524 ± 603.451	2999.208 ± 5 84.489	6479.432 ± 1086.564	5.168	**0.001**
Latency (s) to the first entry in open arms	22.938 ± 5.370	5.790 ± 1.650	3.470 ± 1.034	7.775 ± 1.947	6.152 ± 1.885	15.646 ± 5.217	4.923	**0.001**
Mean velocity (cm/s)	4.066 ± 0.257	3.690 ± 0.149	4.893 ± 0.233	4.893 ± 0.356	4.369 ± 0.257	5.242 ± 0.284	5.114	**0.001**
Protected head-dipping	7.8 ± 0.49	11.091 ± 0.986	11.182 ± 0.903	9.375 ± 0.979	10.182 ± 1.750	8.636 ± 1.28	1.279	0.286
Unprotected head-dipping	3.5 ± 1.186	10.182 ± 2.296	6.546 ± 1.841	6.875 ±s 2.282	3.903 ± 1.221	6.364 ± 1.011	1.929	0.104
Head-dipping	11.3 ± 1.325	21.272 ± 1.893	17.728 ± 1.799	16.25 ± 2.927	14.091 ± 2.343	15 ± 2.074	2.524	**0.039**

In fact, perinatal treatment with BPA in males caused a significant decrease in time spent in closed arms ($p = 0.012$, Fig. 1C) together with an increased number of entries ($p = 0.023$, Fig. 1G) and a reduction in the latency to the first entry ($p = 0.002$, Fig. 1H) in open arms, in which they tended to travel a greater distance ($p = 0.053$, Fig. 1E), compared to control males. On the other hand, BPA treatment in females caused a significant decrease in time spent in open arms ($p = 0.048$, Fig. 1B), together with an increase in the distance traveled ($p = 0.025$, Fig. 1E), compared to control females.

Perinatal treatment with BPS in males is linked, even if not significantly, to the increase in time spent in open arms ($p = 0.471$, Fig. 1B) and to the decrease in time spent in closed arms ($p = 0.667$, Fig. 1C) compared to control males. It also caused a significant decrease in the latency to the first entry in open arms ($p = 0.010$, Fig. 1C), in which they tended to travel a greater distance ($p = 0.058$, Fig. 1E) compared to control males. Conversely, BPS-treated females, compared to control females, seemed to spend less time in open arms ($p = 0.086$, Fig. 1B) and displayed a significant increase in time spent in the closed arms ($p =$

Table 4
Results obtained from the analyzed parameters within the first 5 min of the OF test. Data are reported as Mean ± SEM. Two-way ANOVA (sex and treatment as independent variables) revealed a significant effect for $p \leq 0.05$, highlighted in bold. * Tendency towards significance ($0.05 < p < 0.06$).

Parameter	Oil		BPA		BPS		ANOVA	
	Males	Females	Males	Females	Males	Females	$F_{(5, 57)}$	p
Cumulative duration (s) in:								
Center	12.295 ± 1.909	24.470 ± 3.226	18.109 ± 2.679	19.928 ± 1.83	19.896 ± 3.032	18.376 ± 2.175	2.283	0.058*
Border	287.84 ± 1.91	275.649 ± 3.226	282.928 ± 2.681	280,212 ± 1.831	280.235 ± 3.033	281.758 ± 2.177	2.287	0.058*
Frequency of entrance in:								
Center	11.5 ± 1.551	19.636 ± 2.325	17 ± 2.183	16.778 ± 2.080	18 ± 2.195	18.376 ± 2.175	2.365	0.051*
Border	12.5 ± 1.551	20.545 ± 2.302	17.909 ± 2.125	17.667 ± 2.021	19 ± 2.195	21.273 ± 1.556	2.387	**0.049**
Distance traveled (cm) in:								
Arena	55078.65 ± 1456.051	51255.73 ± 939.741	51996.06 ± 1332.43	49384.66 ± 1625.357	51413.93 ± 1332.43	53713.63 ± 1313.416	2.072	0.082
Center	54216.45 ± 1540.589	49436.682 ± 954.328	51144.028 ± 1428.175	47927.611 ± 1707.159	50050.982 ± 1488.990	52460.318 ± 1414.765	2.366	0.053*
Border	862.203 ± 178.520	1819.045 ± 207.095	852.031 ± 143.619	1457.047 ± 145.799	1362.946 ± 182.690	1253.307 ± 155.909	4.692	**0.001**
Latency to the first entry in the center (s)	40.496 ± 10.606	12.268 ± 4.131	8.589 ± 2.464	10.044 ± 3.961	18.689 ± 3.402	5.716 ± 1.967	6.118	**<0.001**
Mean velocity (cm/s)	11.542 ± 0.514	13.064 ± 0.739	14.021 ± 1.074	11.381 ± 0.785	13.401 ± 0.816	14.328 ± 0.796	2.254	0.061
Grooming	3.3 ± 0.423	2.455 ± 0.718	3.818 ± 0.761	4.222 ± 1.267	4.091 ± 0,623	2.182 ± 0.519	1.373	0.248
Protected rearing	31.2 ± 2.284	33.364 ± 3.619	33.363 ± 2.487	27.667 ± 2.609	34.273 ± 2.512	33.455 ± 2.146	0.765	0.579
Unprotected rearing	3.8 ± 1.254	6.455 ± 1.391	4.273 ± 1.685	6.111 ± 1.670	6.818 ± 2.231	4.455 ± 0.888	0.667	0.650
Rearing	35.9 ± 2.834	39.819 ± 3.968	37.455 ± 2.774	33.778 ± 3.179	41.091 ± 4.564	38.182 ± 2.354	0.573	0.720

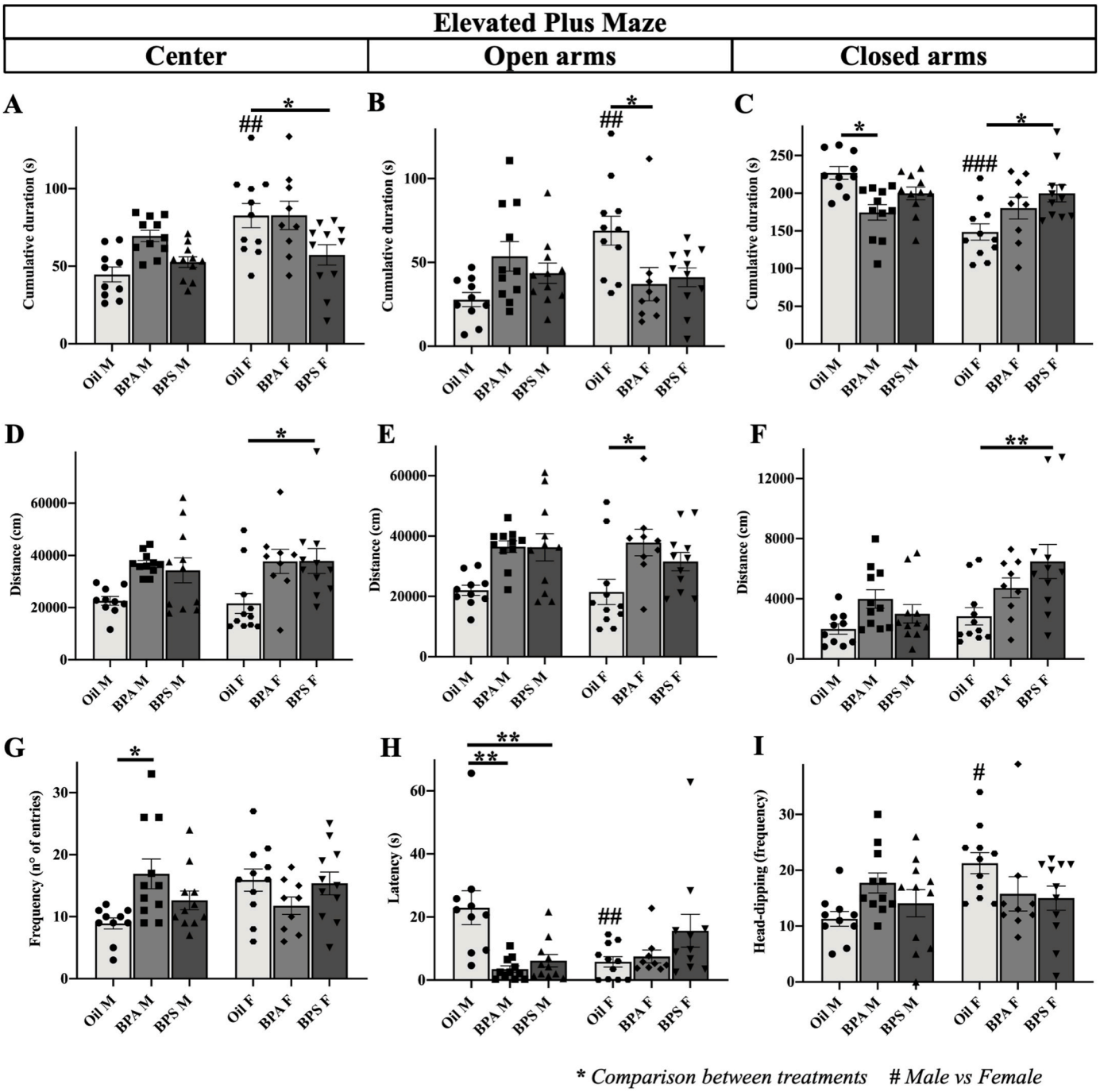

Fig. 1. Analysis of anxiety-related behaviors of control and treated mice through Elevated Plus Maze Test. Time spent in the center **(A)**, in open arms **(B)** or in closed arms **(C)** by oil- (light gray), BPA- (gray) or BPS- (dark gray) treated male mice (left side of the graph) or female mice (right side of the graph). Total distance traveled in the center **(D)**, in open arms **(E)** or in closed arms **(F)** by oil- (light gray), BPA- (gray) or BPS- (dark gray) treated male mice (left side of the graph) or female mice (right side of the graph). **(G)** Number of entries in open arms displayed by oil- (light gray), BPA- (gray) or BPS- (dark gray) treated male mice (left side of the graph) or female mice (right side of the graph) within the first 5 min of the test. **(H)** Latency to first entry in open arms displayed by oil- (light gray), BPA- (gray) or BPS- (dark gray) treated male mice (left side of the graph) or female mice (right side of the graph) within the first 5 min of the test**. (I)** Total number of head-dipping performed by oil- (light gray), BPA- (gray) or BPS- (dark gray) treated male mice (left side of the graph) or female mice (right side of the graph) within the first 5 min of the test. Data are expressed as mean ± SEM. Two-way ANOVA revealed a significant effect for $p \leq 0.05$ (* = comparison between treatments; # = male vs female).

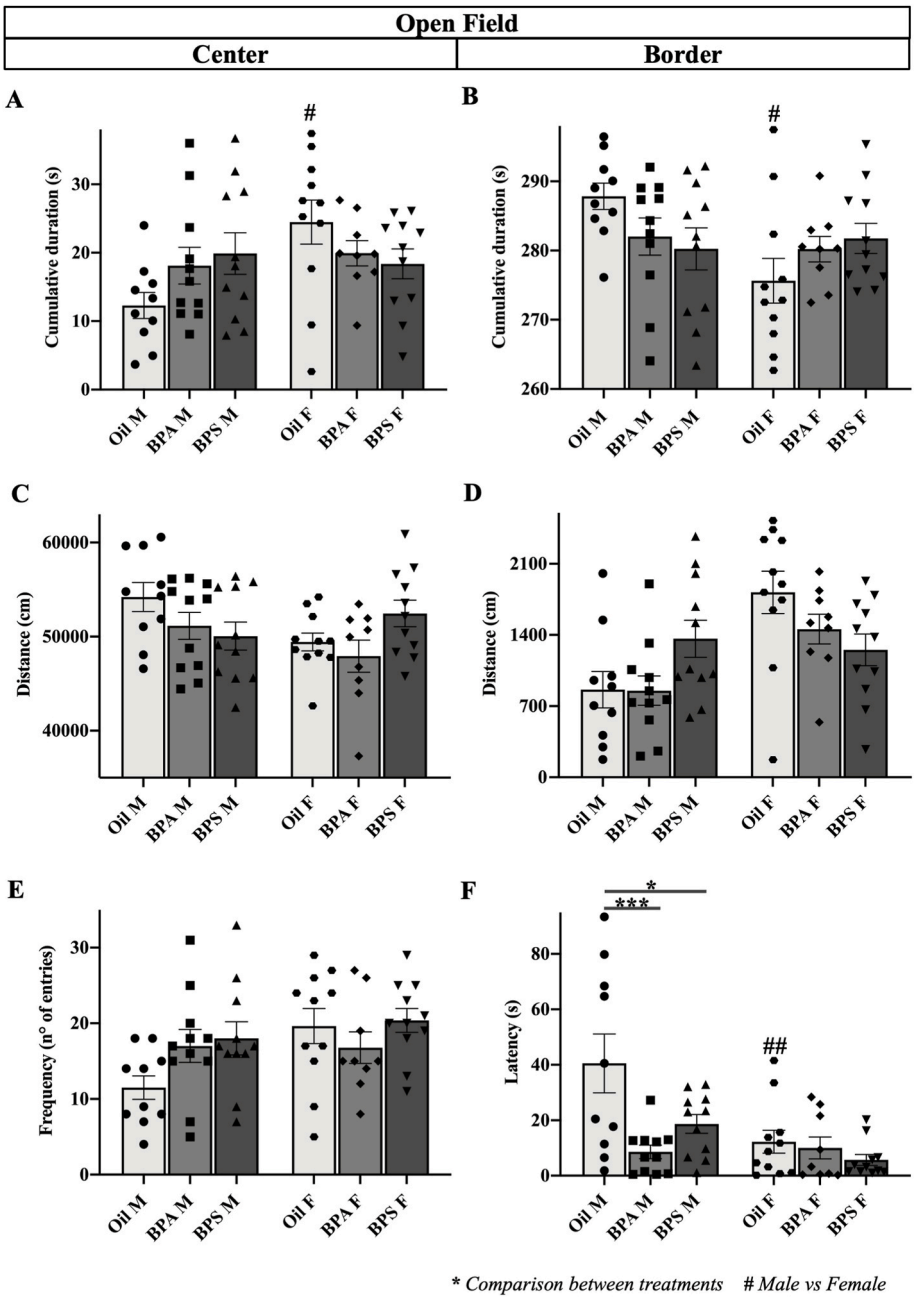

Fig. 2. Analysis of anxiety-related behaviors of control and treated mice through Open Field Test. Time spent in the center **(A)** or along the border **(B)** of the arena by oil- (light gray), BPA- (gray) or BPS- (dark gray) treated male mice (left side of the graph) or female mice (right side of the graph). Total distance traveled in the center **(C)** or along the border **(D)** of the arena by oil- (light gray), BPA- (gray) or BPS- (dark gray) treated male mice (left side of the graph) or female mice (right side of the graph). **(E)** Number of entries in the center of the arena displayed by oil- (light gray), BPA- (gray) or BPS- (dark gray) treated male mice (left side of the graph) or female mice (right side of the graph) within the first 5 min of the test. **(F)** Latency to the first entry in the center of the arena displayed by oil- (light gray), BPA- (gray) or BPS- (dark gray) treated male mice (left side of the graph) or female mice (right side of the graph) within the first 5 min of the test. Data are expressed as mean ± SEM. Two-way ANOVA revealed a significant effect for $p \leq 0.05$ (* = comparison between treatments; # = male vs female).

0.011, Fig. 1C), in which they traveled a greater distance ($p = 0.006$, Fig. 1F).

Finally, both BPs disrupt the sexual differences in the frequency of head-dipping (Fig. 1I).

3.1.2. Open field (OF)

The statistical analysis of the OF highlighted some significant differences among the groups (summarized in Table 4). The analysis confirmed the sex differences, which also emerged in the analysis of the EPM, between control males and females. Males spent less time in the center ($p = 0.019$, Fig. 2A) and more time along the border ($p = 0.019$, Fig. 2B), in which they traveled a shorter distance ($p = 0.003$, Fig. 2D). Additionally, males displayed an increased latency to the first entry in the center ($p = 0.003$, Fig. 2F) and tended to enter the center less frequently ($p = 0.065$, Fig. 1E) compared to females. Moreover, males traveled, in the entire arena, significantly ($p = 0.003$) more distance compared to females (Table 3). Once again, the treatment with both BPs seemed to disrupt these sex-driven differences.

BPA-treated groups showed no significant sexual differences both in time spent in the center ($p = 0.997$, Fig. 2A) nor along the border ($p = 0.997$, Fig. 2B) of the arena. This is related to the fact that, even if not significantly, BPA treatment led to an increase in time spent in the center

and a decrease in time spent along the border in males, while it caused the opposite in females (Fig. 2A-B). Furthermore, BPA-treated males displayed a significant decrease in latency to the first entry in the center ($p = 0.001$, Fig. 2F) compared to control males.

The BPS-treated groups also showed no significant sexual differences both in time spent in the center ($p = 0.998$, Fig. 2A) nor along the border ($p = 0.998$, Fig. 2B) of the arena. In fact, the BPS treatment in males caused, even if not significantly, similar alterations as the BPA treatment, decreasing the time spent in the center (Fig. 2A) and increasing that spent along the border (Fig. 2B), while doing the opposite in the females (Fig. 2A-B). Moreover, BPS-treated males showed a significant decrease in latency to the first entry in the center ($p = 0.041$, Fig. 2F) compared to control males.

Finally, both BPs disrupt the sexual differences in frequency of entries in the center of the arena (Fig. 2E).

3.2. 5-HT-ir analysis

The statistical analysis of the immunoreactivity for 5-HT (summarized in Table 5) revealed that both BPs treatments affected the nuclei, differently in the two sexes.

First, we corroborated the presence of sexual dimorphism within the DR in oil-treated mice, in terms of FA ($p = 0.001$, Fig. 3E), both in the dorsal ($p = 0.050$, Fig. 3F) and in the ventral regions ($p = 0.004$, Fig. 3G) of the nucleus, with males showing lower FA compared to females (Fig. 3A, upper level).

The treatment with BPA caused the highest alterations in males within all the analyzed nuclei (Fig. 3 left side-central level; Fig. 4 central column-upper level). We observed an increase of 5-HT-ir, both in terms of number of cells and FA, in the DR ($p = 0.001$, Fig. 3B; $p < 0.001$, Fig. 3E), in the DRD ($p = 0.007$, Fig. 3C; $p < 0.001$, Fig. 3F), in the DRV ($p = 0.001$, Fig. 3D; $p < 0.001$, Fig. 3G) and also in the MnR ($p = 0.006$, Fig. 4B; $p = 0.003$, Fig. 4C) of BPA-treated males compared to the control males. In BPA-treated females (Fig. 3 right side-central level; Fig. 4 central column-lower level), we found a significant increase in FA in the DRD ($p = 0.009$, Fig. 3F) and in the MnR ($p < 0.001$, Fig. 4C), compared to control females.

In males, the perinatal treatment with BPS caused a significant increase in the FA of the DR ($p = 0.001$, Fig. 3E), due to a significant increase in the ventral component of the nucleus, appreciable both in terms of number of cells ($p = 0.031$, Fig. 3D) and FA ($p = 0.004$, Fig. 3G), compared to controls.

In BPS-treated females (Fig. 3 right side-lower level; Fig. 4 left column-lower level), we found a significant increase in FA in the DRD ($p = 0.020$, Fig. 3F), compared to the control females.

4. Discussion

Nowadays, exposure to BPs represents a matter of concern for human health (Abraham and Chakraborty, 2020; Kahn et al., 2020; Pelch et al., 2019), as they can be easily found in the environment (D. Chen et al., 2016; Vasiljevic and Harner, 2021; Wu et al., 2018) and have already been detected in different human physiological fluids (e.g., plasma, urine) at different concentrations (Abraham and Chakraborty, 2020) depending on the examined cohort, type of sample and detection methods (H.-C. Chen et al., 2022). The relevance of biomonitoring human exposure to such compounds was firmly stated by the Human Biomonitoring for Europe (HBM4EU) project, launched in 2016 and completed in 2022, which aimed to study the human exposure to different chemicals (BPs included) and the possible link to adverse health outcomes (Vaccher et al., 2022).

To date, the effects of BPs on various aspects of endocrine control, such as reproduction (den Braver-Sewradj et al., 2020; Tomza-Marciniak et al., 2018) or metabolism (den Braver-Sewradj et al., 2020; Rubin et al., 2019), are widely debated, but very little is known about the possible effects of potential effects of BPs on anxiety-like behaviors. The results of this study highlight that exposure to both BPA and BPS during early phases of life alters, in a sex-dependent manner, both anxiety-related behaviors and serotonergic populations within the Raphe nucleus, which is involved in the control of these behaviors, in adult animals.

During the EPM test, BPA-treated males showed a significant increase in the time spent in the open arms compared to the control males and a decrease in the latency of the first entry in the open arms, which was also displayed by BPS-treated males. Among females a significant decrease in time spent in open arms was observed in the BPA-treated group, together with an increase in time spent in the closed arms among the BPS-treated females. The OF test showed that both treatments disrupted the sex-dependent differences in the analyzed behaviors, mainly decreasing anxiety-related behaviors in males and increasing them in females. These behavioral alterations suggest different effects of exposure to BPs in the two sexes: anxiolytic in males and anxiogenic in females.

Therefore, we analyzed the serotonergic system in the Raphe complex, which is highly involved in the control of anxiety-related behaviors. We performed an immunohistochemical analysis of the 5-HT-ir, both in terms of the number of cells and fractional area, in the DR distinguishing its dorsal (DRD) and ventral (DRV) components and in the MnR. In control mice, we detected sexual dimorphism of the system in the DR only, with control females showing higher values of 5-HT-ir when compared to control males. BPA-treated males displayed a significant increase of 5-HT-ir in all analyzed nuclei, whereas BPS-treated males showed an increase in DRV only. In females, both BPA- and

Table 5
Results obtained from the analysis of 5-HT-ir in all selected nuclei. Data are reported as Mean ± SEM, both as number of positive cells and fractional area. Two-way ANOVA revealed a significant effect for $p \leq 0.05$, highlighted in bold. DR = dorsal raphe; DRD = dorsal.

Number of serotonin positive cells								
Zone	Oil		BPA		BPS		ANOVA	
	Males	Females	Males	Females	Males	Females	$F_{(5, 18)}$	P
DR	133.75 ± 7.793	150.75 ± 3.613	209.25 ± 8.702	138.5 ± 13.295	158.5 ± 16.795	174.75 ± 4.423	7.106	**0.001**
DRD	101.25 ± 4.644	107.5 ± 4.806	154 ± 7.223	87.5 ± 11.449	111.25 ± 14.585	127.75 ± 7.204	6.576	**0.001**
DRV	32.5 ± 3.663	43.25 ± 2.496	55.25 ± 3.544	51 ± 3.189	47.25 ± 2.358	47 ± 2.858	6.479	**0.001**
MnR	27.5 ± 1.936	39 ± 3.629	46.25 ± 2.780	46.25 ± 5.391	32.25 ± 1.436	37.5 ± 2.062	5.055	**0.005**
Fractional Area (%)								
Zone	**Oil**		**BPA**		**BPS**		**ANOVA**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	$F_{(5, 18)}$	**p**
DR	19.964 ± 0.701	34.835 ± 1.472	47.689 ± 0.907	43.58 ± 4.123	34.657 ± 1.424	43.047 ± 1.813	22.874	**<0.001**
DRD	7.871 ± 0.569	12.881 ± 0.627	20.149 ± 0.761	19.172 ± 1.344	12.694 ± 0.988	18.601 ± 1.832	18.917	**<0.001**
DRV	12.094 ± 0.866	21.954 ± 1.214	27.540 ± 0.567	24.408 ± 3.087	21.963 ± 1.576	24.446 ± 0.51	11.382	**<0.001**
MnR	4.518 ± 0.406	7.795 ± 0.924	10.605 ± 1.456	17.68 ± 1.163	6.992 ± 0.462	5.145 ± 0.861	26.013	**<0.001**

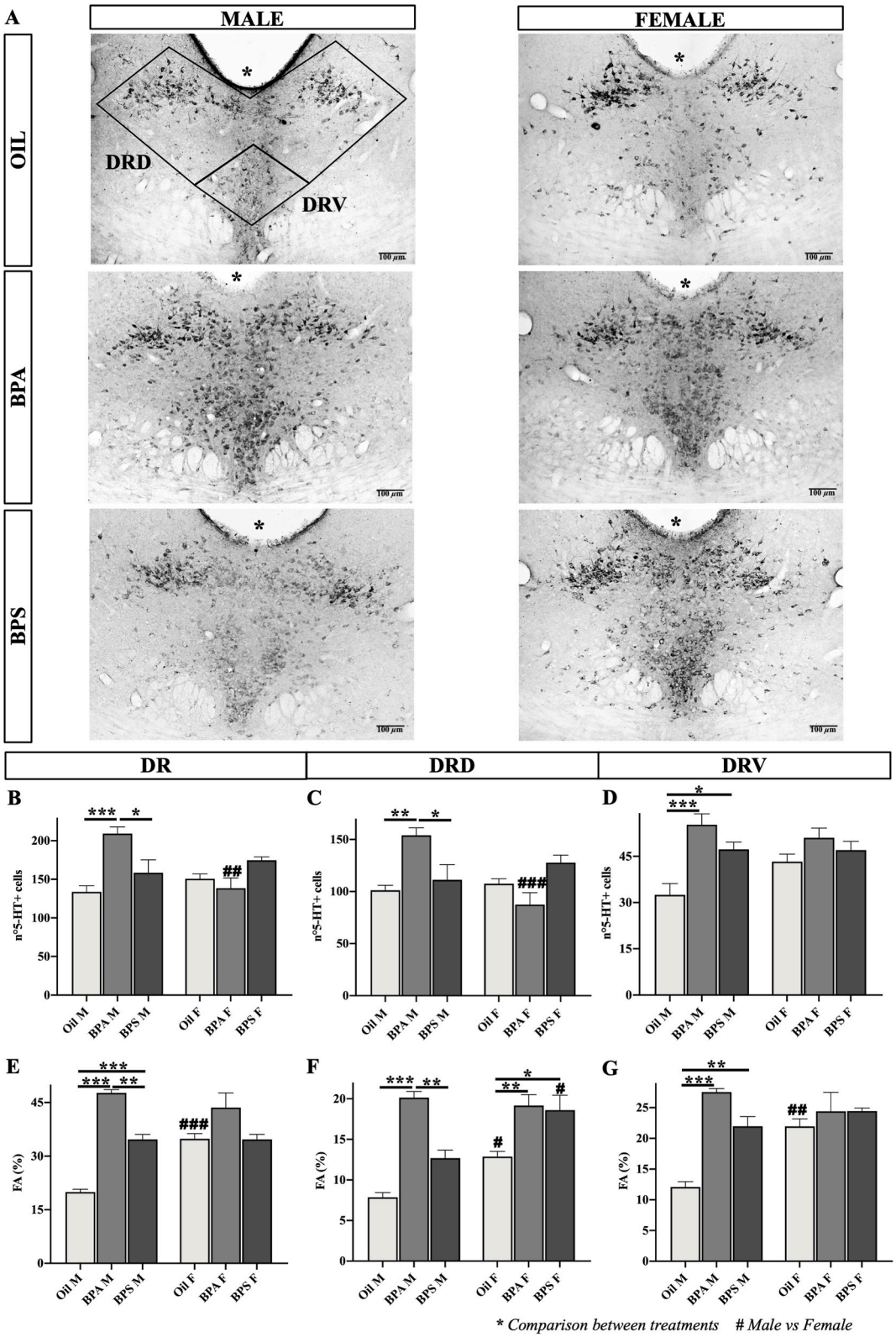

Fig. 3. Serotonin immunoreactivity in the DR of control and treated mice. (A) Representative image of serotonin immunoreactivity in a coronal section of DR of oil-treated (upper level), BPA- (central level) or BPS- (lower level) treated male (left) or female (right) mice. Analysis of 5-HT-ir in total DR (as **(B)** number of 5-HT + cells and **(E)** fractional area), in DRD (as **(C)** number of 5-HT + cells and **(F)** fractional area) and in DRV (as **(D)** number of 5-HT + cells and **(G)** fractional area). In the histograms (B, C, D, E, F, G) the oil-treated mice are shown in light gray, BPA-treated mice are shown in gray, and BPS-ones are shown in dark gray. Male mice are shown on the left side of the graph, while females are on the right side. Data are expressed as mean ± SEM. Two-way ANOVA revealed a significant effect for $p \leq 0.05$ (* = comparison between treatments; # = male vs female). Scale bar = 100 μm. 5-HT = serotonin; ir = immunoreactivity; FA = fractional area; DR = dorsal raphe; DRD = dorsal region of dorsal raphe; DRV = ventral region of dorsal raphe; * = cerebral aqueduct.

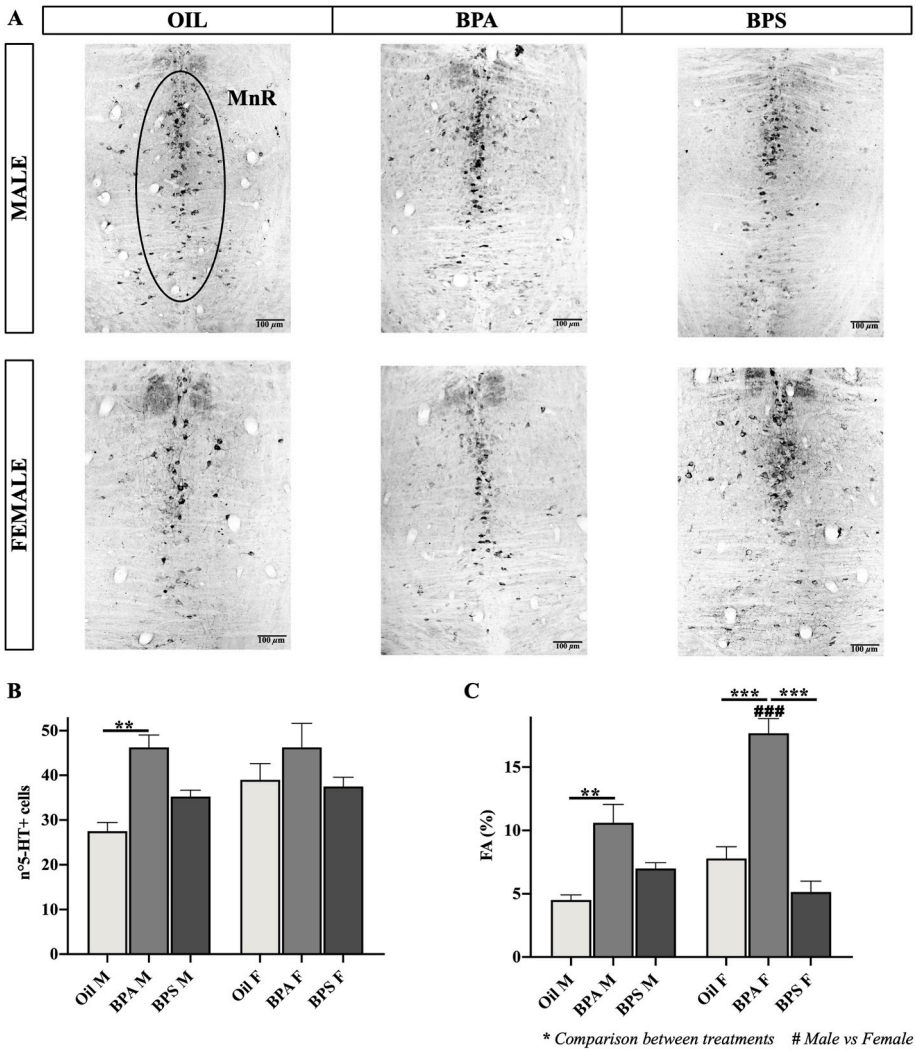

Fig. 4. Serotonin immunoreactivity in the MnR of control and treated mice. (A) Representative image of serotonin immunoreactivity in a coronal section of MnR of oil-treated (left), BPA- (center) or BPS- (right) treated male (up) or female (down) mice. Analysis of 5-HT-ir in MnR, expressed both as **(B)** number of 5-HT + cells and **(C)** fractional area. In the histograms **(B, C)** the oil-treated mice are shown in light gray, BPA-treated mice are shown in gray, and BPS-ones are shown in dark gray. Male mice are shown on the left side of the graph, while females are on the right side. Data are expressed as mean ± SEM. Two-way ANOVA revealed a significant effect for $p \leq 0.05$ (* = comparison between treatments). Scale bar = 100 μm. 5-HT = serotonin; ir = immunoreactivity; FA = fractional area; MnR = median raphe.

BPS-treated groups showed a significant increase of 5-HT-ir in DRD compared to controls, and BPA-treated females also showed a significant increase in MnR. Interestingly, both BP treatments caused alterations within the analyzed nuclei, increasing 5-HT-ir. However, while in males, the increase seemed to be due to a greater number of 5-HT-positive cells, in females, the alterations were appreciable only in terms of fractional area.

The EPM test allows evaluation of different aspects of anxiety-related behaviors, leaning on the balance between the natural tendency of rodents to avoid open or elevated spaces and their innate curiosity to explore unknown new areas (Carola et al., 2002; Lezak et al., 2017). A less anxious mouse will spend more time in the open arms compared to a more anxious one (Carola et al., 2002; Lezak et al., 2017). In the OF test, mice with lower anxiety tend to spend more time in the center of the arena compared to the border to explore the open space (Carola et al., 2002; Lezak et al., 2017).

Our results, at first, confirmed previous evidence of sexual dimorphism in anxiety-related behaviors (Cover et al., 2014; Donner and Lowry, 2013). In fact, both the EPM and the OF test showed that control females, in estrus, displayed less anxious behaviors, basically spending more time in open and unprotected zones compared to control males. The perinatal treatment with both BPs disrupted these sex-driven behavioral differences. Treated males seemed to be less anxious and more explorative compared to control males; whereas treated females became more anxious compared to control females. These results suggest an anxiolytic effect of tested BPs in males and an anxiogenic one in females. It has already been shown that exposure to BPA during critical periods alters anxiety-related behaviors. In particular, males exposed in utero to low-dose BPA displayed a decrease in anxiety-related behavior (Kundakovic et al., 2013), while some anxiogenic effects have been shown in females exposed in utero (Kundakovic et al., 2013) or during the pre- or postnatal periods (Gioiosa et al., 2013) to low-dose BPA. Our data corroborated previous results, in which perinatal exposure to low-dose BPA has anxiolytic effects in males and anxiogenic effects in females. Interestingly, we noticed that the same effects are also mediated by BPS. Until now, BPS effects on anxiety in rodents have been poorly investigated. There is some evidence of anxiogenic effects of BPS exposure in females (Hu et al., 2022), while available studies suggest an increase in anxiety-related behaviors also in males (da Silva et al., 2019; Hu et al., 2022; Kim et al., 2015; McDonough et al., 2021; Mornagui et al., 2019). However, this discrepancy could be due to the different timing, dose, and way of BPS administration, which are known to be

particularly accountable for the observable effects mediated by EDCs (Gioiosa et al., 2013).

The analysis of the serotonin system within the DR and MnR showed some significant effects of both BPs. The development of this system is particularly complex and includes mechanisms that specify the 5-HT molecular identity, the structural/regional identity, and the network wiring (Deneris and Gaspar, 2018). In mice, the primary development of 5-HT neurons starts during embryonic life (around embryonic day 9.5). At birth, 5-HT neurons are still immature and their maturation begins simultaneously with the initiation of 5-HT synthesis and continues to at least the third week of life (Deneris and Gaspar, 2018). Thus, our window of exposure fully covers the development of this system.

At first, we corroborated previous results that described sexual dimorphism in rodents' DR (Domínguez et al., 2003; Rubinow et al., 1998), with control females showing higher 5-HT-ir compared to control males. Then, we demonstrated that perinatal exposure to either low-dose BPA or BPS causes an increase in 5-HT-ir in the analyzed nuclei, which differentially impacts the two sexes.

We observed an increase in both the number of cells and FA in all analyzed nuclei in the BPA-treated males, while the increase was limited to the DRV in the BPS-treated males. These results provide the framework for a mechanism by which BPs exert anxiolytic effects in males. In fact, 5-HT neurons located in the DR are particularly involved in regulating the anxiety response, mainly promoting a decrease in anxiety-like behaviors (Bocchio et al., 2016; Ren et al., 2018), as the DRD sends its fibers to subcortical regions and the DRV sends its fibers to cortical regions (Ren et al., 2018). Moreover, also the increase in the 5-HT-ir in the MnR observed in the BPA-treated males may be responsible for the anxiolytic effects, acting similarly to a 5-HT antagonist and thereby activating negative feedback in presynaptic serotonergic neurons located within the nucleus (Domínguez et al., 2003).

In both BPA- and BPS-treated females the effects were appreciable only in terms of FA. In fact, we observed an increase in 5-HT-ir in the DRD in both BPA- and BPS-treated females and also in the MnR of the BPA-treated females, compared to controls. Since we did not notice any significant alterations in terms of the number of cells, the increase in FA seems to be mainly due to an increase in serotonergic fibers and dendritic branching. In the literature, the impact of sex hormones on brain development and plasticity, particularly the structural effects on neurite outgrowth, synaptogenesis, and dendritic branching mediated, especially in females, by estrogen and progesterone is well documented (Barth et al., 2015; Giannini et al., 2019). It could be possible that the opposite effect on anxiety-related behaviors of BPA and BPS in the two sexes is linked to a differential targeting of the serotonin neurons involving the cell bodies in males and the fibers and the dendritic branching in the females. It has been observed that perinatal BPA exposure can alter 5-HT metabolism and signaling: it increases 5-HT production in the DR in both male and female rats (Nakamura et al., 2010), and it induces a 5-HT increase in the hippocampus of female mice, accompanied by increased fear memory (Matsuda et al., 2013). However, the mechanisms by which BPA alters 5-HT expression have not been resolved, but some studies suggest dysregulation of tryptophan hydroxylase (TPH) (Castro et al., 2015; Yao et al., 2020). To date, the effects of BPS on serotonin are not widely reported. Despite this, we showed that BPS mediates similar effects, suggesting that it could act similarly to BPA. Moreover, both BPA and BPS are known to act on a wide set of hormone receptors (Delfosse et al., 2012; den Braver-Sewradj et al., 2020; Murata and Kang, 2018; Park et al., 2020; Rochester and Bolden, 2015), detectable in brain areas involved in the control of anxiety-related behaviors (Landgraf, 2001; Nomura et al., 2005; Walf and Frye, 2006). Considering this, the observed effects could be linked to the high presence of ERβ (Mitra et al., 2003; Nomura et al., 2005), which is efficiently targeted by both BPA and BPS (X. Liu et al., 2019; Molina-Molina et al., 2013; Naderi and Kwong, 2020), within Raphe nuclei and especially the DR (Sheng et al., 2004). In fact, ERβ plays a crucial role in the regulation of anxiety-related behaviors in mice (Borrow and Handa, 2017; Imwalle et al., 2005). Furthermore, it is known that the expression of ERβ is sexually dimorphic (Mogi et al., 2015; Zhang et al., 2002) and that in utero exposure to BPA could lead to region-specific changes in the expression of genes encoding estrogen receptors within the brain (Kawai et al., 2007; Kundakovic et al., 2013). Interestingly, ERβ gene expression decreases in the hypothalamus of female mice exposed to low-dose BPA, but it increases in males (Kundakovic et al., 2013). Thus, we can speculate that both BPA and BPS might cause a decrease in the expression of ERβ in female Raphe nuclei, which is known to lead to an increase in anxiety-like behaviors (Imwalle et al., 2005), but they might cause an increase in ERβ expression in males along with a consequent decrease in the anxiety. The potential alterations of ERβ expression within the Raphe nuclei could particularly impact the serotonergic neurons, given the high co-expression (Nomura et al., 2005). However, in the MnR, we observed some effects only in the BPA-treated animals of both sexes but not in the BPS-treated animals. Moreover, the effects of BPS observed in the DR occur to a lesser degree compared to those mediated by BPA. This could be due to the fact that BPS, even if it displays lower affinity to ERα compared to BPA, acts more specifically on ERα, showing lesser effects on ERβ (Kojima et al., 2019; Y Li et al., 2018).

The serotonergic neurons in raphe nuclei are richly connected to other brain regions, and in particular to several hypothalamic nuclei (Ogawa et al., 2014). Particularly, corticotropin-releasing hormone (CRH) neurons located in the paraventricular nucleus of the hypothalamus (PVN) are extremely integrated with serotonin signaling, influencing stress-related responses which are fundamental in the control of anxiety (Jørgensen et al., 2002). The potential disruption of the stress axis, a well-known target of BPs' action (Michael Caudle, 2016), is another mechanism possibly involved in the observed behavioral alterations. It is known that developmental stressors can cause alterations in central stress system responses that result in long-term changes in adaptive responses to stress (Wiersielis et al., 2020). Particularly, BPs could act by altering the number of CRH neurons in brain regions that regulate stress response during the perinatal period. As different neurotransmitter systems, such as the serotonergic one, are known to be affected by both exposure to BPs (especially BPA) and CRH signaling, this could lead to sex-specific impairments in the stress response (Funabashi et al., 2004; Lama et al., 2023; Wiersielis et al., 2020).

4.1. Limitations of the study

In this last paragraph, we report some limitations of our study. First of all, we are aware that, in general, the effects observed in studies carried out on EDCs are strictly dependent on the animal model used, and in particular, the mouse strain, the sex, the methods and period of exposure, and the administered dose. These parameters could be responsible for some discrepancies in the outcomes of our work compared to others already published in the literature.

Animals: The number of animals used to perform the study is limited, but sufficient (Bonaldo et al., 2021, 2022, 2023; Gioiosa et al., 2013). The number of experimental animals to be used in behavioral studies varies and depends on different parameters such as the living conditions (e.g., wildlife vs animal facility) or species (e.g., vertebrate vs nonvertebrate) (Taborsky, 2010).

Period of exposure: It is known that the impact of EDCs exposure is more dangerous if it occurs during specific "critical periods" of life (such as the perinatal one) when organisms are particularly sensitive to hormonal disruption. Exposure during such critical windows of development could lead to long-term impairments (Frye et al., 2012). We performed a perinatal treatments of BPs and observed behavioral and immunohistochemical changes in the adult animals. Indeed, exposure to BPs during other periods of life could lead to different outcomes (Ji et al., 2023).

Dose: Selecting a single dose to test represents a limit of the study. However, the selected dose is particularly relevant. In the literature

(Misme-Aucouturier et al., 2022; Naule et al., 2014; Sun et al., 2023; Wang et al., 2022), there are works testing the human BPA TDI (which may vary in different countries) in rodents, without any conversion. It is known that rodents are less sensitive than humans, mainly because of their metabolic rate (Demetrius, 2005). Thus, the same dose could potentially lead to even more dangerous effects in humans. Furthermore, TDI doses tested in rodents would usually be higher than those set for humans (Lambré et al., 2023). There are also works testing even lower doses (Kimber et al., 2022), as the EFSA is evaluating whether to lower the BPA TDI at 0.2 ng/kg BW/day (Lambré et al., 2023). Interestingly, the HBM4EU reported human biomonitoring guidance values that are under the current BPA TDI but are still above the recently proposed value. Furthermore, although BPS is one of the most used BPA substitutes, and has already been detected in environmental and human samples (Catenza et al., 2021; J. Liu et al., 2021; Vaccher et al., 2022), no user guidelines are available. Last, it is extremely difficult to assess the actual environmental level of the two BPs, as these are strictly dependent on the measurement methods, country, and type of analyzed sample (e.g., soil, water, etc.) (Careghini et al., 2015; Corrales et al., 2015; Czarny-Krzymińska et al., 2023; Ying and Kookana, 2005).

Performed analysis: We observed behavioral and immunohistochemical changes in adult animals after performing a perinatal treatment of BPs. However, we have no data on how early during development these changes can be observed, how long they may last throughout life, or if there may be transgenerational effects. Furthermore, we did not investigate the potential impairment of functionality or biomolecular changes on the serotonin system, or possible alterations in behavioral responses to chronic stress in those animals. Thus, further studies are required to clarify these aspects of BPs exposure.

5. Conclusions

In this study, we suggest that perinatal exposure to both BPA and BPS, even at a low dose, leads to a disruption of sexual differences in anxiety-related behaviors, which could be partially linked to alterations of the serotonergic system within the dorsal and median Raphe nuclei. Moreover, our results support the idea that exposure to BPs must be avoided, or at least limited, by new and more stringent regulations on the use of these compounds, such as the one recently proposed by the EFSA, which aims to reduce the TDI for BPA from 4 μg/kg BW/day to 0.2 ng/kg BW/day (Lambré et al., 2023). Finally, as the current EFSA TDI for BPA appears not to be completely safe and the regulation of BPS use is still not available, our results underline the need to further regulate the use of both BPs, not only BPA, to limit or avoid health implications.

CRediT authorship contribution statement

Brigitta Bonaldo: Conceptualization, Methodology, Investigation, Writing - original draft. **Antonino Casile:** Investigation, Methodology. **Marialaura Teresa Ostuni:** Investigation. **Martina Bettarelli:** Investigation. **Sofia Nasini:** Investigation. **Marilena Marraudino:** Investigation. **GianCarlo Panzica:** Writing - review & editing, Funding acquisition. **Stefano Gotti:** Supervision, Funding acquisition.

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.

Data availability

Data will be made available on request.

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References

Abraham, A., Chakraborty, P., 2020. A review on sources and health impacts of bisphenol A. Rev. Environ. Health 35 (2), 201–210. https://doi.org/10.1515/reveh-2019-0034.

Albani, S.H., Andrawis, M.M., Abella, R.J.H., Fulghum, J.T., Vafamand, N., Dumas, T.C., 2015. Behavior in the elevated plus maze is differentially affected by testing conditions in rats under and over three weeks of age. Front. Behav. Neurosci. 9, 31. https://doi.org/10.3389/fnbeh.2015.00031.

Andersson, N., Arena, M., Auteri, D., Barmaz, S., Grignard, E., Kienzler, A., Lepper, P., Lostia, A.M., Munn, S., Parra Morte, J.M., Pellizzato, F., Tarazona, J., Terron, A., Van der Linden, S., 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal. European Food Safety Authority 16 (6), e05311. https://doi.org/10.2903/j.efsa.2018.5311.

Bakoyiannis, I., Kitraki, E., Stamatakis, A., 2021. Endocrine-disrupting chemicals and behaviour: a high risk to take? Best Pract. Res. Clin. Endocrinol. Metabol. 35 (5), 101517 https://doi.org/10.1016/j.beem.2021.101517.

Barth, C., Villringer, A., Sacher, J., 2015. Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. Front. Neurosci. 9, 37. https://doi.org/10.3389/fnins.2015.00037.

Bocchio, M., McHugh, S.B., Bannerman, D.M., Sharp, T., Capogna, M., 2016. Serotonin, amygdala and fear: assembling the puzzle. Front. Neural Circ. 10, 24. https://doi.org/10.3389/fncir.2016.00024.

Bonaldo, B., Marraudino, M., Casile, A., Bettarelli, M., Gotti, S., Panzica, G.C., 2020. Effects of chronic exposure to Bisphenol-A in pregnant female mice. Eur. J. Histochem. 64, 11. https://doi.org/10.4081/ejh.2020.3200.

Bonaldo, B., Casile, A., Bettarelli, M., Gotti, S., Panzica, G., Marraudino, M., 2021. Effects of chronic exposure to bisphenol A in adult female mice on social behavior, vasopressin system, and estrogen membrane receptor (GPER1). Eur. J. Histochem. 65 (s1) https://doi.org/10.4081/ejh.2021.3272.

Bonaldo, B., Gioisa, L., Panzica, G., Marraudino, M., 2022. Exposure to either bisphenol A or S represents a risk for crucial behaviors for pup survival, such as spontaneous maternal behavior in mice. Neuroendocrinology. https://doi.org/10.1159/000526074.

Bonaldo, B., Casile, A., Montarolo, F., Bettarelli, M., Napoli, F., Gotti, S., Panzica, G., Marraudino, M., 2023. Effects of perinatal exposure to bisphenol A or S in EAE model of multiple sclerosis. Cell Tissue Res. https://doi.org/10.1007/s00441-023-03746-w.

Borrow, A.P., Handa, R.J., 2017. Estrogen receptors modulation of anxiety-like behavior. Vitam. Horm. 103, 27–52. https://doi.org/10.1016/bs.vh.2016.08.004.

Careghini, A., Mastorgio, A.F., Saponaro, S., Sezenna, E., 2015. Bisphenol A, nonylphenols, benzophenones, and benzotriazoles in soils, groundwater, surface water, sediments, and food: a review. Environ. Sci. Pollut. Res. Int. 22 (8), 5711–5741. Epub 2014 Dec 30, 10.1007/s11356-014-3974-510.1007/s11356-014-3974-5.

Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., Renzi, P., 2002. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav. Brain Res. 134 (1–2), 49–57. https://doi.org/10.1016/s0166-4328(01)00452-1.

Castro, B., Sánchez, P., Miranda, M.T., Torres, J.M., Ortega, E., 2015. Identification of dopamine- and serotonin-related genes modulated by bisphenol A in the prefrontal cortex of male rats. Chemosphere 139, 235–239. https://doi.org/10.1016/j.chemosphere.2015.06.061.

Catenza, C.J., Farooq, A., Shubear, N.S., Donkor, K.K., 2021. A targeted review on fate, occurrence, risk and health implications of bisphenol analogues. Chemosphere 268, 129273. https://doi.org/10.1016/j.chemosphere.2020.129273.

Chang, H., Wang, M., Xia, W., Chen, T., Huo, W., Mao, Z., Zhu, Y., Li, Y., Xu, S., 2016. Perinatal exposure to low-dose bisphenol A disrupts learning/memory and DNA methylation of estrogen receptor alpha in the hippocampus. Toxicology Research 5 (3), 828–835. https://doi.org/10.1039/c5tx00449g.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure,

and toxicity-A review. Environ. Sci. Technol. 50 (11), 5438–5453. https://doi.org/10.1021/acs.est.5b05387.
Chen, H.-C., Chang, J.-W., Sun, Y.-C., Chang, W.-T., Huang, P.-C., 2022. Determination of parabens, bisphenol A and its analogs, triclosan, and benzophenone-3 levels in human urine by isotope-dilution-UPLC-MS/MS method followed by supported liquid extraction. Toxics 10 (1). https://doi.org/10.3390/toxics10010021.
Corrales, J., Kristofco, L.A., Steele, W.B., Yates, B.S., Breed, C.S., Williams, E.S., Brooks, B.W., 2015. Global assessment of bisphenol A in the environment: review and analysis of its occurrence and bioaccumulation. Dose Response : A Publication of International Hormesis Society 13 (3), 1559325815598308. https://doi.org/10.1177/1559325815598308.
Cover, K.K., Maeng, L.Y., Lebrón-Milad, K., Milad, M.R., 2014. Mechanisms of estradiol in fear circuitry: implications for sex differences in psychopathology. Transl. Psychiatry 4 (8), e422. https://doi.org/10.1038/tp.2014.67.
Czarny-Krzymińska, K., Krawczyk, B., Szczukocki, D., 2023. Bisphenol A and its substitutes in the aquatic environment: occurrence and toxicity assessment. Chemosphere 315, 137763. https://doi.org/10.1016/j.chemosphere.2023.137763.
da Silva, B.S., Pietrobon, C.B., Bertasso, I.M., Lopes, B.P., Carvalho, J.C., Peixoto-Silva, N., Santos, T.R., Claudio-Neto, S., Manhaes, A.C., Oliveira, E., de Moura, E.G., Lisboa, P.C., 2019. Short and long-term effects of bisphenol S (BPS) exposure during pregnancy and lactation on plasma lipids, hormones, and behavior in rats. Environ. Pollut. 250, 312–322. https://doi.org/10.1016/j.envpol.2019.03.100.
Davidson, T.L., Jarrard, L.E., 2004. The hippocampus and inhibitory learning: a “Gray” area? Neurosci. Biobehav. Rev. 28 (3), 261–271. https://doi.org/10.1016/j.neubiorev.2004.02.001.
Deakin, J.F., Graeff, F.G., 1991. 5-HT and mechanisms of defence. J. Psychopharmacol. 5 (4), 305–315. https://doi.org/10.1177/026988119100500414.
Delfosse, V., Grimaldi, M., Pons, J.L., Boulahtouf, A., le Maire, A., Cavailles, V., Labesse, G., Bourguet, W., Balaguer, P., 2012. Structural and mechanistic insights into bisphenols action provide guidelines for risk assessment and discovery of bisphenol A substitutes. Proc. Natl. Acad. Sci. U. S. A. 109 (37), 14930–14935. https://doi.org/10.1073/pnas.1203574109.
Demetrius, L., 2005. Of mice and men. When it comes to studying ageing and the means to slow it down, mice are not just small humans. EMBO Rep. 6 (Suppl. 1), S39–S44. https://doi.org/10.1038/sj.embor.7400422. Spec No.
den Braver-Sewradj, S.P., van Spronsen, R., Hessel, E.V.S., 2020. Substitution of bisphenol A: a review of the carcinogenicity, reproductive toxicity, and endocrine disruption potential of alternative substances. Crit. Rev. Toxicol. 50 (2), 128–147. https://doi.org/10.1080/10408444.2019.1701986.
Deneris, E., Gaspar, P., 2018. Serotonin neuron development: shaping molecular and structural identities. Wiley Interdisciplinary Reviews. Developmental Biology 7 (1). https://doi.org/10.1002/wdev.301.
Domínguez, R., Cruz-Morales, S.E., Carvalho, M.C., Xavier, M., Brandao, M.L., 2003. Sex differences in serotonergic activity in dorsal and median raphe nucleus. Physiol. Behav. 80 (2–3), 203–210. https://doi.org/10.1016/j.physbeh.2003.07.012.
Donner, N.C., Lowry, C.A., 2013. Sex differences in anxiety and emotional behavior. Pflueg. Arch. Eur. J. Physiol. 465 (5), 601–626. https://doi.org/10.1007/s00424-013-1271-7.
EFSA (European Food Safety Authority), 2006. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2,2-bis(4hydroxyphenyl)propane. EFSA J. 428, 1–75. https://doi.org/10.2903/j.efsa.2007.428.
EFSA (European Food Safety Authority), 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 13 (1), 3978. https://doi.org/10.2903/j.efsa.2015.3978.
Eladak, S., Grisin, T., Moison, D., Guerquin, M.J., N'Tumba-Byn, T., Pozzi-Gaudin, S., Benachi, A., Livera, G., Rouiller-Fabre, V., Habert, R., 2015. A new chapter in the bisphenol a story: bisphenol S and bisphenol F are not safe alternatives to this compound. In: Fertility and Sterility, vol. 103, pp. 11–21. https://doi.org/10.1016/j.fertnstert.2014.11.005. Issue 1.
European Commission, 2011. Commission Directive 2011/8/Eu of 28 January 2011 Amending Directive 2002/72/Ec as regards the restriction of use of bisphenol a in plastic infant feeding bottles. Off. J. Eur. Union 26, 11–14.
Frye, C.A., Bo, E., Calamandrei, G., Calza, L., Dessi-Fulgheri, F., Fernandez, M., Fusani, L., Kah, O., Kajta, M., Le Page, Y., Patisaul, H.B., Venerosi, A., Wojtowicz, A. K., Panzica, G.C., 2012. Endocrine disrupters: a review of some sources, effects, and mechanisms of actions on behaviour and neuroendocrine systems. J. Neuroendocrinol. 24 (1), 144–159. https://doi.org/10.1111/j.1365-2826.2011.02229.x.
Funabashi, T., Kawaguchi, M., Furuta, M., Fukushima, A., Kimura, F., 2004. Exposure to bisphenol A during gestation and lactation causes loss of sex difference in corticotropin-releasing hormone-immunoreactive neurons in the bed nucleus of the stria terminalis of rats. Psychoneuroendocrinology 29 (4), 475–485. https://doi.org/10.1016/s0306-4530(03)00055-6.
García-González, D., Khodosevich, K., Watanabe, Y., Rollenhagen, A., Lübke, J.H.R., Monyer, H., 2017. Serotonergic projections govern postnatal neuroblast migration. Neuron 94 (3), 534–549.e9. https://doi.org/10.1016/j.neuron.2017.04.013.
Giannini, A., Caretto, M., Genazzani, A.R., Simoncini, T., 2019. Optimizing quality of life through sex steroids by their effects on neurotransmitters. Climacteric : The Journal of the International Menopause Society 22 (1), 55–59. https://doi.org/10.1080/13697137.2018.1543265.
Gioiosa, L., Parmigiani, S., Vom Saal, F.S., Palanza, P., 2013. The effects of bisphenol A on emotional behavior depend upon the timing of exposure, age and gender in mice. Horm. Behav. 63 (4), 598–605. https://doi.org/10.1016/j.yhbeh.2013.02.016.
Gold, P.W., 2015. The organization of the stress system and its dysregulation in depressive illness. Mol. Psychiatr. 20 (1), 32–47. https://doi.org/10.1038/mp.2014.163.
Gordon, J.A., Hen, R., 2004. The serotonergic system and anxiety. NeuroMolecular Med. 5 (1), 27–40. https://doi.org/10.1385/NMM:5:1:027.
Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. Executive summary to EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36 (6), 593–602. https://doi.org/10.1210/er.2015-1093.
Gramec Skledar, D., Peterlin Masic, L., 2016. Bisphenol A and its analogs: do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199. https://doi.org/10.1016/j.etap.2016.09.014.
Griebel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol. Ther. 65 (3), 319–395. https://doi.org/10.1016/0163-7258(95)98597-j.
Hohoff, C., 2009. Anxiety in mice and men: a comparison. J. Neural. Transm. 116 (6), 679–687. https://doi.org/10.1007/s00702-009-0215-z. Vienna, Austria : 1996.
Hornung, J.-P., 2010. Chapter 1.3 - the neuronatomy of the serotonergic system. In: Müller, C.P., Jacobs, B.L. (Eds.), Handbook of Behavioral Neuroscience, vol. 21. Elsevier, pp. 51–64. https://doi.org/10.1016/S1569-7339(10)70071-0.
Hu, F., Liang, W., Zhang, L., Wang, H., Li, Z., Zhou, Y., 2022. Hyperactivity of basolateral amygdala mediates behavioral deficits in mice following exposure to bisphenol A and its analogue alternative. Chemosphere 287 (Pt 1), 132044. https://doi.org/10.1016/j.chemosphere.2021.132044.
Imwalle, D.B., Gustafsson, J.-A., Rissman, E.F., 2005. Lack of functional estrogen receptor beta influences anxiety behavior and serotonin content in female mice. Physiol. Behav. 84 (1), 157–163. https://doi.org/10.1016/j.physbeh.2004.11.002.
Ji, R., Cui, M., Zhou, D., Pan, X., Xie, Y., Wu, X., Liang, X., Zhang, H., Song, W., 2023. Adulthood bisphenol A exposure induces anxiety in male mice via downregulation of alpha-1D adrenergic receptor in paraventricular thalamus. Ecotoxicol. Environ. Saf. 262, 115205 https://doi.org/10.1016/j.ecoenv.2023.115205.
Jørgensen, H., Knigge, U., Kjaer, A., Møller, M., Warberg, J., 2002. Serotonergic stimulation of corticotropin-releasing hormone and pro-opiomelanocortin gene expression. J. Neuroendocrinol. 14 (10), 788–795. https://doi.org/10.1046/j.1365-2826.2002.00839.x.
Kahn, L.G., Philippat, C., Nakayama, S.F., Slama, R., Trasande, L., 2020. Endocrine-disrupting chemicals: implications for human health. Lancet Diabetes Endocrinol. 8 (8), 703–718. https://doi.org/10.1016/S2213-8587(20)30129-7.
Kawai, K., Murakami, S., Senba, E., Yamanaka, T., Fujiwara, Y., Arimura, C., Nozaki, T., Takii, M., Kubo, C., 2007. Changes in estrogen receptors alpha and beta expression in the brain of mice exposed prenatally to bisphenol A. Regul. Toxicol. Pharmacol. : RTP (Regul. Toxicol. Pharmacol.) 47 (2), 166–170. https://doi.org/10.1016/j.yrtph.2006.04.002.
Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 8 (6), e1000412 https://doi.org/10.1371/journal.pbio.1000412.
Kim, B., Colon, E., Chawla, S., Vandenberg, L.N., Suvorov, A., 2015. Endocrine disruptors alter social behaviors and indirectly influence social hierarchies via changes in body weight. Environ. Health 14, 64. https://doi.org/10.1186/s12940-015-0051-6.
Kimber, I., Woeffen, N., Sondenheimer, K., 2022. Bisphenol A, T(H)17 cells, and allergy: a commentary. J. Immunot. 19 (1), 93–99. https://doi.org/10.1080/1547691X.2022.2113842.
Kojima, H., Takeuchi, S., Sanoh, S., Okuda, K., Kitamura, S., Uramaru, N., Sugihara, K., Yoshinari, K., 2019. Profiling of bisphenol A and eight its analogues on transcriptional activity via human nuclear receptors. Toxicology 413, 48–55. https://doi.org/10.1016/j.tox.2018.12.001.
Kraeuter, A.-K., Guest, P.C., Sarnyai, Z., 2019. The elevated plus maze test for measuring anxiety-like behavior in rodents. Methods Mol. Biol. 1916, 69–74. https://doi.org/10.1007/978-1-4939-8994-2_4.
Kulesskaya, N., Voikar, V., 2014. Assessment of mouse anxiety-like behavior in the light-dark box and open-field arena: role of equipment and procedure. Physiol. Behav. 133, 30–38. https://doi.org/10.1016/j.physbeh.2014.05.006.
Kundakovic, M., Gudsnuk, K., Franks, B., Madrid, J., Miller, R.L., Perera, F.P., Champagne, F.A., 2013. Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. Proc. Natl. Acad. Sci. U. S. A. 110 (24), 9956–9961. https://doi.org/10.1073/pnas.1214056110.
Kuruto-Niwa, R., Nozawa, R., Miyakoshi, T., Shiozawa, T., Terao, Y., 2005. Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system. Environ. Toxicol. Pharmacol. 19 (1), 121–130. https://doi.org/10.1016/j.etap.2004.05.009.
Lama, A., Del Piano, F., Annunziata, C., Comella, F., Opallo, N., Melini, S., Grumetto, L., Pirozzi, C., Mattace Raso, G., Meli, R., Ferrante, M.C., 2023. Bisphenol A exacerbates anxiety-like behavior and neuroinflammation in prefrontal cortex of adult obese mice. Life Sci. 313, 121301 https://doi.org/10.1016/j.lfs.2022.121301.
Lambré, C., Barat Baviera, J.M., Bolognesi, C., Chesson, A., Cocconcelli, P.S., Crebelli, R., Gott, D.M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Silano Until December, V., Steffensen, I.-L., Tlustos, C., Vernis, L., Zorn, H., Batke, M., Bignami, M., et al., 2023. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal. European Food Safety Authority 21 (4), e06857. https://doi.org/10.2903/j.efsa.2023.6857.
Landgraf, R., 2001. Neuropeptides and anxiety-related behavior. Endocr. J. 48 (5), 517–533. https://doi.org/10.1507/endocrj.48.517.
Lezak, K.R., Missig, G., Carlezon, W.A.J., 2017. Behavioral methods to study anxiety in rodents. Dialogues Clin. Neurosci. 19 (2), 181–191. https://doi.org/10.31887/DCNS.2017.19.2/wcarlezon.

Li, Y., Perera, L., Coons, L.A., Burns, K.A., Tyler Ramsey, J., Pelch, K.E., Houtman, R., van Beuningen, R., Teng, C.T., Korach, K.S., 2018. Differential in vitro biological action, coregulator interactions, and molecular dynamic analysis of bisphenol A (BPA), BPAF, and BPS ligand-ERalpha complexes. Environ. Health Perspect. 126 (1), 17012 https://doi.org/10.1289/EHP2505.

Li, Yi, He, X., Kawaguchi, R., Zhang, Y., Wang, Q., Monavarfeshani, A., Yang, Z., Chen, B., Shi, Z., Meng, H., Zhou, S., Zhu, J., Jacobi, A., Swarup, V., Popovich, P.G., Geschwind, D.H., He, Z., 2020. Microglia-organized scar-free spinal cord repair in neonatal mice. Nature 587 (7835), 613–618. https://doi.org/10.1038/s41586-020-2795-6.

Liu, X., Sakai, H., Nishigori, M., Suyama, K., Nawaji, T., Ikeda, S., Nishigouchi, M., Okada, H., Matsushima, A., Nose, T., Shimohigashi, M., Shimohigashi, Y., 2019. Receptor-binding affinities of bisphenol A and its next-generation analogs for human nuclear receptors. Toxicol. Appl. Pharmacol. 377, 114610 https://doi.org/10.1016/j.taap.2019.114610.

Liu, J., Zhang, L., Lu, G., Jiang, R., Yan, Z., Li, Y., 2021. Occurrence, toxicity and ecological risk of Bisphenol A analogues in aquatic environment - a review. Ecotoxicol. Environ. Saf. 208, 111481 https://doi.org/10.1016/j.ecoenv.2020.111481.

Longo, A., Mele, P., Bertocchi, I., Oberto, A., Bachmann, A., Bartolomucci, A., Palanza, P., Sprengel, R., Eva, C., 2014. Conditional inactivation of neuropeptide Y Y1 receptors unravels the role of Y1 and Y5 receptors coexpressing neurons in anxiety. Biol. Psychiatr. 76 (11), 840–849. https://doi.org/10.1016/j.biopsych.2014.01.009.

Matsuda, S., Saika, S., Amano, K., Shimizu, E., Sajiki, J., 2010. Changes in brain monoamine levels in neonatal rats exposed to bisphenol A at low doses. Chemosphere 78 (7), 894–906. https://doi.org/10.1016/j.chemosphere.2009.11.010.

Matsuda, S., Matsuzawa, D., Ishii, D., Tomizawa, H., Sajiki, J., Shimizu, E., 2013. Perinatal exposure to bisphenol A enhances contextual fear memory and affects the serotoninergic system in juvenile female mice. Horm. Behav. 63 (5), 709–716. https://doi.org/10.1016/j.yhbeh.2013.03.016.

McDonough, C.M., Xu, J., Guo, T.L., 2021. Behavioral changes and hyperglycemia in NODEF mice following bisphenol S exposure are affected by diets. Neurotoxicology 85, 209–221. https://doi.org/10.1016/j.neuro.2021.06.001.

McLean, A.C., Valenzuela, N., Fai, S., Bennett, S.A.L., 2012. Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for mouse estrous cycle staging identification. J. Vis. Exp. 67, 4389. https://doi.org/10.3791/4389.

Michael Caudle, W., 2016. This can't be stressed enough: the contribution of select environmental toxicants to disruption of the stress circuitry and response. Physiol. Behav. 166, 65–75. https://doi.org/10.1016/j.physbeh.2015.09.021.

Misme-Aucouturier, B., De Carvalho, M., Delage, E., Dijoux, E., Klein, M., Brosseau, C., Bodinier, M., Guzylack-Piriou, L., Bouchaud, G., 2022. Oral exposure to bisphenol A exacerbates allergic inflammation in a mouse model of food allergy. Toxicology 472, 153188. https://doi.org/10.1016/j.tox.2022.153188.

Mitra, S.W., Hoskin, E., Yudkovitz, J., Pear, L., Wilkinson, H.A., Hayashi, S., Pfaff, D.W., Ogawa, S., Rohrer, S.P., Schaeffer, J.M., McEwen, B.S., Alves, S.E., 2003. Immunolocalization of estrogen receptor beta in the mouse brain: comparison with estrogen receptor alpha. Endocrinology 144 (5), 2055–2067. https://doi.org/10.1210/en.2002-221069.

Mogi, K., Takanashi, H., Nagasawa, M., Kikusui, T., 2015. Sex differences in spatiotemporal expression of AR, ERα, and ERβ mRNA in the perinatal mouse brain. Neurosci. Lett. 584, 88–92. https://doi.org/10.1016/j.neulet.2014.10.028.

Molina-Molina, J.M., Amaya, E., Grimaldi, M., Saenz, J.M., Real, M., Fernandez, M.F., Balaguer, P., Olea, N., 2013. In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. Toxicol. Appl. Pharmacol. 272 (1), 127–136. https://doi.org/10.1016/j.taap.2013.05.015.

Mornagui, B., Rezg, R., Repond, C., Pellerin, L., 2019. Effects of bisphenol S, a major substitute of bisphenol A, on neurobehavioral responses and cerebral monocarboxylate transporters expression in mice. Food Chem. Toxicol. 132, 110670 https://doi.org/10.1016/j.fct.2019.110670.

Murata, M., Kang, J.H., 2018. Bisphenol A (BPA) and cell signaling pathways. Biotechnol. Adv. 36 (1), 311–327. https://doi.org/10.1016/j.biotechadv.2017.12.002.

Mustieles, V., D'Cruz, S.C., Couderq, S., Rodriguez-Carrillo, A., Fini, J.B., Hofer, T., Steffensen, I.L., Dirven, H., Barouki, R., Olea, N., Fernandez, 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.

Naderi, M., Kwong, R.W.M., 2020. A comprehensive review of the neurobehavioral effects of bisphenol S and the mechanisms of action: new insights from in vitro and in vivo models. Environ. Int. 145, 106078 https://doi.org/10.1016/j.envint.2020.106078.

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. https://doi.org/10.1016/j.neulet.2010.08.021.

Naule, L., Picot, M., Martini, M., Parmentier, C., Hardin-Pouzet, H., Keller, M., Franceschini, I., Mhaouty-Kodja, S., 2014. Neuroendocrine and behavioral effects of maternal exposure to oral bisphenol A in female mice. J. Endocrinol. 220 (3), 375–388. Print 2014 Mar, 10.1530/JOE-13-060710.1530/JOE-13-0607.

Nomura, M., Akama, K.T., Alves, S.E., Korach, K.S., Gustafsson, J.-A., Pfaff, D.W., Ogawa, S., 2005. Differential distribution of estrogen receptor (ER)-alpha and ER-beta in the midbrain raphe nuclei and periaqueductal gray in male mouse: predominant role of ER-beta in midbrain serotonergic systems. Neuroscience 130 (2), 445–456. https://doi.org/10.1016/j.neuroscience.2004.09.028.

Ogawa, S.K., Cohen, J.Y., Hwang, D., Uchida, N., Watabe-Uchida, M., 2014. Organization of monosynaptic inputs to the serotonin and dopamine neuromodulatory systems. Cell Rep. 8 (4), 1105–1118. https://doi.org/10.1016/j.celrep.2014.06.042.

Palanza, P., Morellini, F., Parmigiani, S., vom Saal, F.S., 2002. Ethological methods to study the effects of maternal exposure to estrogenic endocrine disrupters: a study with methoxychlor. Neurotoxicol. Teratol. 24 (1), 55–69. https://doi.org/10.1016/s0892-0362(01)00191-x.

Park, C., Song, H., Choi, J., Sim, S., Kojima, H., Park, J., Iida, M., Lee, Y., 2020. The mixture effects of bisphenol derivatives on estrogen receptor and androgen receptor. Environ. Pollut. 260, 114036 https://doi.org/10.1016/j.envpol.2020.114036.

Patisaul, H.B., Sullivan, A.W., Radford, M.E., Walker, D.M., Adewale, H.B., Winnik, B., Coughlin, J.L., Buckley, B., Gore, A.C., 2012. Anxiogenic effects of developmental bisphenol A exposure are associated with gene expression changes in the juvenile rat amygdala and mitigated by soy. PLoS One 7 (9), e43890. https://doi.org/10.1371/journal.pone.0043890.

Paxinos, G., Franklin, K.B.J., 2001. The Mouse Brain in Stereotaxic Coordinates, second ed. Academic Press.

Pelch, K., Wignall, J.A., Goldstone, A.E., Ross, P.K., Blain, R.B., Shapiro, A.J., Holmgren, S.D., Hsieh, J.H., Svoboda, D., Auerbach, S.S., Parham, F.M., Masten, S. A., Walker, V., Rooney, A., Thayer, K.A., 2019. A scoping review of the health and toxicological activity of bisphenol A (BPA) structural analogues and functional alternatives. Toxicology 424, 152235. https://doi.org/10.1016/j.tox.2019.06.006.

Poimenova, A., Markaki, E., Rahiotis, C., Kitraki, E., 2010. Corticosterone-regulated actions in the rat brain are affected by perinatal exposure to low dose of bisphenol A. Neuroscience 167 (3), 741–749. https://doi.org/10.1016/j.neuroscience.2010.02.051.

Rebolledo-Solleiro, D., Castillo Flores, L.Y., Solleiro-Villavicencio, H., 2021. Impact of BPA on behavior, neurodevelopment and neurodegeneration. Front. Biosci. 26, 363–400. https://doi.org/10.2741/4898.

Ren, J., Friedmann, D., Xiong, J., Liu, C.D., Ferguson, B.R., Weerakkody, T., DeLoach, K. E., Ran, C., Pun, A., Sun, Y., Weissbourd, B., Neve, R.L., Huguenard, J., Horowitz, M. A., Luo, L., 2018. Anatomically defined and functionally distinct dorsal raphe serotonin sub-systems. Cell 175 (2), 472–487.e20. https://doi.org/10.1016/j.cell.2018.07.043.

Ren, J., Isakova, A., Friedmann, D., Zeng, J., Grutzner, S.M., Pun, A., Zhao, G.Q., Kolluru, S.S., Wang, R., Lin, R., Li, P., Li, A., Raymond, J.L., Luo, Q., Luo, M., Quake, S.R., Luo, L., 2019. Single-cell transcriptomes and whole-brain projections of serotonin neurons in the mouse dorsal and median raphe nuclei. Elife 8. https://doi.org/10.7554/eLife.49424.

Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol A substitutes. Environ. Health Perspect. 123 (7), 643–650. https://doi.org/10.1289/ehp.1408989.

Rubin, B.S., Schaeberle, C.M., Soto, A.M., 2019. The case for BPA as an obesogen: contributors to the controversy. Front. Endocrinol. 10, 30. https://doi.org/10.3389/fendo.2019.00030.

Rubinow, D.R., Schmidt, P.J., Roca, C.A., 1998. Estrogen-serotonin interactions: implications for affective regulation. Biol. Psychiatr. 44 (9), 839–850. https://doi.org/10.1016/s0006-3223(98)00162-0.

Schmitt, U., Hiemke, C., 1998. Combination of open field and elevated plus-maze: a suitable test battery to assess strain as well as treatment differences in rat behavior. Prog. Neuro Psychopharmacol. Biol. Psychiatr. 22 (7), 1197–1215. https://doi.org/10.1016/s0278-5846(98)00051-7.

Seibenhener, M.L., Wooten, M.C., 2015. Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. J. Vis. Exp. 96, e52434 https://doi.org/10.3791/52434.

Sheng, Z., Kawano, J., Yanai, A., Fujinaga, R., Tanaka, M., Watanabe, Y., Shinoda, K., 2004. Expression of estrogen receptors (alpha, beta) and androgen receptor in serotonin neurons of the rat and mouse dorsal raphe nuclei; sex and species differences. Neurosci. Res. 49 (2), 185–196. https://doi.org/10.1016/j.neures.2004.02.011.

Shoji, H., Miyakawa, T., 2021. Effects of test experience, closed-arm wall color, and illumination level on behavior and plasma corticosterone response in an elevated plus maze in male C57BL/6J mice: a challenge against conventional interpretation of the test. Mol. Brain 14 (1), 34. https://doi.org/10.1186/s13041-020-00721-2.

Sun, F., Huang, Y., Chen, H., Huang, J., Zhang, L., Wei, S., Liu, F., Chen, D., Huang, W., 2023. BPA and its alternatives BPF and BPAF exaggerate hepatic lipid metabolism disorders in male mice fed a high fat diet. Sci. Total Environ. 867, 161521 https://doi.org/10.1016/j.scitotenv.2023.161521.

Taborsky, M., 2010. Sample size in the study of behaviour. Ethology 116 (3), 185–202. https://doi.org/10.1111/j.1439-0310.2010.01751.x.

Thoene, M., Dzika, E., Gonkowski, S., Wojtkiewicz, J., 2020. Bisphenol S in food causes hormonal and obesogenic effects comparable to or worse than bisphenol A: a literature review. Nutrients 12 (2). https://doi.org/10.3390/nu12020532.

Tomza-Marciniak, A., Stepkowska, P., Kuba, J., Pilarczyk, B., 2018. Effect of bisphenol A on reproductive processes: a review of in vitro, in vivo and epidemiological studies. J. Appl. Toxicol. 38 (1), 51–80. https://doi.org/10.1002/jat.3480.

Vaccher, V., Lopez, M.E., Castaño, A., Mol, H., Haji-Abbas-Zarrabi, K., Bury, D., Koch, H. M., Dvorakova, D., Hajslova, J., Nübler, S., Kaur Sakhi, A., Thomsen, C., Vorkamp, K., Göen, T., Antignac, J.-P., 2022. European interlaboratory comparison investigations (ICI) and external quality assurance schemes (EQUAS) for the analysis of bisphenol A, S and F in human urine: results from the HBM4EU project. Environ. Res. 210, 112933 https://doi.org/10.1016/j.envres.2022.112933.

Vasiljevic, T., Harner, T., 2021. Bisphenol A and its analogues in outdoor and indoor air: properties, sources and global levels. Sci. Total Environ. 789, 148013 https://doi.org/10.1016/j.scitotenv.2021.148013.

Viglietti-Panzica, C., Aste, N., Balthazart, J., Panzica, G.C., 1994. Vasotocinergic innervation of sexually dimorphic medial preoptic nucleus of the male Japanese quail: influence of testosterone. Brain Res. 657 (1–2), 171–184, 10.1016/0006-8993(94)90965-210.1016/0006-8993(94)90965-2.

Walf, A.A., Frye, C.A., 2006. A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology 31 (6), 1097–1111. https://doi.org/10.1038/sj.npp.1301067.

Walf, A.A., Frye, C.A., 2007. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat. Protoc. 2 (2), 322–328. https://doi.org/10.1038/nprot.2007.44.

Wang, Y., Wu, Y., Zhang, S., 2022. Impact of bisphenol-A on the spliceosome and meiosis of sperm in the testis of adolescent mice. BMC Vet. Res. 18 (1), 278. https://doi.org/10.1186/s12917-022-03336-y.

Watson Jr., R.E., Wiegand, S.J., Clough, R.W., Hoffman, G.E., 1986. Use of cryoprotectant to maintain long-term peptide immunoreactivity and tissue morphology. Peptides 7 (1), 155–159. https://doi.org/10.1016/0196-9781(86)90076-8.

Wiersielis, K.R., Samuels, B.A., Roepke, T.A., 2020. Perinatal exposure to bisphenol A at the intersection of stress, anxiety, and depression. Neurotoxicol. Teratol. 79, 106884 https://doi.org/10.1016/j.ntt.2020.106884.

Wu, L.H., Zhang, X.M., Wang, F., Gao, C.J., Chen, D., Palumbo, J.R., Guo, Y., Zeng, E.Y., 2018. Occurrence of bisphenol S in the environment and implications for human exposure: a short review. Sci. Total Environ. 615, 87–98. https://doi.org/10.1016/j.scitotenv.2017.09.194.

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, 126715. https://doi.org/10.1016/j.chemosphere.2020.126715.

Ying, G.G., Kookana, R.S., 2005. Sorption and degradation of estrogen-like-endocrine disrupting chemicals in soil. Environ. Toxicol. Chem. 24 (10), 2640–2645. https://doi.org/10.1897/05-074r.1.

Zangrossi, H.J., Graeff, F.G., 2014. Serotonin in anxiety and panic: contributions of the elevated T-maze. Neurosci. Biobehav. Rev. 46 (Pt 3), 397–406. https://doi.org/10.1016/j.neubiorev.2014.03.007.

Zhang, J.Q., Cai, W.Q., Zhou, D.S., Su, B.Y., 2002. Distribution and differences of estrogen receptor beta immunoreactivity in the brain of adult male and female rats. Brain Res. 935 (1–2), 73–80. https://doi.org/10.1016/s0006-8993(02)02460-5.

Zhang, X., Beaulieu, J.-M., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., 2004. Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science (New York, N. Y.) 305 (5681), 217. https://doi.org/10.1126/science.1097540.

Zhou, R., Chen, F., Feng, X., Zhou, L., Li, Y., Chen, L., 2015. Perinatal exposure to low-dose of bisphenol A causes anxiety-like alteration in adrenal axis regulation and behaviors of rat offspring: a potential role for metabotropic glutamate 2/3 receptors. J. Psychiatr. Res. 64, 121–129. https://doi.org/10.1016/j.jpsychires.2015.02.018.