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Supplementary Information for

Maternal glyphosate exposure causes autism-like behaviors in offspring through increased expression of soluble epoxide hydrolase

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SI Materials and Methods

Animals and animal care. Pregnant ddY mice (embryo at the 5th day (E5), 9-10 weeks old) were purchased from Japan SLC Inc. (Hamamatsu, Shizuoka, Japan). Pregnant mice in each clear polycarbonate cage (22.5 × 33.8 × 14.0 cm) were housed singly under controlled temperatures and 12 hour light/dark cycles (lights on between 07:00–19:00 h), with ad libitum food (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water. The protocol was approved by the Chiba University Institutional Animal Care and Use Committee. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA.

Treatment of glyphosate in drinking water into pregnant mice. In this study, we used commercially available Roundup® Maxload [48% (w/v) glyphosate (*N*-phosphonomethylglycine) potassium salt, 52% other ingredients such as water and surfactant. Lot number: 11946898. Nissan Chemical Corporation, Tokyo, Japan]. Previous studies used drinking water containing 0.38% (w/v) glyphosate (expressed as free base: 1% Roundup®) during pregnancy and lactation, equivalent to 50 mg/kg/day of glyphosate (1,2). This corresponded with 1/20 of the glyphosate no-observed-adverse-effect level, as described previously (3). Therefore, water or formulated glyphosate [or 0.1, 0.25, 0.50, 0.75, 1.0 % Roundup®] were given to the pregnant mice from E5 to P21 (weaning). The male offspring were separated from their mothers at weaning (P21), and mice were caged each three - five in the groups in clear polycarbonate cage (22.5 × 33.8 × 14.0 cm). Mice were housed under controlled temperatures and 12 hour light/dark cycles (lights on between 07:00–19:00 h), with ad libitum food and water.

Measurement of glyphosate in the blood. Water or 0.098% (w/v) formulated glyphosate was given to pregnant mice from E5 to P21, as described above. At weaning (P21), mothers and male offspring mice were deeply anesthetized with isoflurane and plasma was collected. The plasma samples were stored at -80°C before assay. Measurement of glyphosate in the plasma was performed using LC/MS/MS at UC Davis.

The 40 μL of internal standard (2 μg/mL of glyphosate-2-^{13}C solution in methanol) and 40 μL of methanol were added to 20 μL of plasma. The spiked sample was vortexed for 5 minutes and then centrifuged at 16,100 g/min for another 5 minutes. The supernatants were transferred for the following LC/MS/MS measurement, which used a Waters Acquity UPLC system (Waters, Milford, MA) interfaced with a QTRAP 6500+ mass spectrometer (Sciex, Redwood City, CA) using an electrospray source. The separation was achieved on a Waters Acquity BEH C18 50 × 2.1 mm 1.7 μm column with mobile phases of water with 0.1% of formic acid as mobile phase A and acetonitrile with 0.1% of formic acid as mobile phase B. The gradient was shown in **Table S7**. All the parameters on the mass spectrometer were optimized with pure standards of glyphosate and glyphosate-2-^{13}C (purchased from Millipore Sigma, Burlington MA) under positive MRM mode. The detailed parameters were given in **Table S8**.

Table S7. The liquid chromatography gradient used for the analysis of glyphosate.

Time	Flow Rate	%A	%B	Curve
Initial	0.35	75	25	Initial
0.5	0.35	75	25	6
2	0.35	10	90	6
3	0.35	10	90	6
3.1	0.35	75	25	6
5	0.35	75	25	6

Table S8. The optimization of the mass transitions of mass spectrometer for glyphosate.

Compounds	Q1	Q3	DP	CE	CXP
Glyphosate	169.9	87.9	60	11	10
Glyphosate_qualify	169.9	60	60	21	8
glyphosate-2-^{13}C	170.9	88.9	60	14	15
glyphosate-2-^{13}C quality	170.9	61	60	28	9

Collection of blood and brain samples and oxylipin analysis. Water or 0.098% (w/v) formulated glyphosate was given to pregnant mice from E5 to P21, as described above. The male offspring were separated from their mothers at weaning (P21). At juvenile (P28) stage, mice were deeply anesthetized with isoflurane and plasma was collected. Subsequently, brains were removed from the skulls. For Western blot analysis, brain regions such as prefrontal cortex (PFC), hippocampus, and striatum, were dissected from brain on ice. The samples were stored at -80℃ before assay. For oxylipin analysis, plasma was collected after isoflurane anesthesia at a juvenile (P28) stage. Subsequently, PFC, hippocampus, and striatum were dissected from brain on ice, and the samples were stored at -80℃ before assay. Measurement of eicosanoids in the plasma and brain regions was performed at UC Davis using the previously described method (4).

Western blot analysis. Western blot analysis was performed as reported previously (5-7). Basically, the tissue samples were homogenized in Laemmli lysis buffer. 50 µg of protein were measured using the DC protein assay kit (Bio-Rad), and incubated for 5 min at 95℃, with an equal volume of 125 mM Tris-HCl, pH6.8, 20% glycerol, 0.1% bromophenol blue, 10% β-mercaptoethanol, 4% sodium dodecyl sulfate, and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis, using 7.5% or AnyKD mini-gels (Mini-PROTEAN® TGX™ Precast Gel; Bio-Rad, CA, USA). Proteins were transferred onto polyvinylidenedifluoride (PVDF) membranes using a Trans Blot Mini Cell (Bio-Rad). For immunodetection, the blots were blocked with 2% BSA in TBST (TBS + 0.1% Tween-20) for 1 h at room temperature (RT), and kept with primary sEH rabbit polyclonal antibody (prepared at UC Davis) overnight at 4°C. The next day, blots were washed three times in TBST and incubated with horseradish peroxidase conjugated anti-rabbit or anti-mouse antibody 1 hour, at RT. After final three washes with TBST, bands were detected using the enhanced chemiluminescence (ECL) prime Western Blotting Detection system (GE Healthcare Bioscience). Images were captured with a ChemDoc imaging system (Bio-Rad), and the immunoreactive bands were analyzed by Image Lab software.

Gene expression analysis by quantitative real-time PCR. At juvenile (P28) stage, mice were sacrificed, and their brains were removed for measurement of gene expression of *Ephx2* mRNA. Brain regions such as PFC, hippocampus, and striatum were dissected from the brains on ice. A quantitative RT-PCR system (Step One Plus, Thermo Fisher Scientific, Yokohama, Japan) was used to measure mRNAs. The specific mRNA transcripts were quantified by TaqManGene Expression assays (Thermo Fisher Scientific, Yokohama, Japan). Expression levels of *Ephx2* (Mm01313813_m1) was measured in brain tissue. Total RNA was extracted by use of an RNeasy Mini Kit (Qiagen, Hilden, Germany). The purity of total RNA was assessed by Biophotometer plus (Eppendorf, Hamburg, Germany). The RNA samples were used in the first strand cDNA synthesis with High Capacity cDNA Reverse Transcription Kit (#4368813 Thermo Fisher Scientific, Yokohama, Japan). All samples were tested in triplicate and average values were used for quantification. The average values were normalized to Vic-labeled *Actb* mRNA (Cat#4352341E: pre-developed TaqMan Assay Reagents, Thermo Fisher Scientific, Yokohama, Japan).

Treatment of TPPU. TPPU was dissolved in polyethylene glycol 400 (PEG 400: Tokyo Chemical Industry Co., Ltd, Tokyo, Japan). TPPU (3 mg/kg/day) or vehicle (5 ml/kg, PEG 400) were administered orally in the pregnant mice from E5 to P21. Behavioral tests of offspring were performed during juvenile stage (P28–P35) after maternal glyphosate exposure (**Fig. 1A**).

Behavioral analysis. Locomotion, the novel object recognition test (NORT), and prepulse inhibition (PPI) test were performed as reported previously (5,7-13).

Locomotor Activity**:** Both horizontal and rearing activity were monitored by an infrared ray passive sensor system (SCANET-SV10, Melquest Ltd., Toyama, Japan), and activity was integrated every minute. Individual mice were placed in activity chambers and allowed 1 hour of free exploration as spontaneous activity.

Novel Object Recognition Test (NORT): Mice were habituated for 10 minutes in the test box for 3 straight days. On 4th day, two objects (differing in shape and color but of similar size) were placed in the box 35.5 cm apart (symmetrically), and each animal was allowed to explore in the box for 5 minutes. The animals were considered to be exploring the object when the head of the animal was both facing and within 2.54 cm of the object or when any part of the body, except for the tail was touching the object. The time that mice spent exploring each object was recorded. After training, mice were immediately returned to their home cages, and the box and objects were cleaned with 75% ethanol, to avoid any possible instinctive odorant cues. Retention tests were carried out at one-day intervals, following the respective training. During the retention test, each mouse was reintroduced into their original test box, and one of the training objects was replaced by a novel object. The mice were then allowed to explore freely for 5 minutes, and the time spent exploring each object was recorded. Throughout the experiments, the objects were counter-balanced, in terms of their physical complexity and emotional neutrality. A preference index, that is, the ratio of time spent exploring either of the two objects (training session) or the novel object (retention test session) over the total time spent exploring both objects, was used.

PPI: The offspring mice were tested for their acoustic startle reactivity (ASR) in a startle chamber (SR-LAB; San Diego Instruments, San Diego, CA, USA) using the standard methods described previously (9,10). The test sessions were begun after an initial 10-min acclimation period in the chamber. The mice were subjected to one of six trials: (1) pulse alone, as a 40 ms broadband burst; a pulse (40 ms broadband burst) preceded by 100 ms with a 20 ms prepulse that was (2) 4 dB, (3) 8 dB, (4) 12 dB, or (5) 16 dB over background (65 dB); and (6) background only (no stimulus). The amount of prepulse inhibition (PPI) was expressed as the percentage decrease in the amplitude of the startle reactivity caused by presentation of the prepulse (% PPI). The PPI test lasted 20 min in total.

Three-chamber Social Interaction Test: The three-chamber social interaction test was performed to investigate sociability and preference for social novelty in mice, as reported previously (7). The apparatus consisted of a rectangular, three-chambered box and a lid with a video camera (BrainScience Idea, Co., Ltd, Osaka, Japan). Each chamber (20 cm × 40 cm × 20 cm) was divided by a clear plastic wall with a small square opening (5 cm × 8 cm). First, each subject mouse was placed in the box and allowed to explore for 10 min to habituate the environment. During the session, an empty wire cage (10 cm in diameter, 17.5 cm in height, with vertical bars 0.3 cm apart) was located in the center of left and right chamber. Next, an unfamiliar ddY male mouse (stranger 1) that had no prior contact with the subject mouse was put into a wire cage that was placed into one of the side chambers. To assess sociability, the subject mouse was allowed to explore the box for an additional 10-min session. Finally, to evaluate social preference for a new stranger, a second stranger male mouse (stranger 2) was placed into the wire cage that had been empty during the first 10-min session (social novelty preference test). Thus, the subject mouse had a choice between the first, non-familiar mouse (stranger 1) and the novel unfamiliar mouse (stranger 2). The time spent in each chamber and the time spent around each cage was recorded on video.

Grooming test: The test was performed as previously described (14,15). Each mouse was put individually in a clean standard mouse cage and allowed to acclimate for 10 min. A video camera (C920r HD Pro, Japan) was set up two meters in front of the cage to record the mice behavior for the next 10 min, following the habituation time. After the experiment, the cumulative time spent in self-grooming was counted by an experimenter through watching these videos. A stopwatch was used for scoring cumulative time spent grooming during the 10 min test session.

PV-immunohistochemistry. Immunohistochemistry of PV was performed as reported previously (7,13,16,17). Mice were anesthetized with 5% isoflurane and sodium pentobarbital (50 mg/kg), and perfused transcardially with 10 mL of saline, followed by 30 mL of ice-cold 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Brains were removed from the skulls and post fixed overnight at 4°C in the same fixative. For the immunohistochemical analysis, 50 μm-thick serial, coronal sections of brain tissue were cut in ice-cold 0.01M phosphate buffered saline (pH 7.4) using a vibrating blade microtome (VT1000s, Leica Microsystems, Tokyo, Japan). Mounted on gelatinized slides brain sections were washed by PBS for three times and then blocked in PBS containing 0.3% Triton X-100 (PBST) and 3% normal serum for 1 h at room temperature. The samples were then incubated for 24 h at 4°C with mouse polyclonal anti-parvalbumin (PV) antibody (1:100, abcam, ab11427) in PBST with 1% normal serum. After that the sections were washed three times in PBS and then incubated for 2 h in room temperature with Alexa Fluor 488 Polyclonal Antibody (1:1000, Invitrogen, A11094). Then, sections were washed three times in PBS containing 0.1% Triton X-100 and cover slipped under VECTASHIELD (Vector Laboratories, Inc. Burlingame, CA, USA). The PV-immunofluorescent-positive cells in the inflalimbic (IL) and prelimbic (PrL) regions (0.05 mm^2) of mPFC was analyzed using a fluorescence microscope with a CCD camera (Olympus IX70, Tokyo, Japan) and the SCION IMAGE software package. Images of sections within mPFC region were captured using a CFI PLan APO Lambda 40× objective with a Keyence BZ-X710 microscope (Keyence Corporation, Osaka, Japan).

Measurement of amino acids. On P28, mice were deeply anesthetized with isoflurane and plasma was collected. Subsequently, prefrontal cortex (PFC), hippocampus and striatum were quickly dissected on ice from whole brain. The dissected tissues were weighed and stored at -80℃ until assayed. Levels of amino acids (glutamate, glutamine, glycine, L-serine, D-serine, GABA) were measured using high performance liquid chromatography system (Shimadzu Corporation, Kyoto, Japan), as reported previously (12,18,19).

16S rRNA analysis and measurement of short-chain fatty acids of fecal samples. On P28, we collected fresh fecal samples from each mouse at around 10:00 in order to avoid circadian effects on the microbiome. The fecal samples were put into a sterilized screw cap microtube immediately after defecation, and these samples were stored at -80°C until use. DNA extraction from mouse feces and 16S rRNA analysis of fecal samples were performed by MyMetagenome Co, Ltd. (Tokyo, Japan), as reported previously (20,21).

Measurement of short-chain fatty acids—acetic acid, propionic acid, butyric acid, lactic acid, and succinic acid—in fecal samples was performed by the TechnoSuruga Laboratory, Co., Ltd. (Shizuoka, Japan).

Statistical analysis. Analysis of the data was performed using GraphPad Prism (La Jolla, CA). Comparisons between two groups were performed using Student t-test. The PPI data were analyzed using multivariable analysis of variance (MANOVA). Comparisons among four groups were performed using the repeated measure two-way analysis of variance (ANOVA), two-way ANOVA or three-way ANOVA, followed by Fisher's LSD test. The P-values of less than 0.05 were considered statistically significant.

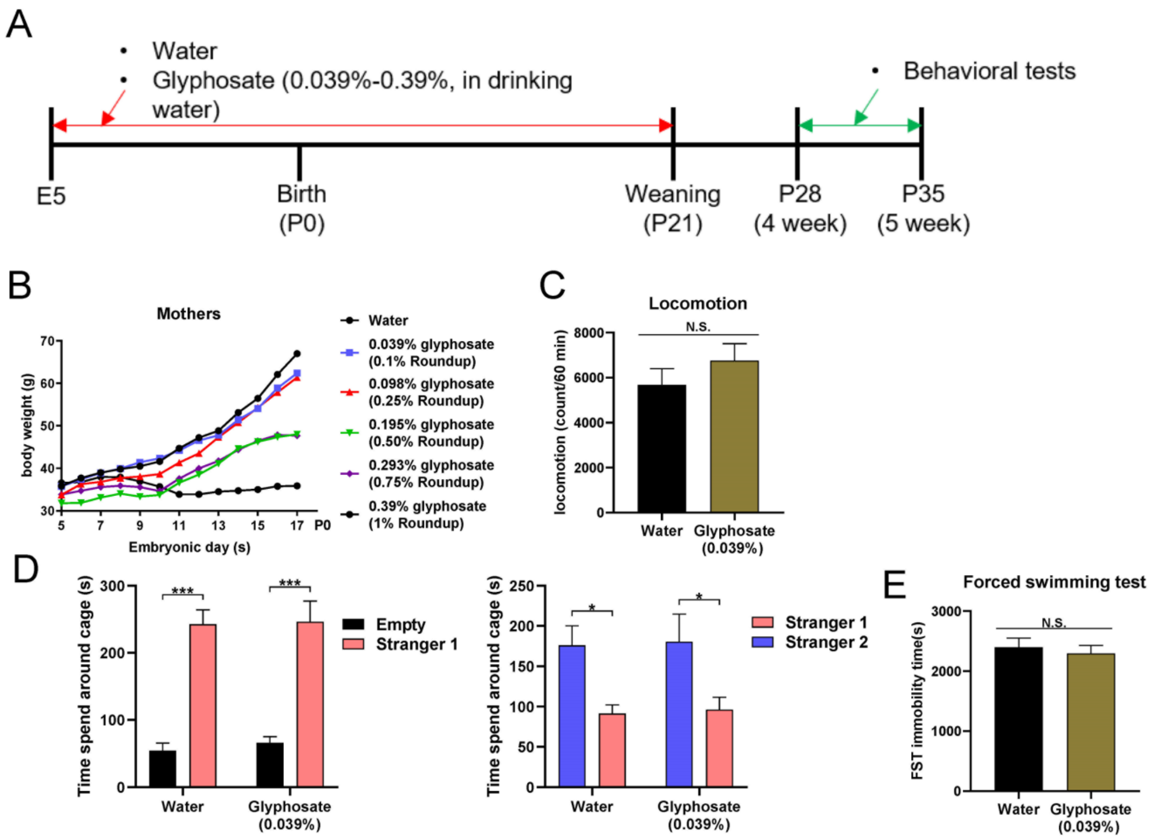

Figure S1. General and behavioral data of mother and juvenile offspring after maternal glyphosate exposure. (A): Schedule of treatment and behavioral tests. Water or formulated glyphosate [0.039% (w/v) (or 0.1% Roundup®) - 0.39% (w/v) (or 1.0% Roundup®)] were given to pregnant mice. (B): Change of body weight of mothers (n = 3 - 6). (C): Locomotion. Data are shown as mean ± S.E.M. (n = 10). (D): Three chamber social interaction test. Left: Two-way ANOVA (glyphosate: $F_{1,20} = 0.147$, $P = 0.706$; stranger: $F_{1,20} = 84.33$, $P < 0.001$; interaction (glyphosate × stranger): $F_{1,20} = 0.038$, $P = 0.848$). Right: Two-way ANOVA (glyphosate: $F_{1,20} = 0.051$, $P = 0.823$; stranger: $F_{1,20} = 16.87$, $P < 0.001$; interaction (glyphosate × stranger): $F_{1,20} < 0.001$, $P = 0.998$). Data are shown as mean ± S.E.M. (n = 6). (E): Forced swimming test. Data are shown as mean ± S.E.M. (n = 10). N.S.: not significant.

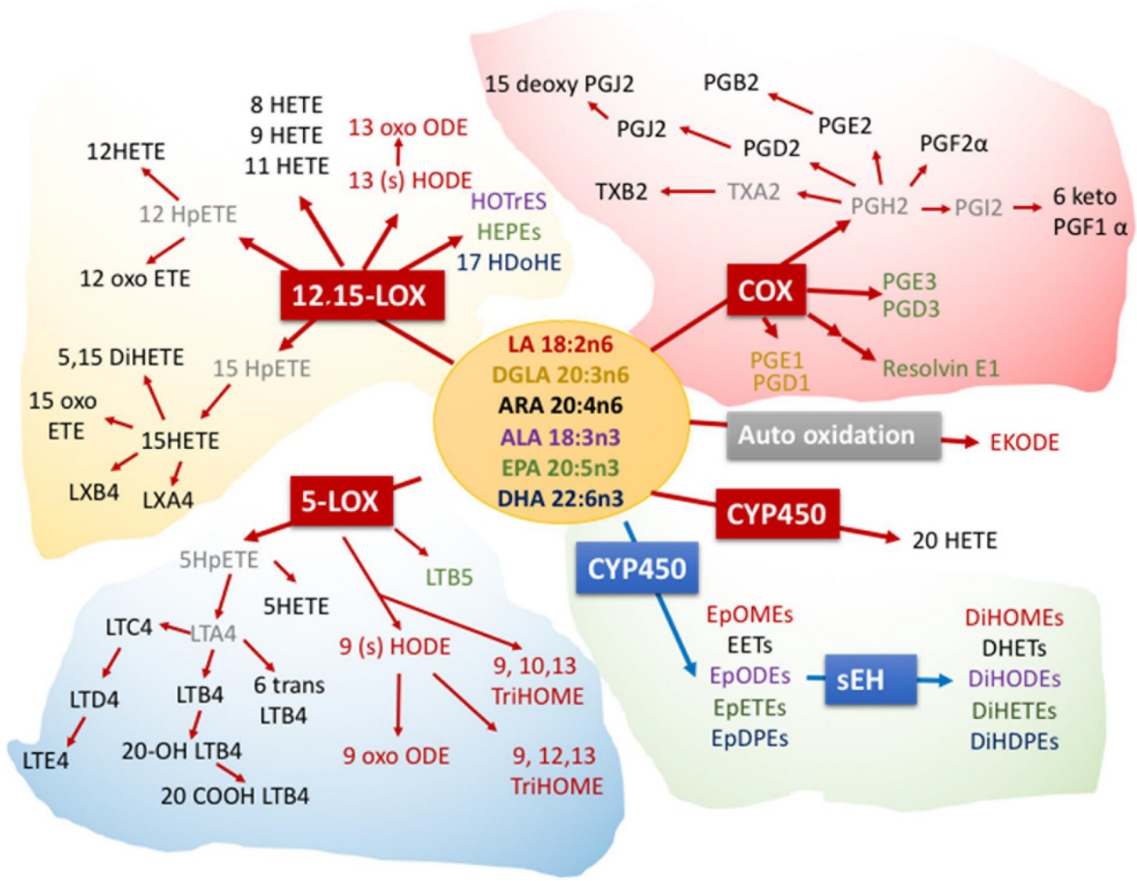

Figure S2. Eicosanoids measured in the blood and brain regions from male juvenile offspring after maternal glyphosate exposure (7).

Table S1. General and behavioral data of mother and juvenile offspring after maternal glyphosate exposure.

Concentration	Number of mothers used	Alive number of mothers on P0	maternal mortality	number of offspring born	Alive number of offspring on P21	offspring mortality	Behavioral abnormality
0.39% glyphosate (1% Roundup®)	4	0	100%	0	0	-	-
0.293% glyphosate (0.75% Roundup®)	4	3	25%	32	4	87.5%	-
0.195% glyphosate (0.50% Roundup®)	5	4	20%	43	2	95.3%	-
0.098% glyphosate (0.25% Roundup®)	3	3	0%	30	24	20%	observed
0.039% glyphosate (0.1% Roundup®)	3	3	0%	33	33	0%	Not observed

The concentration of glyphosate in the table is shown as the free base.

Table S2. Oxylipin analysis in plasma

Compounds	Control (nmol/L)			Glyphosate (nmol/L)			P value
6-keto-PGF1a	**0.408**	**±**	**0.047**	**0.252**	**±**	**0.043**	**0.025**
TXB2	0.776	±	0.120	0.548	±	0.069	0.116
9,12,13-TriHOME	17.372	±	2.022	12.758	±	1.096	0.060
9,10,13-TriHOME	**7.900**	**±**	**0.981**	**5.220**	**±**	**0.403**	**0.021**
PGF2a	**0.705**	**±**	**0.055**	**0.462**	**±**	**0.048**	**0.004**
PGE2	0.725	±	0.013	0.704	±	0.010	0.219
PGD2	**0.193**	**±**	**0.012**	**0.138**	**±**	**0.010**	**0.002**
11,12-,15-TriHETrE	**0.724**	**±**	**0.131**	**0.300**	**±**	**0.072**	**0.011**
19,20-DiHDPE	**10.277**	**±**	**0.591**	**6.507**	**±**	**0.444**	**0.000**
14,15-DiHETrE	0.686	±	0.055	0.575	±	0.051	0.156
LTB3	2.586	±	0.110	2.540	±	0.525	0.942
16,17-DiHDPE	**2.591**	**±**	**0.196**	**1.955**	**±**	**0.201**	**0.036**
11,12-DiHETrE	0.414	±	0.034	0.394	±	0.045	0.726
13,14-DiHDPE	**0.731**	**±**	**0.036**	**0.530**	**±**	**0.056**	**0.007**
EKODE	23.079	±	11.409	12.405	±	2.140	0.370
5,6-DiHETrE	**0.588**	**±**	**0.031**	**0.417**	**±**	**0.047**	**0.007**
8-HEPE	7.786	±	0.622	7.652	±	0.970	0.909
12-HEPE	163.602	±	36.795	92.380	±	15.026	0.090
5-HEPE	11.079	±	1.083	8.117	±	1.251	0.090
4,5-DiHDPE	**1.967**	**±**	**0.134**	**1.527**	**±**	**0.112**	**0.022**
13-HODE	**108.971**	**±**	**15.254**	**66.733**	**±**	**9.200**	**0.029**
9-HODE	**27.135**	**±**	**3.659**	**17.293**	**±**	**2.482**	**0.039**
15(16)-EpODE	**27.169**	**±**	**3.147**	**14.493**	**±**	**1.929**	**0.004**
15-HETE	2.590	±	0.326	1.985	±	0.228	0.146
17(18)-EpETE	**9.343**	**±**	**1.042**	**4.845**	**±**	**0.480**	**0.002**
17-HDoHE	3948.481	±	1343.355	1386.287	±	316.090	0.080
11-HETE	**2.434**	**±**	**0.172**	**1.949**	**±**	**0.131**	**0.038**
15-oxo-ETE	0.966	±	0.150	0.746	±	0.105	0.244
14(15)-EpETE	**5.921**	**±**	**0.937**	**2.316**	**±**	**0.258**	**0.003**
8-HETE	8.502	±	0.959	7.349	±	0.718	0.349
12-HETE	134.957	±	20.785	90.069	±	11.494	0.075
11(12)-EpETE	**7.002**	**±**	**1.143**	**2.579**	**±**	**0.284**	**0.002**
8(9)-EpETE	**4.572**	**±**	**0.577**	**0.863**	**±**	**0.138**	**0.000**
9-HETE	0.087	±	0.014	0.056	±	0.012	0.120
15(S)-HETrE	1.229	±	0.163	0.743	±	0.167	0.052
12-oxo-ETE	831.657	±	164.846	500.173	±	99.031	0.102
5-HETE	**3.641**	**±**	**0.230**	**2.137**	**±**	**0.335**	**0.002**
19(20)-EpDPE	**49.406**	**±**	**6.516**	**19.092**	**±**	**1.307**	**0.000**
12(13)-EpOME	**94.456**	**±**	**12.455**	**38.512**	**±**	**4.378**	**0.001**
14(15)-EpETrE	**8.183**	**±**	**1.404**	**4.038**	**±**	**0.637**	**0.019**
9(10)-EpOME	**63.958**	**±**	**12.021**	**28.326**	**±**	**4.243**	**0.016**
16(17)-EpDPE	**19.080**	**±**	**3.183**	**7.978**	**±**	**0.799**	**0.005**
13(14)-EpDPE	**18.937**	**±**	**3.242**	**8.177**	**±**	**0.873**	**0.007**
5-oxo-ETE	29.432	±	4.666	20.987	±	2.839	0.151
10(11)-EpDPE	**29.647**	**±**	**4.960**	**13.285**	**±**	**1.234**	**0.007**
11(12)-EpETrE	**12.482**	**±**	**2.192**	**6.360**	**±**	**1.002**	**0.026**
7(8)-EpDPE	**402.209**	**±**	**66.364**	**195.343**	**±**	**16.718**	**0.010**
8(9)-EpETrE	**33.331**	**±**	**7.604**	**13.892**	**±**	**2.938**	**0.042**
5(6)-EpETrE	**41.145**	**±**	**6.192**	**24.279**	**±**	**3.524**	**0.035**

The value (nmol/L blood) are the mean ±SEM (n = 8 to 10). The bold is statistically significant.
The green color means the compound decreased in glyphosate offspring compared with control.

Table S3. Oxylipin analysis in PFC

Compounds	Control (pmol/g)			Glyphosate (pmol/g)			P value
6-keto-PGF1a	71.859	±	10.966	88.691	±	7.208	0.216
TXB2	**137.151**	**±**	**16.944**	**246.160**	**±**	**16.777**	**0.000**
9,12,13-TriHOME	40.375	±	8.257	52.749	±	6.979	0.267
9,10,13-TriHOME	22.337	±	4.483	31.772	±	4.440	0.152
PGF2a	**244.053**	**±**	**34.499**	**431.431**	**±**	**34.510**	**0.001**
PGE2	**90.158**	**±**	**13.679**	**137.389**	**±**	**14.051**	**0.027**
PGD2	**322.348**	**±**	**26.200**	**439.532**	**±**	**37.011**	**0.019**
11,12-,15-TriHETrE	**14.229**	**±**	**1.496**	**27.925**	**±**	**2.262**	**0.000**
19,20-DiHDPE	7.162	±	0.421	7.491	±	0.703	0.693
14,15-DiHETrE	**1.256**	**±**	**0.120**	**2.498**	**±**	**0.243**	**0.000**
LTB3	45.708	±	18.777	53.423	±	26.072	0.816
16,17-DiHDPE	**0.707**	**±**	**0.082**	**1.154**	**±**	**0.110**	**0.004**
11,12-DiHETrE	**0.863**	**±**	**0.055**	**1.383**	**±**	**0.124**	**0.001**
13,14-DiHDPE	**0.431**	**±**	**0.037**	**0.851**	**±**	**0.076**	**0.000**
EKODE	**9.547**	**±**	**0.671**	**12.663**	**±**	**1.143**	**0.030**
5,6-DiHETrE	**0.404**	**±**	**0.039**	**0.699**	**±**	**0.113**	**0.024**
8-HEPE	1.054	±	0.155	0.959	±	0.080	0.592
12-HEPE	3.702	±	1.166	6.850	±	1.105	0.066
5-HEPE	1.554	±	0.180	1.760	±	0.234	0.494
4,5-DiHDPE	0.957	±	0.210	1.083	±	0.242	0.700
13-HODE	40.531	±	2.810	45.239	±	3.261	0.288
9-HODE	25.933	±	2.089	32.184	±	2.752	0.087
15(16)-EpODE	**1.095**	**±**	**0.204**	**0.500**	**±**	**0.121**	**0.022**
15-HETE	**197.804**	**±**	**29.559**	**313.016**	**±**	**24.240**	**0.007**
17(18)-EpETE	**0.588**	**±**	**0.139**	**3.729**	**±**	**1.017**	**0.007**
17-HDoHE	**3452.039**	**±**	**903.839**	**6200.527**	**±**	**739.211**	**0.030**
11-HETE	**188.165**	**±**	**23.250**	**275.604**	**±**	**19.707**	**0.010**
15-oxo-ETE	**6.740**	**±**	**0.540**	**10.104**	**±**	**1.289**	**0.027**
14(15)-EpETE	0.590	±	0.140	1.633	±	0.487	0.054
8-HETE	5.970	±	1.038	7.904	±	0.840	0.165
12-HETE	**125.575**	**±**	**61.553**	**448.365**	**±**	**69.003**	**0.003**
11(12)-EpETE	**0.665**	**±**	**0.211**	**2.775**	**±**	**0.733**	**0.048**
8(9)-EpETE	1.752	±	0.434	1.992	±	0.638	0.760
9-HETE	0.893	±	0.173	1.826	±	0.587	0.145
15(S)-HETrE	**5.611**	**±**	**0.977**	**11.900**	**±**	**1.029**	**0.000**
12-oxo-ETE	**1331.981**	**±**	**114.969**	**2140.116**	**±**	**142.081**	**0.000**
5-HETE	**13.471**	**±**	**1.447**	**18.560**	**±**	**1.797**	**0.041**
19(20)-EpDPE	**89.030**	**±**	**26.621**	**353.450**	**±**	**99.156**	**0.019**
12(13)-EpOME	**14.229**	**±**	**3.214**	**67.240**	**±**	**21.629**	**0.026**
14(15)-EpETrE	**369.147**	**±**	**105.708**	**1037.632**	**±**	**291.994**	**0.045**
9(10)-EpOME	**11.898**	**±**	**2.490**	**57.775**	**±**	**18.915**	**0.027**
16(17)-EpDPE	**49.169**	**±**	**14.279**	**195.454**	**±**	**58.684**	**0.026**
13(14)-EpDPE	**44.704**	**±**	**12.681**	**190.175**	**±**	**59.161**	**0.027**
5-oxo-ETE	144.718	±	35.486	142.497	±	16.459	0.955
10(11)-EpDPE	**61.132**	**±**	**17.665**	**270.138**	**±**	**86.641**	**0.030**
11(12)-EpETrE	364.169	±	88.308	1128.684	±	354.042	0.051
7(8)-EpDPE	**790.350**	**±**	**192.868**	**3640.707**	**±**	**1175.101**	**0.028**
8(9)-EpETrE	**843.5**	**±**	**202.2**	**31.8**	**±**	**17.1**	**0.001**
5(6)-EpETrE	1312.892	±	297.163	3862.881	±	1353.639	0.082

The value (pmol/g tissue) are the mean ±SEM (n = 8 to 10). The bold is statistically significant.
The green color means the compound decreased in glyphosate offspring compared with control.
The orange color means the compound increased in glyphosate offspring compared with control.

Table S4. Oxylipin analysis in the hippocampus

Compounds	Control (pmol/g)			Glyphosate (pmol/g)			P value
6-keto-PGF1a	**210.973**	**±**	**17.307**	**113.103**	**±**	**15.509**	**0.001**
TXB2	175.011	±	15.917	198.203	±	24.835	0.442
9,12,13-TriHOME	46.966	±	7.670	51.599	±	10.194	0.721
9,10,13-TriHOME	27.122	±	4.287	29.123	±	5.450	0.776
PGF2a	**463.947**	**±**	**37.827**	**314.752**	**±**	**30.178**	**0.006**
PGE2	91.922	±	10.680	68.947	±	7.203	0.091
PGD2	**439.270**	**±**	**24.157**	**349.020**	**±**	**34.288**	**0.045**
11,12-,15-TriHETrE	**26.992**	**±**	**2.050**	**20.278**	**±**	**1.789**	**0.024**
19,20-DiHDPE	**7.827**	**±**	**0.484**	**5.359**	**±**	**0.322**	**0.000**
14,15-DiHETrE	1.375	±	0.208	1.183	±	0.095	0.412
LTB3	81.636	±	17.998	13.669	±	n.d.	n.d.
16,17-DiHDPE	0.753	±	0.064	0.675	±	0.093	0.500
11,12-DiHETrE	1.120	±	0.106	0.914	±	0.053	0.099
13,14-DiHDPE	0.493	±	0.044	0.516	±	0.089	0.823
EKODE	12.888	±	1.489	11.362	±	1.014	0.408
5,6-DiHETrE	0.561	±	0.047	0.470	±	0.051	0.205
8-HEPE	1.151	±	0.086	1.415	±	0.182	0.205
12-HEPE	15.321	±	3.628	9.094	±	3.984	0.263
5-HEPE	1.900	±	0.242	2.917	±	0.708	0.191
4,5-DiHDPE	1.510	±	0.192	1.572	±	0.704	0.933
13-HODE	59.332	±	3.170	54.648	±	8.929	0.627
9-HODE	35.987	±	1.888	31.282	±	6.681	0.506
15(16)-EpODE	**0.726**	**±**	**0.163**	**2.750**	**±**	**0.907**	**0.033**
15-HETE	313.210	±	26.117	263.402	±	29.963	0.226
17(18)-EpETE	4.252	±	3.461	1.592	±	0.499	0.457
17-HDoHE	5370.298	±	389.810	5997.276	±	762.508	0.474
11-HETE	296.258	±	22.047	261.315	±	27.149	0.331
15-oxo-ETE	9.112	±	0.576	8.232	±	0.652	0.325
14(15)-EpETE	2.515	±	2.018	0.712	±	0.184	0.385
8-HETE	**10.308**	**±**	**0.983**	**5.383**	**±**	**0.796**	**0.001**
12-HETE	304.652	±	79.838	131.250	±	40.185	0.068
11(12)-EpETE	0.470	±	0.147	1.254	±	0.537	0.159
8(9)-EpETE	**0.912**	**±**	**0.145**	**3.435**	**±**	**0.784**	**0.008**
9-HETE	1.007	±	0.194	2.073	±	0.532	0.076
15(S)-HETrE	9.162	±	1.121	7.994	±	1.430	0.528
12-oxo-ETE	1524.203	±	95.043	1245.436	±	206.406	0.221
5-HETE	19.187	±	0.993	16.705	±	0.992	0.094
19(20)-EpDPE	42.255	±	7.441	55.856	±	8.656	0.251
12(13)-EpOME	8.394	±	1.398	11.355	±	1.474	0.164
14(15)-EpETrE	213.663	±	39.064	241.703	±	26.592	0.561
9(10)-EpOME	7.129	±	1.163	9.131	±	1.032	0.216
16(17)-EpDPE	22.265	±	4.447	29.112	±	4.286	0.284
13(14)-EpDPE	19.371	±	4.044	27.748	±	3.955	0.158
5-oxo-ETE	**271.915**	**±**	**35.187**	**94.461**	**±**	**9.488**	**0.000**
10(11)-EpDPE	**24.579**	**±**	**4.314**	**40.179**	**±**	**5.185**	**0.034**
11(12)-EpETrE	190.566	±	34.419	254.371	±	33.701	0.204
7(8)-EpDPE	335.530	±	58.928	460.824	±	81.977	0.232
8(9)-EpETrE	**146.5**	**±**	**47.0**	**2.4**	**±**	**1.2**	**0.012**
5(6)-EpETrE	731.031	±	123.125	917.184	±	134.826	0.323

The value (pmol/g tissue) are the mean ±SEM (n = 8 to 10). The bold is statistically significant.
The green color means the compound decreased in glyphosate offspring compared with control.
The orange color means the compound increased in glyphosate offspring compared with control.

Table S5 oxylipin analysis in striatum

Compounds	Control (pmol/g)			Glyphosate (pmol/g)			P value
6-keto-PGF1a	80.263	±	6.685	70.151	±	4.942	0.240
TXB2	**143.486**	**±**	**9.763**	**219.314**	**±**	**15.121**	**0.001**
9,12,13-TriHOME	43.712	±	6.665	59.110	±	9.487	0.201
9,10,13-TriHOME	25.621	±	3.779	35.219	±	5.532	0.169
PGF2a	327.993	±	20.071	367.138	±	24.248	0.230
PGE2	62.314	±	6.350	61.254	±	3.057	0.882
PGD2	335.832	±	20.834	404.389	±	30.557	0.080
11,12-,15-TriHETrE	**19.365**	**±**	**1.301**	**28.513**	**±**	**2.432**	**0.004**
19,20-DiHDPE	8.381	±	0.316	8.734	±	0.672	0.640
14,15-DiHETrE	**1.607**	**±**	**0.058**	**2.399**	**±**	**0.255**	**0.007**
LTB3	66.165	±	17.957	74.130	±	28.033	0.807
16,17-DiHDPE	**0.943**	**±**	**0.039**	**1.427**	**±**	**0.114**	**0.001**
11,12-DiHETrE	1.230	±	0.075	1.461	±	0.114	0.108
13,14-DiHDPE	0.691	±	0.043	0.849	±	0.088	0.125
EKODE	15.864	±	2.271	14.223	±	1.256	0.535
5,6-DiHETrE	0.628	±	0.069	0.655	±	0.058	0.766
8-HEPE	1.323	±	0.159	1.198	±	0.106	0.519
12-HEPE	11.726	±	2.476	16.777	±	3.707	0.272
5-HEPE	2.635	±	0.337	1.966	±	0.253	0.130
4,5-DiHDPE	1.376	±	0.224	1.331	±	0.320	0.910
13-HODE	57.138	±	6.208	61.551	±	3.988	0.557
9-HODE	36.146	±	2.792	42.211	±	2.648	0.132
15(16)-EpODE	0.811	±	0.270	0.837	±	0.335	0.954
15-HETE	**254.134**	**±**	**17.641**	**312.592**	**±**	**16.237**	**0.025**
17(18)-EpETE	**1.025**	**±**	**0.244**	**7.282**	**±**	**2.840**	**0.042**
17-HDoHE	6589.296	±	752.871	7832.089	±	1004.299	0.335
11-HETE	**221.438**	**±**	**13.362**	**263.508**	**±**	**14.043**	**0.044**
15-oxo-ETE	6.911	±	0.402	15.203	±	3.961	0.052
14(15)-EpETE	**0.760**	**±**	**0.203**	**2.368**	**±**	**0.640**	**0.023**
8-HETE	6.054	±	1.093	7.063	±	1.134	0.530
12-HETE	254.764	±	57.123	402.213	±	68.023	0.114
11(12)-EpETE	**1.203**	**±**	**0.270**	**2.950**	**±**	**0.707**	**0.030**
8(9)-EpETE	1.662	±	0.234	2.863	±	1.693	0.491
9-HETE	1.098	±	0.293	2.001	±	0.403	0.086
15(S)-HETrE	**7.618**	**±**	**0.587**	**13.863**	**±**	**0.898**	**0.000**
12-oxo-ETE	**966.895**	**±**	**140.198**	**2383.322**	**±**	**205.470**	**0.000**
5-HETE	**15.975**	**±**	**1.179**	**21.009**	**±**	**1.874**	**0.035**
19(20)-EpDPE	**58.240**	**±**	**11.472**	**373.365**	**±**	**128.458**	**0.025**
12(13)-EpOME	**11.880**	**±**	**1.980**	**75.548**	**±**	**26.478**	**0.028**
14(15)-EpETrE	**206.025**	**±**	**37.616**	**991.031**	**±**	**327.438**	**0.028**
9(10)-EpOME	**10.352**	**±**	**2.057**	**64.909**	**±**	**24.089**	**0.037**
16(17)-EpDPE	**30.359**	**±**	**6.931**	**186.178**	**±**	**73.985**	**0.050**
13(14)-EpDPE	**27.801**	**±**	**5.702**	**196.586**	**±**	**72.402**	**0.032**
5-oxo-ETE	**256.279**	**±**	**31.908**	**177.806**	**±**	**23.342**	**0.063**
10(11)-EpDPE	**39.670**	**±**	**9.816**	**283.118**	**±**	**111.688**	**0.044**
11(12)-EpETrE	**208.934**	**±**	**39.997**	**1056.655**	**±**	**383.807**	**0.041**
7(8)-EpDPE	**528.009**	**±**	**139.936**	**3684.482**	**±**	**1464.079**	**0.046**
8(9)-EpETrE	**318.5**	**±**	**109.4**	**18.8**	**±**	**7.2**	**0.019**
5(6)-EpETrE	**821.143**	**±**	**187.923**	**2286.424**	**±**	**589.971**	**0.024**

The value (pmol/g tissue) are the mean ±SEM (n = 9 to 10). The bold is statistically significant.
The green color means the compound decreased in glyphosate offspring compared with control.
The orange color means the compound increased in glyphosate offspring compared with control.

Abbreviations in Table S2 - Table S5

Abbreviation	Formal Name
6-keto-PGF1a	6-oxo-9S,11R,15S-trihydroxy-13E-prostenoic acid
TXB2	9S,11,15S-trihydroxy-thromboxa-5Z,13E-dien-1-oic acid
9,12,13-TriHOME	9S,12S,13S-trihydroxy-10E-octadecenoic acid
9,10,13-TriHOME	9S,10,13-Trihydroxy-11-octadecenoic acid
PGF2a	7-(3,5-Dihydroxy-2-(3-hydroxy-1-octenyl)cyclopentyl)-5-heptenoic acid
PGE2	(5Z,11alpha,13E,15S)-11,15-dihydroxy-9-oxoprosta-5,13-dien-1-oic acid
PGD2	(5Z,13E,15S)-9alpha,15-Dihydroxy-11-oxoprosta-5,13-dienoate
11,12-,15-TriHETrE	(8E,11Z,13E)-11,12,15-trihydroxyicosa-8,11,13-trienoic acid
19,20-DiHDPE	19,20-dihydroxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid
14,15-DiHETrE	(5Z,8Z,11Z)-14,15-Dihydroxyicosa-5,8,11-trienoic acid
LTB3	(5R,6E,8Z,10E,12S)-5,12-dihydroxyicosa-6,8,10-trienoic acid
16,17-DiHDPE	16,17-dihydroxy-4Z,7Z,10Z,13Z,19Z-docosapentaenoic acid
11,12-DiHETrE	(5Z,8Z,14Z)-11,12-Dihydroxyicosa-5,8,14-trienoic acid
13,14-DiHDPE	13,14-dihydroxy-4Z,7Z,10Z,16Z,19Z-docosapentaenoic acid
EKODE	9-Oxo-11-(3-pentyl-2-oxiranyl)-10E-undecenoic acid
5,6-DiHETrE	(8Z,11Z,14Z)-5,6-Dihydroxyicosa-8,11,14-trienoic acid
8-HEPE	8-hydroxy-5Z,9E,11Z,14Z,17Z-eicosapentaenoic acid
12-HEPE	12-hydroxy-5Z,8Z,10E,14Z,17Z-eicosapentaenoic acid
5-HEPE	5-Hydroxyeicosapentaenoic acid
4,5-DiHDPE	7,8-dihydroxy-4Z,10Z,13Z,16Z,19Z-docosapentaenoic acid
13-HODE	(9Z,11E)-13-hydroxyoctadeca-9,11-dienoic acid
9-HODE	9-hydroxy-10E,12Z-octadecadienoic acid
15(16)-EpODE	15(16)-epoxy-9Z,12Z-octadecadienoic acid
15-HETE	15-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid
17(18)-EpETE	17(18)-epoxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid
17-HDoHE	17-Hydroxy-4,7,10,13,15,19-docosahexaenoic acid
11-HETE	11-Hydroxy-5Z,8Z,12E,14Z-eicosatetraenoic acid
15-oxo-ETE	(5Z,8Z,11Z,13E)-15-oxoicosa-5,8,11,13-tetraenoic acid
14(15)-EpETE	14(15)-Epoxy-5Z,8Z,11Z,17Z-eicosatetraenoic acid
8-HETE	8-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoate
12-HETE	12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid
11(12)-EpETE	11(12)-epoxy-5Z,8Z,14Z,17Z-eicosatetraenoic acid
8(9)-EpETE	8(9)-Epoxy-5Z,11Z,14Z,17Z-eicosatetraenoic acid
9-HETE	9-hydroxy-5Z,7E,11Z,14Z-eicosatetraenoic acid
15(S)-HETrE	15-hydroxy-(8Z,11Z,13E)-eicosatrienoic acid
12-oxo-ETE	12-oxo-5Z,8Z,10E,14Z-eicosatetraenoic acid
5-HETE	5-Hydroxyeicosatetraenoic acid
19(20)-EpDPE	19(20)-epoxy-4Z,7Z,10Z,13Z,16Z-docosapentaenoic acid
12(13)-EpOME	(9Z)-12,13-epoxyoctadecenoic acid
14(15)-EpETrE	14,15-epoxy-5Z,8Z,11Z-eicosatrienoic acid
9(10)-EpOME	9,10-epoxy-12Z-octadecenoic acid
16(17)-EpDPE	16(17)-epoxy-4Z,7Z,10Z,13Z,19Z-docosapentaenoic acid
13(14)-EpDPE	13(14)-epoxy-4Z,7Z,10Z,16Z,19Z-docosapentaenoic acid
5-oxo-ETE	5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid
10(11)-EpDPE	10(11)-epoxy-4Z,7Z,13Z,16Z,19Z-docosapentaenoic acid
11(12)-EpETrE	(5Z,8Z,14Z)-11,12-Epoxyicosa-5,8,14-trienoate
7(8)-EpDPE	7(8)-epoxy-4Z,10Z,13Z,16Z,19Z-docosapentaenoic acid
8(9)-EpETrE	8,9-Epoxy-5,11,14-icosatrienoic acid
5(6)-EpETrE	4-(3-tetradeca-2,5,8-trienyloxiran-2-yl)butanoic acid

Table 6. Levels of NMDAR-related amino acids in the plasma, PFC, hippocampus, and striatum of offspring

	Glutamate	Glutamine	Glycine	L-Serine	D-Serine	GABA
Plasma (nmol/ml)						
Control	60.047± 5.976	508.014 ± 13.285	333.402 ± 13.215	148.271 ± 6.711	3.841 ± 0.293	
Glyphosate	**38.003 ± 1.974****	496.268 ± 15.748	354.185 ± 9.806	144.824 ± 4.622	**7.522 ± 0.578*****	
PFC (nmol/mg tissue)						
Control	11.827 ± 0.293	5.486 ± 0.194	0.804 ± 0.033	0.712 ± 0.032	0.345 ± 0.017	2.243± 0.048
Glyphosate	**10.392 ± 0.377****	5.053 ± 0.243	**0.705 ± 0.023***	**0.622 ± 0.016***	0.313 ± 0.010	**2.060 ± 0.058***
Hippocampus (nmol/mg tissue)						
Control	11.477 ± 0.295	4.979 ± 0.147	0.962 ± 0.143	0.764 ± 0.041	0.277 ± 0.011	2.736 ± 0.306
Glyphosate	**9.350 ± 0.282*****	4.765 ± 0.137	0.725 ± 0.030	0.662 ± 0.037	0.259 ± 0.017	2.469 ± 0.097
Striatum (nmol/mg tissue)						
Control	8.712 ± 0.348	5.756 ± 0.302	0.757 ± 0.041	0.771± 0.056	0.309 ± 0.022	2.925 ± 0.177
Glyphosate	**7.745 ± 0.141***	5.735 ± 0.319	0.755 ± 0.039	0.715 ± 0.032	0.281 ± 0.010	3.076 ± 0.151

Data are expressed as the mean ± SEM (Control: n = 9, Glyphosate: n = 10).The bold is statistically significant. *P < 0.05, **P < 0.01, ***P < 0.001 compared to control group (Student's t test).

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