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Supplemental Material

Evaluation of Neurotoxicity in BALB/c Mice following Chronic Exposure to Polystyrene Microplastics

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Figure S4. The average swim speed of mice on days 1-5 in Morris water maze test is shown. Circle (Control), square (100 μg/L), triangle (1000 μg/L). (A) Control and 0.5 μm PS-MPs exposure groups. (B) Control and 4 μm PS-MPs exposure groups. (C) Control and 10 μm PS-MPs exposure groups. The results are expressed as means ± SD (n = 10 mice/per group). The mean and SD summary data for quantification are shown in Table S3. *P*-Values for all comparisons are reported in Table S4. Data were detected by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.

Figure S5. The total path length to find the hidden platform of mice on days 1-5 in Morris water maze test are shown. Circle (Control), square (100 μg/L), triangle (1000 μg/L). (A) Control and 0.5 μm PS-MPs exposure groups. (B) Control and 4 μm PS-MPs exposure groups. (C) Control and 10 μm PS-MPs exposure groups. The results are expressed as means ± SD (n = 10 mice/per group). The mean and SD summary data for quantification are shown in Table S3. *P*-Values for all comparisons are reported in Table S4. **P* < 0.05 vs. control, as detected by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.

Figure S6. The recognition scores (time spent (s) investigating one object/time spent (s) investigating both objects in total) of mice on day 1 (A) and day 2 (B) of novel object recognition (NOR) experiment were examined (n = 10 mice/per group). The mean and SD summary data for quantification are shown in Table S3. *P*-Values for all comparisons are reported in Table S4.

Figure S7. The total distance moved (A) and average movement speed (B) of mice in testing day (day 3) of the novel object recognition test are shown. The results are expressed as means ± SD (n = 10 mice/per group). The mean and SD summary data for quantification are shown in Table S3. *P*-Values for all comparisons are reported in Table S4. **P* < 0.05 vs. control, as detected by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.

Figure S8. The numbers of neurons in the hippocampal cornu ammonis 1 (CA1), cornu ammonis 3 (CA3), and dentate gyrus (DG) sections were counted (n = 3 mice/group, n = 3 slides/mice). Data are shown as mean ± SD. The mean and SD summary data for quantification are shown in Table S3. *P*-Values for all comparisons are reported in Table S4. **P* < 0.05 compared with control, as detected by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.

Figure S9. The mRNA levels of *caspase 3* (A), the *Bax/Bcl-2* mRNA ratio (B) in the hippocampus were tested with quantitative real-time PCR (qRT-PCR) by normalizing to *Gapdh*. The results are expressed as means ± SD (n = 3, N = 3 mice/group). The mean and SD summary data for quantification are shown in Table S3. *P*-Values for all comparisons are reported in Table S4. **P* < 0.05 and ***P* < 0.01 compared with the control, as detected by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.

Figure S10. The expression of synapsin 1, synaptophysin, and PSD 95 protein in the hippocampus was measured via western blotting. The western blotting results were quantified and statistically analyzed, as shown in (Figure 7C) as mean ± SD (n = 3, N = 3 mice/group). The mean and SD summary data for quantification of western blotting are shown in Table S3. *P*-Values for all comparisons are reported in Table S4.

Figure S11. The mRNA expression levels of *Gap43*(A), *Syt 4* (B), and *Ncam*(C) were detected by qRT-PCR in the hippocampus of mice exposed to PS-MPs and control mice. Data are shown as mean ± SD (n = 3). The mean and SD summary data for quantification are shown in Table S3. *P*-Values for all comparisons are reported in Table S4. $^{**}P < 0.01$ compared with control, as detected by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.

Figure S12. (A) A confocal fluorescence microscope was used to detect colocalization of Alexa Fluor 594-labled MCP-1 (red)/TNF-α (red) and DAPI (blue) (scale bar = 20 μm, n = 3) in the hippocampus of mice exposed to PS-MPs and control mice. (B) Percent of positivity was calculated based on the percentage of MCP-1 positive cells out of the total number of cells in an image. (C) Percent of positivity was calculated based on the percentage of TNF-α positive cells out of the total number of cells in an image. Data are shown as mean ± SD (n = 3). The mean and SD summary data for quantification are shown in Table S3. *P*-Values for all comparisons are reported in Table S4. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control, as detected by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests.