Inhibition of NADPH oxidase by apocynin prevents learning and memory deficits in a mouse Parkinson's disease model

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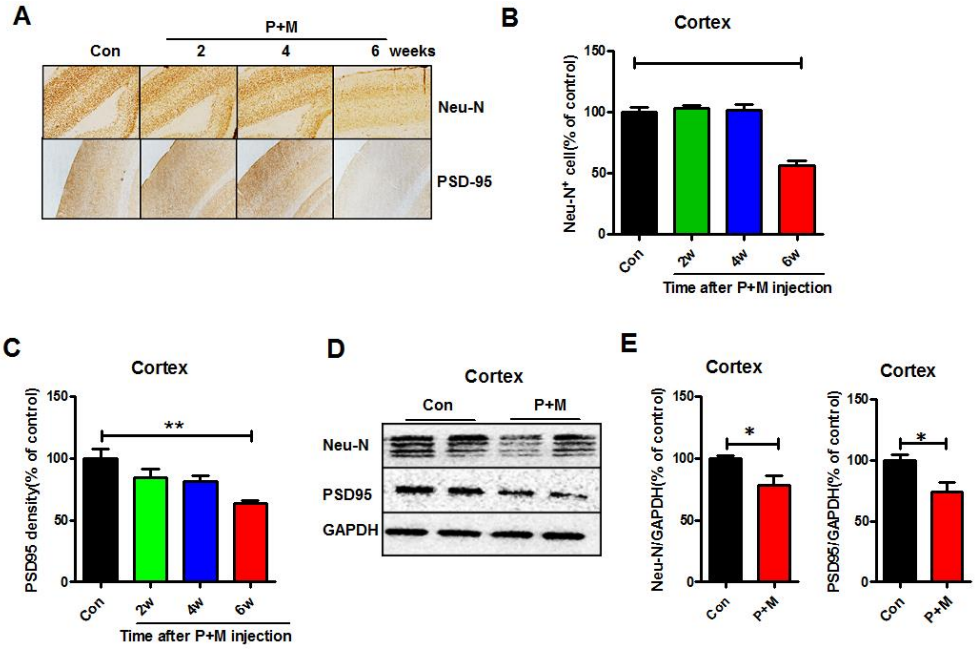
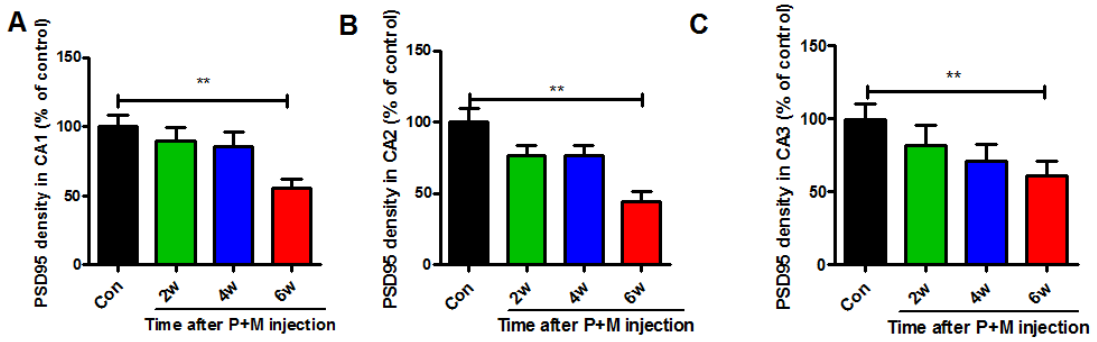
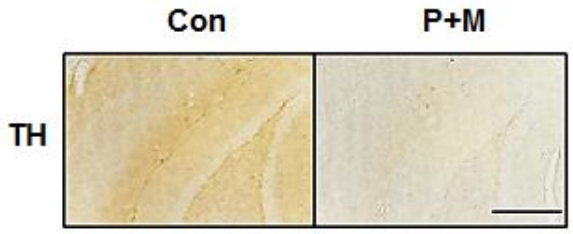


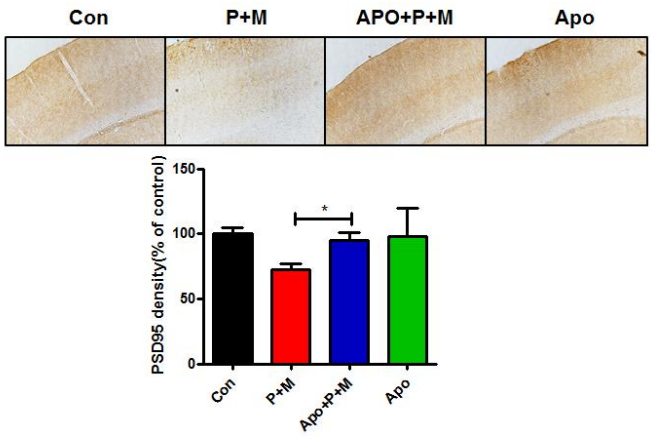
Supplementary Fig. S1. P+M exposure induces neurodegeneration and reduction of PSD-95 expression in the cortex. (A) After 2, 4 and 6 weeks of initial P+M treatment, neuronal nuclei and synpase in the cortex were immunostained with antibodies against Neu-N and PSD-95, respectively, and the representative images were shown. (B) The number of Neu-N^+ cells in the cortex was quantified by automated counting. (C) The density of PSD-95 immunostaining in the cortex was quantified. (D) The expressions of Neu-N and PSD-95 in the cortex were detected by using Western blot and the representative blots were shown. GAPDH was used as an internal control. (E) The band density of Neu-N and PSD-95 blots was quantified. *p<0.05, **p<0.01; Scale bar = 200 μm.



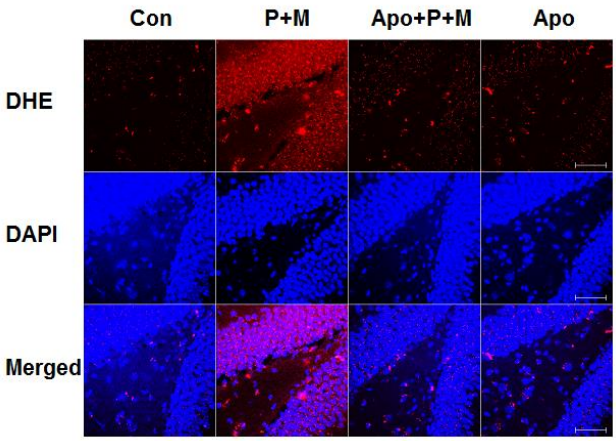
Supplementary Fig. S2. P+M exposure induces reduction of PSD-95 in the hippocampus. After 2, 4, and 6 weeks of initial P+M treatment, synpase in the hippocampus were immunostained with antibody against PSD-95. The density of PSD-95 immunostaining in the CA1 (A), CA2 (B) and CA3 (C) regions of hippocampus was quantified. ** $p<0.01$.



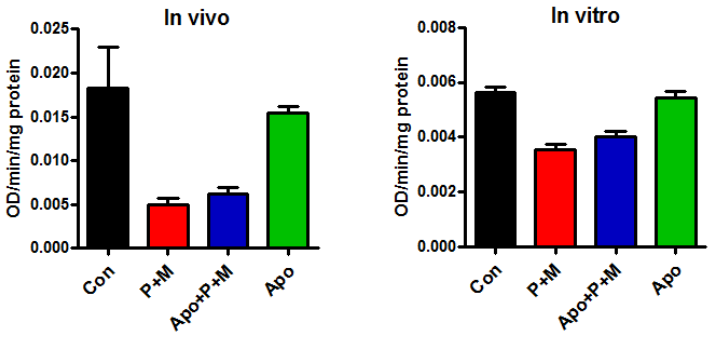
Supplementary Fig. S3. P+M exposure induces reduction of TH expression in the hippocampus. After 6 weeks of initial P+M treatment, the projections of dopaminergic neurons in the hippocampus was immunostained with antibody against TH and the representative images were shown.



Supplementary Fig. S4. Apocynin mitigates P+M-induced reduction of PSD-95 in the cortex. Mice were administrated with apocynin 2 days prior to P+M treatment. After 6 weeks of initial P+M exposure, neuronal synpase in the cortex were immunostained with anti-PSD-95 antibody and the density of PSD-95 immunostaining was quantified.



Supplementary Fig. S5. Apocynin mitigates P+M-induced increase in DHE oxidation in the hippocampus of mice. After 6 weeks of initial P+M exposure with or without apocynin pre-treatment, the production of superoxide in the hippocampus of mice was assessed by DHE and the representative images were shown.



Supplementary Fig. S6. Apocynin fails to interfere with mitochondria complex I activity in P+M-treated mice and BV2 microglial cells. (A) After 6 weeks of initial P+M exposure with or without apocyni pre-treatment, the activities of mitochondrial complex I in the hippocampus of mice were measured by using commerical assay kits. (B) BV2 microglial cells were pre-treated with apocynin for 30 mins prior to P+M exposure. After 1h of P+M exposure, the activities of mitochondrial complex I were measured by using commerical assay kits.