Exendin-4 Improves Long-Term Potentiation and Neuronal Dendritic Growth in High-Fat Diet Mice and Neurons Under Metabolic Imbalance Conditions

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Research Article

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Abstract

Metabolic syndrome, which increases the risk of obesity and type 2 diabetes, has emerged as a significant issue worldwide. Metabolic syndrome can occur due to diverse factors such as genetic background, lifestyle changes, food intake, and aging. Recent studies have highlighted the relationship between metabolic imbalance and neurological pathologies, such as synaptic dysfunction and memory loss. Glucagon-like peptide 1 (GLP-1) secreted from gut L-cells, and specific brain nuclei play multiple roles, including glucose metabolism, regulation of insulin sensitivity, inflammation control, synaptic plasticity improvement, and neuronal protection. Even though GLP-1 and GLP-1 receptor agonists (GLP-1RA) appear to have neuroprotective functions, the specific mechanisms of GLP-1 and GLP-1RA in brain function have remained unclear. Here, we investigated whether exendin-4 improves cognitive function and brain insulin resistance in metabolic imbalanced high-fat diet mice brain as a GLP-1RA, using electrophysiological experiments. Further, we identified the neuroprotective effect of exendin-4 in primary cultured hippocampal and cortical neurons under an in vitro metabolic imbalance condition, including neuronal structure improvement. This study provides significant findings on the effects of exendin-4 in synaptic plasticity, long-term potentiation (LTP), neuroinflammation, and neural structure. We suggest that GLP-1 may be vital to treating neuropathology caused by metabolic imbalance.

Full Text

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Figures

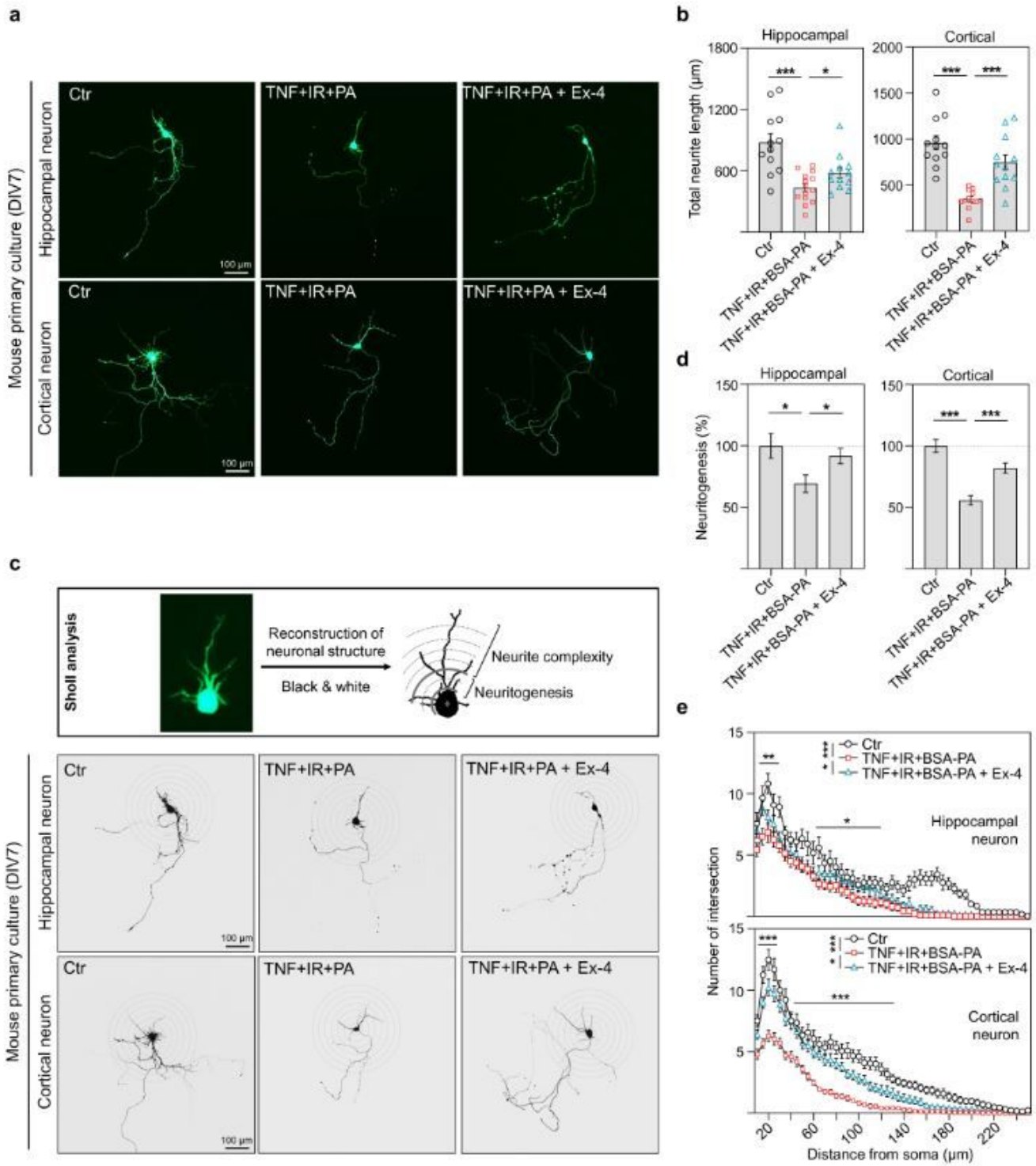

Figure 1

Exendin-4 improved neural complexity in in vitro neurons under metabolic imbalance conditions (a) Representative images (40×) of GFP-positive primary hippocampal and cortical neurons at DIV 7 after the treatment of inducer combination of metabolic imbalances such as TNF-α, insulin, glucose, palmitate, and a therapeutic agent exendin-4. (b) The histogram for total neurite length changes after the treatment of metabolic imbalance factors and exendin-4 in mouse primary hippocampal and cortical neurons at

DIV 7. (c) Representative images of reconstructed primary hippocampal and cortical neurons of panel A. A Sholl analysis was conducted after converting the GFP signal to a black and white reconstruction. The diameter of the first sholl covering the soma was 10 um, and the radius of the sholl increased by 5 um. (d) The histogram for neuritogenesis changes after the treatment of metabolic imbalance factors and exendin-4 in mouse primary hippocampal and cortical neurons at DIV 7. (e) Histogram of the neurite complexity changes after treating metabolic imbalance factors and exendin-4 in mouse primary hippocampal and cortical neurons at DIV 7. Data information: Ctr.: control, TNF: 25 ng/ml TNF-α, IR: insulin resistance (treatment of 100 nM insulin and 4.5 g/L D-glucose), BSA-PA: 50 μM BSA-conjugated palmitate, Ex-4: 10 nM exendin-4. The duration and method of drug treatment are described in the method section. The length of the scale bar is included in the representative images. Data are expressed as the group mean ± standard error of the mean. In (b), (d), and (e), data are expressed from 12 neurons per independent group in triplicate. Statistical analyses examined the relative significance between each group ((b) and (d): unpaired two-tail t-test with Welch's correction; (e) Two-way ANOVA with Bonferroni post-test to compare the replicate means by distance from the soma). n.sP < 0.05, *P < 0.05, **P < 0.01, and ***P < 0.001.

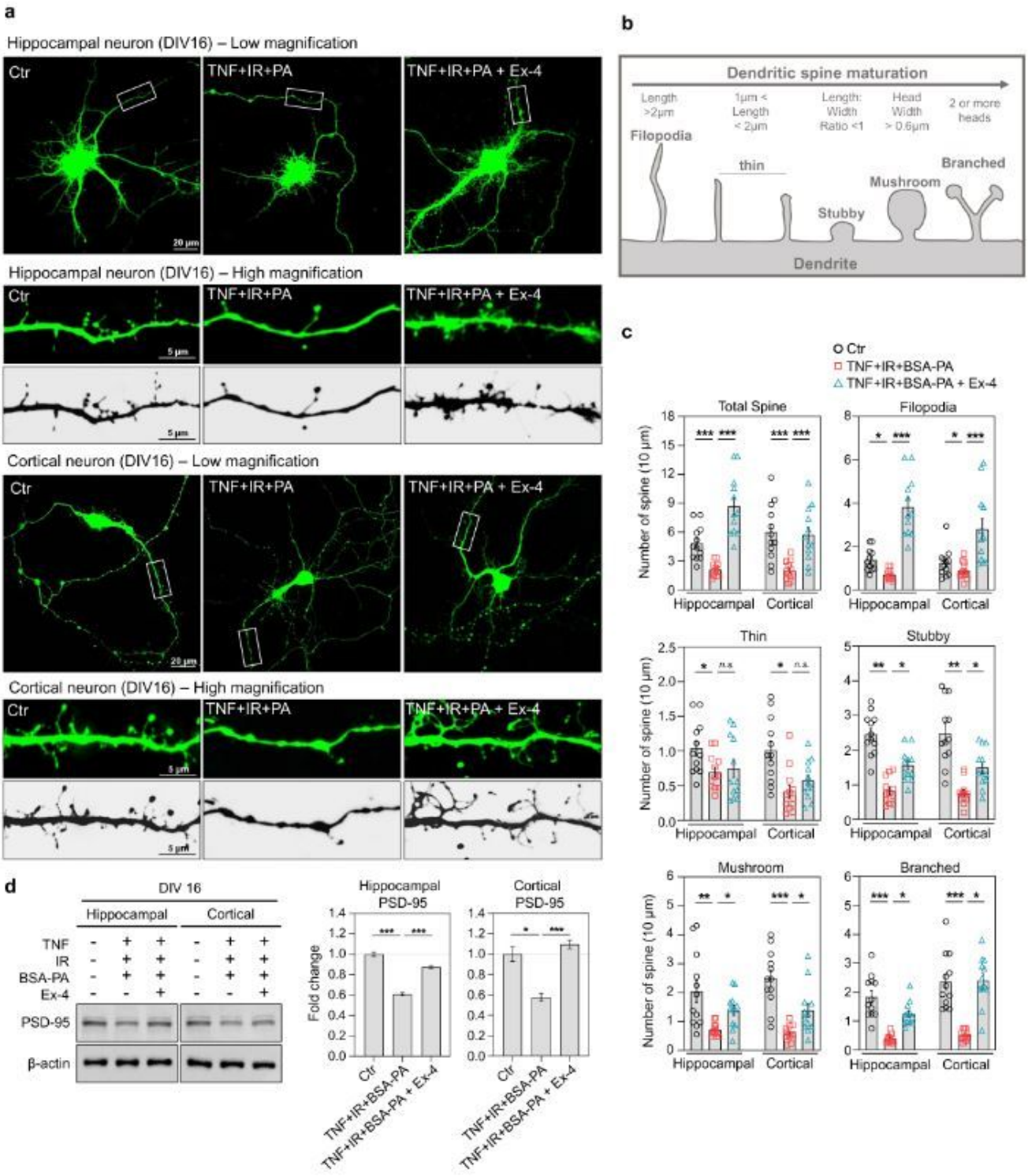

Figure 2

changes the dendritic spine shape and protein dynamics related to synaptic function in neurons under metabolic imbalance conditions (a) Representative images at low- (40×) and high-magnification (120×) of GFP-positive primary hippocampal and cortical neurons at DIV 16 after the treatment of the metabolic imbalance inducing factors such as TNF-α, insulin, glucose, palmitate, and a therapeutic agent exendin-4. The high-magnification images' original location is indicated by the rectangular box outlined in white in

the low magnification images. (b) Schematic drawing for the dendritic spine maturation process. Parameters such as the length and width for the definition of the spine shape are indicated. (c) The histogram for the changes in the shape of the dendritic spine after the treatment of the metabolic imbalance inducing factors and a therapeutic agent exendin-4 into the primary hippocampal and cortical neurons at DIV 16. The number of spine types in the dendrite of 10 um length was counted to represent the mean. (d) The measurement and histogram of the expression of a protein related to synaptic function, PSD-95, after the treatment of the metabolic imbalance inducing factors and a therapeutic agent exendin-4 into the primary hippocampal and cortical neurons at DIV 16. Data information: Ctr.: control, TNF: 25 ng/ml TNF-α, IR: insulin resistance (treatment of 100 nM insulin and 4.5 g/L D-glucose), BSA-PA: 50 μM BSA-conjugated palmitate, Ex-4: 10 nM exendin-4. The duration and method of the drug treatment are described in the Methods section. The length of the scale bar is included in the representative images. Data are expressed as the group mean ± standard error of the mean. In (s), data are expressed from 12 neurons per independent group in triplicate. In (d), data are expressed from three independent experiments in triplicate. Statistical analyses examined the relative significance between each group (unpaired two-tail t-test with Welch's correction). n.s.P > 0.05, *P < 0.05, **P < 0.01, and ***P < 0.001.

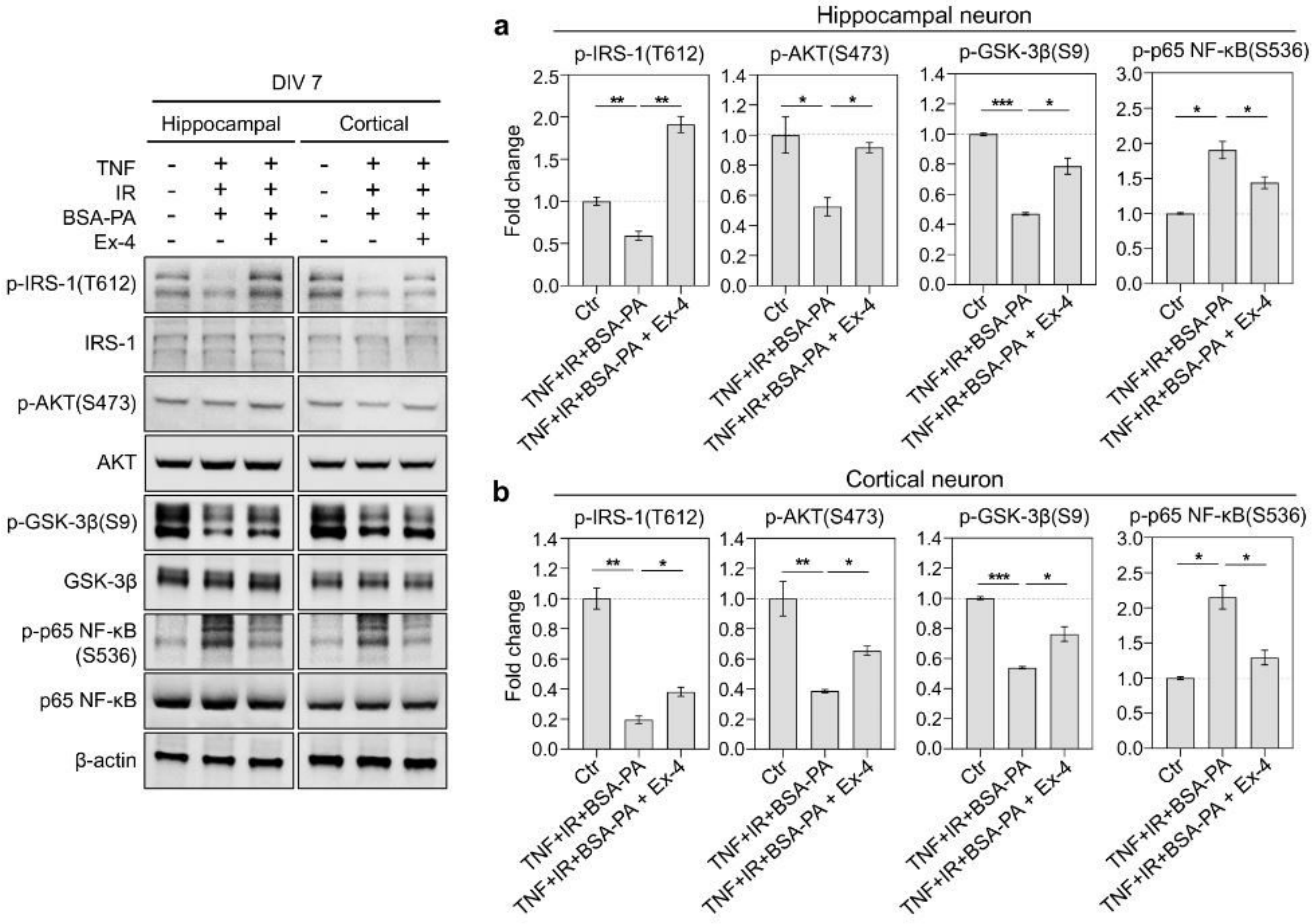

Figure 3

Exendin-4 regulates the phosphorylation of proteins related to insulin signaling and neuroinflammation in in vitro neurons under metabolic imbalance conditions (a) The measurement of phosphorylated and native protein levels after treating an inducer combination of metabolic imbalances such as TNF-α, insulin, glucose, palmitate, and a therapeutic agent exendin-4 into the primary hippocampal neurons of mice at DIV 7. (b) The measurement of phosphorylated and native protein levels after treating an inducer combination of metabolic imbalances such as TNF-α, insulin, glucose, palmitate, and a therapeutic agent exendin-4 into mouse primary cortical neurons at DIV 7. Data information: Cont.: control, TNF: 25 ng/ml TNF-α, IR: insulin resistance (treatment of 100 nM insulin and 4.5 g/L D-glucose), BSA-PA: 50 μM BSA-conjugated palmitate, Ex-4: 10 nM exendin-4. The duration and method of drug treatment are described in the Methods section. Data are expressed as the group mean ± standard error of the mean. Data are expressed from three independent experiments in triplicate. Their native protein normalized a phosphorylated protein, and the corresponding value was expressed as a fold change in the histogram. Statistical analyses examined the relative significance between each group (unpaired two-tail t-test with Welch's correction). *P < 0.05, **P < 0.01, and ***P < 0.001.

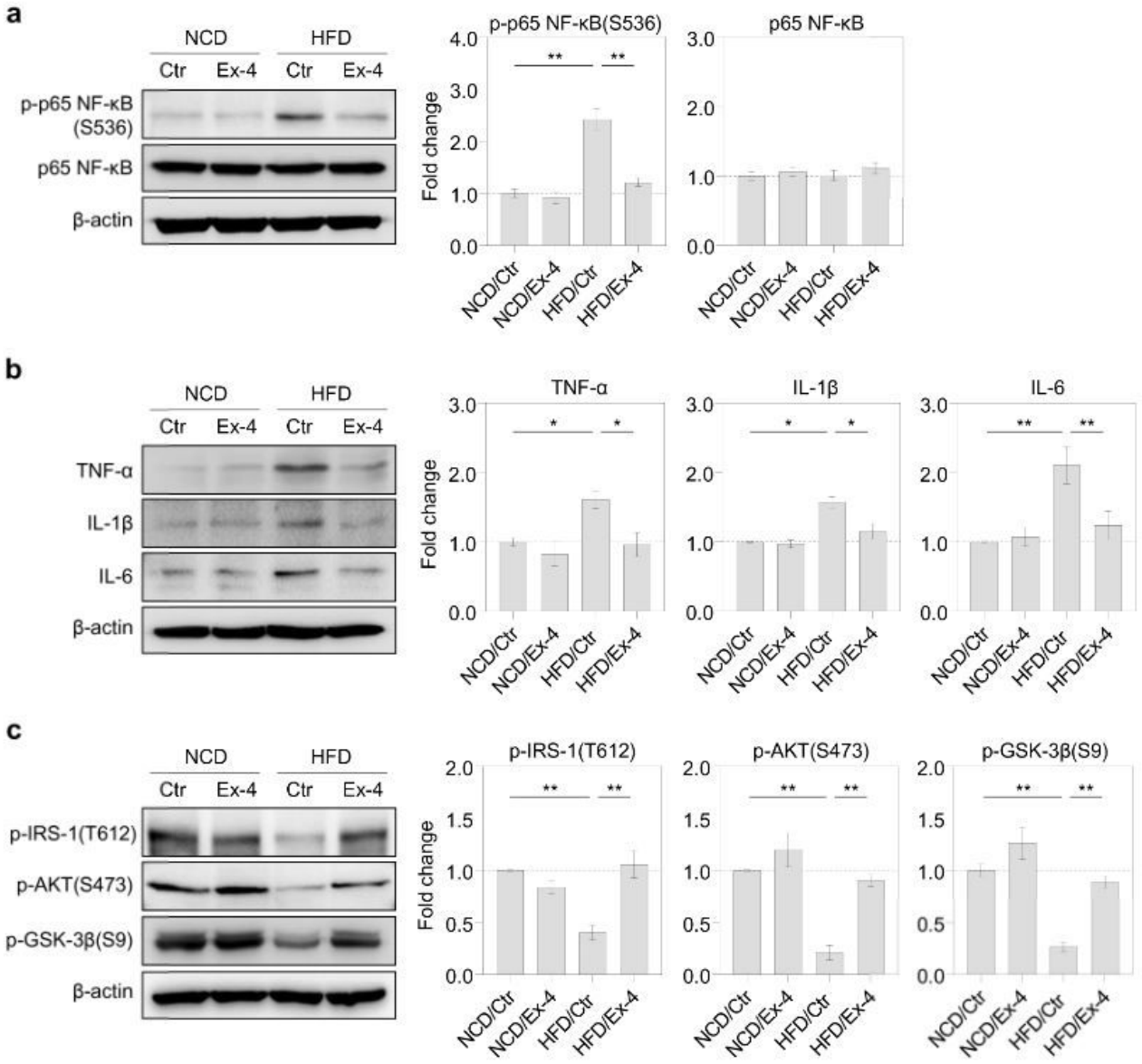

Figure 4

Exendin-4 suppressed neuroinflammation in the HFD mice brain (a) p-p65 NF-κB (S536) and p65 NF-κB protein levels in the hippocampus between the control and exendin-4 groups in either the NCD or HFD-fed mice evaluated by western blot analysis and quantified by densitometric analysis, n=4, equal to the number of animals. (b) TNF-α, IL-1β, and IL-6 protein levels in the hippocampus between the control and exendin-4 groups in either the NCD or HFD-fed mice evaluated by western blot analysis and quantified by densitometric analysis, n=3–4, equal to the number of animals. (c) p-IRS-1 (T612), p-AKT (S473), p-GSK-3β (S9) protein levels in the hippocampus between the control and exendin-4 groups in either NCD or HFD-fed mice evaluated by western blot analysis and quantified by densitometric analysis, n=4, equal to the number of animals. Data information: Ctr.: control; Ex-4: exendin-4; NCD: normal chow diet; HFD: high-fat diet. Data are expressed as the group mean ± standard error of the mean. Data are expressed from

three or four independent experiments in triplicate. Statistical analyses examined the relative significance between each group by ANOVA with post-hoc Tukey HSD Test or Games-Howell Test. Differences were considered significant at *P < 0.05, **P < 0.01.

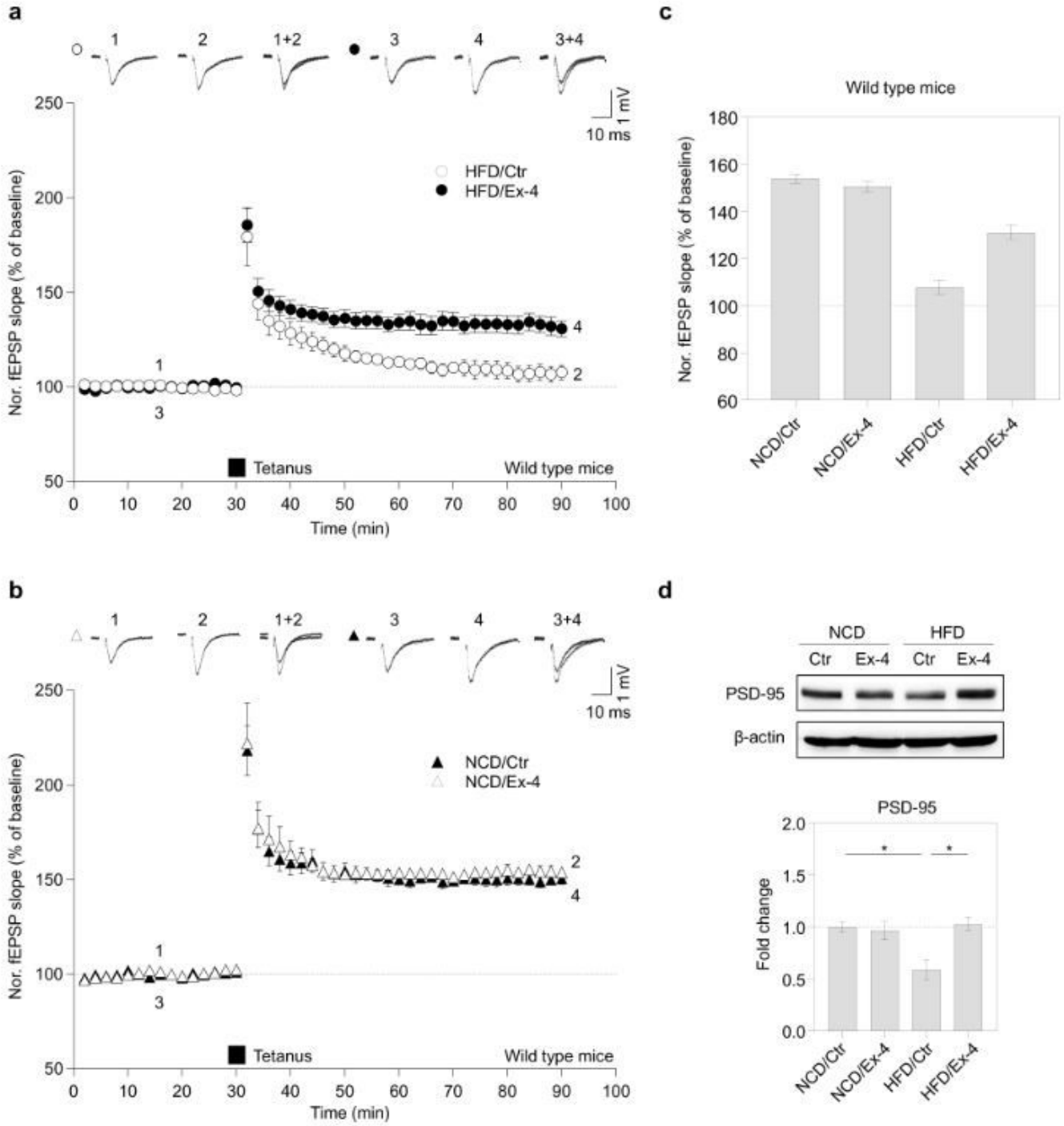

Figure 5

Exendin-4 enhanced LTP in the HFD mice brain Figure 5. Exendin-4 enhanced 810 LTP in the HFD mice brain (a) HFD-fed wild-type mouse hippocampal LTP was assessed in acute slices of the control group

(open circle) and exendin-4 injected group (closed circle), n=10 per group from 10 animals. (b) NCD-fed wild-type mouse hippocampal LTP was assessed in acute slices of the control group (open circle) and exendin-4 injected group (closed circle), n=10 per group from 10 animals. (c) Bar graphs of the sEPSPs slope after tetanus stimulation between the control and exendin-4 groups in either the NCD or HFD-fed mice hippocampus. (d) PSD-95 protein levels in the hippocampus between the control and exendin-4 groups in either NCD or HFD-fed mice evaluated by western blot analysis and quantified by densitometric analysis, n=4, equal to the number of animals. Data information: Ctr.: control; Ex-4: exendin-4; NCD: normal chow diet; HFD: high-fat diet. All data are expressed as the group mean ± standard error of the mean. Data from western blotting are expressed from four independent experiments in triplicate. Statistical analyses examined the relative significance between each group by ANOVA with post-hoc Tukey HSD Test. Differences were considered significant at *P < 0.05.

Supplementary Files

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- 20201120SupplementaryinformationfulllengthblotsFinal.pdf