Shen et al., https://doi.org/10.1083/jcb.201605046

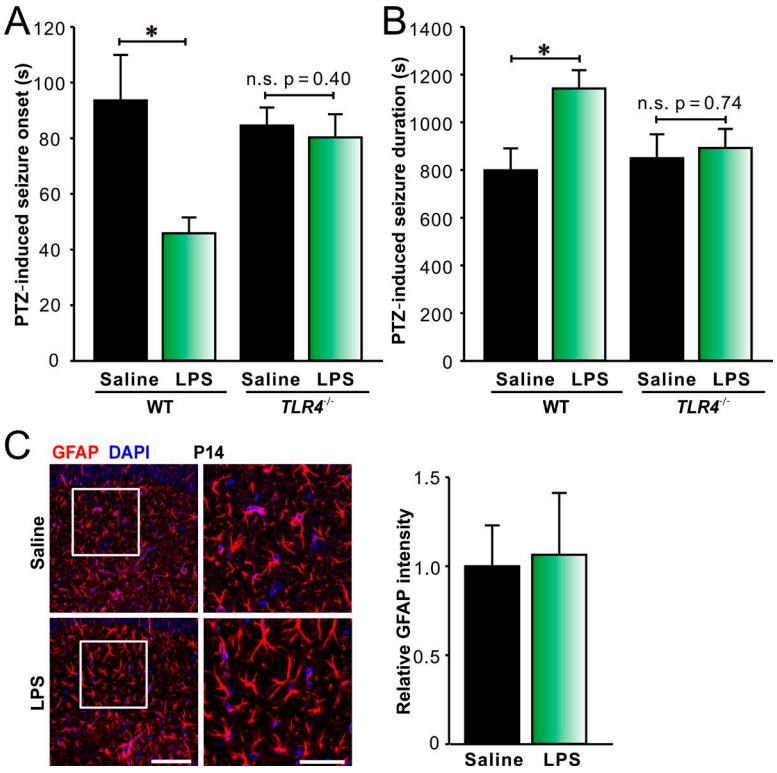


Figure S1. **The effects of LPS on PTZ-induced seizure susceptibility and astrocyte activation are attenuated in *TLR4*$^{-/-}$ mice during a critical developmental period.** Histograms showing LPS challenges on PTZ-induced seizure onset (A) and duration (B) in *TLR4*$^{-/-}$ and WT littermate mice. $n = 7$ and 10 for WT and *TLR4*$^{-/-}$ groups, respectively. (C) Representative images of immunohistostaining of GFAP (red) and DAPI (blue) in hippocampal CA1 area of 2-wk-old *TLR4*$^{-/-}$ mice treated with saline or LPS (left) and quantification of relative intensity (right) of GFAP. Bars: (left) 100 µm; (right) 50 µm. $n = 10$ for each group. *, $P < 0.05$ (*t* test). Data are mean ± SEM.

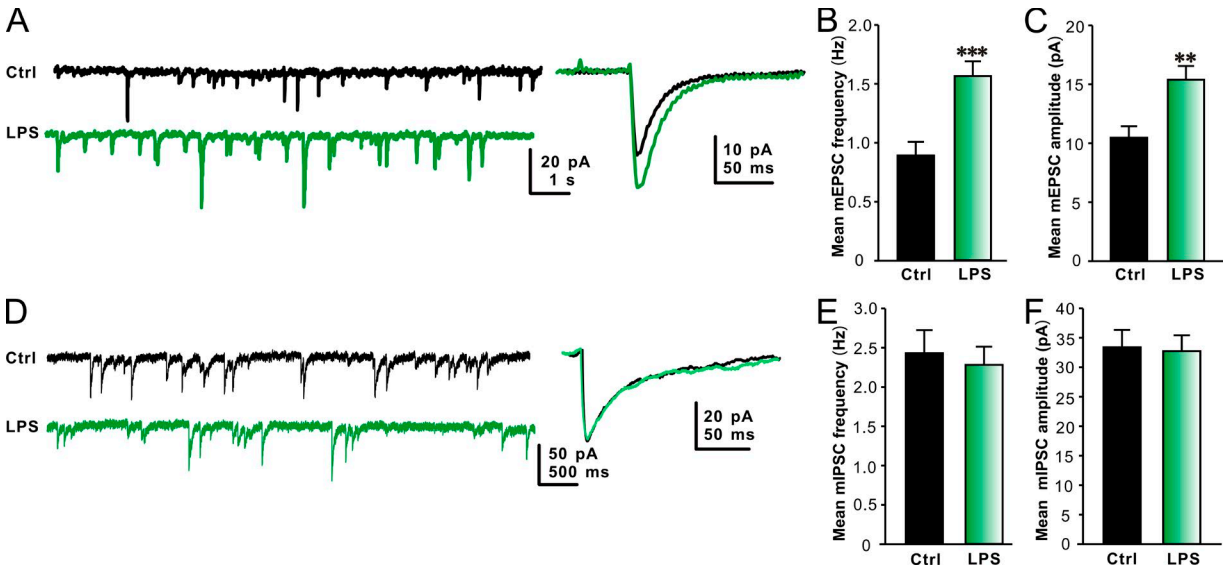


Figure S2. **Effect of LPS on mEPSCs and mIPSCs in hippocampal neurons co-cultured with astrocytes.** LPS was added at DIV13 to -14, and recordings were done at DIV15. (A) Representative traces of mEPSCs recorded from neruons in co-cultures. Histograms showing LPS increased mEPSC frequency (B) and amplitude (C) in co-cultured neurons. *n* = 10 for each group. (D) Representative traces of mIPSCs recorded from neurons in co-cultures. Histograms showing LPS did not change mIPSC frequency (E) and amplitude (F) in neurons co-cultured with astrocytes. *n* = 9 for each group. **, P < 0.01; ***, P < 0.001 (*t* test). Data are mean ± SEM.

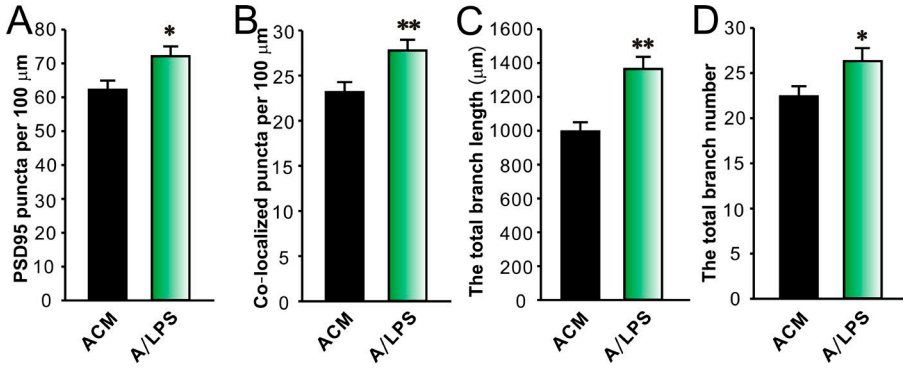


Figure S3. **A/LPS promotes synaptogenesis and dendritic branching in cultured hippocampal neurons.** Neurons at DIV4 to -5 were cultured with ACM. Half of the medium was replaced at DIV8 with fresh ACM. At DIV13, half of the medium was replaced with A/LPS or ACM. Immunocytostainings were done at DIV15. Histograms showing A/LPS increased PSD95 puncta (A) and PSD95/synaptophysin colocalized puncta (B) in cultured neurons. Histograms showing A/LPS increased the total length (C) and number (D) of dendritic branches. *n* = 30 for each group. *, P < 0.05; **, P < 0.01 (*t* test). Data are mean ± SEM.

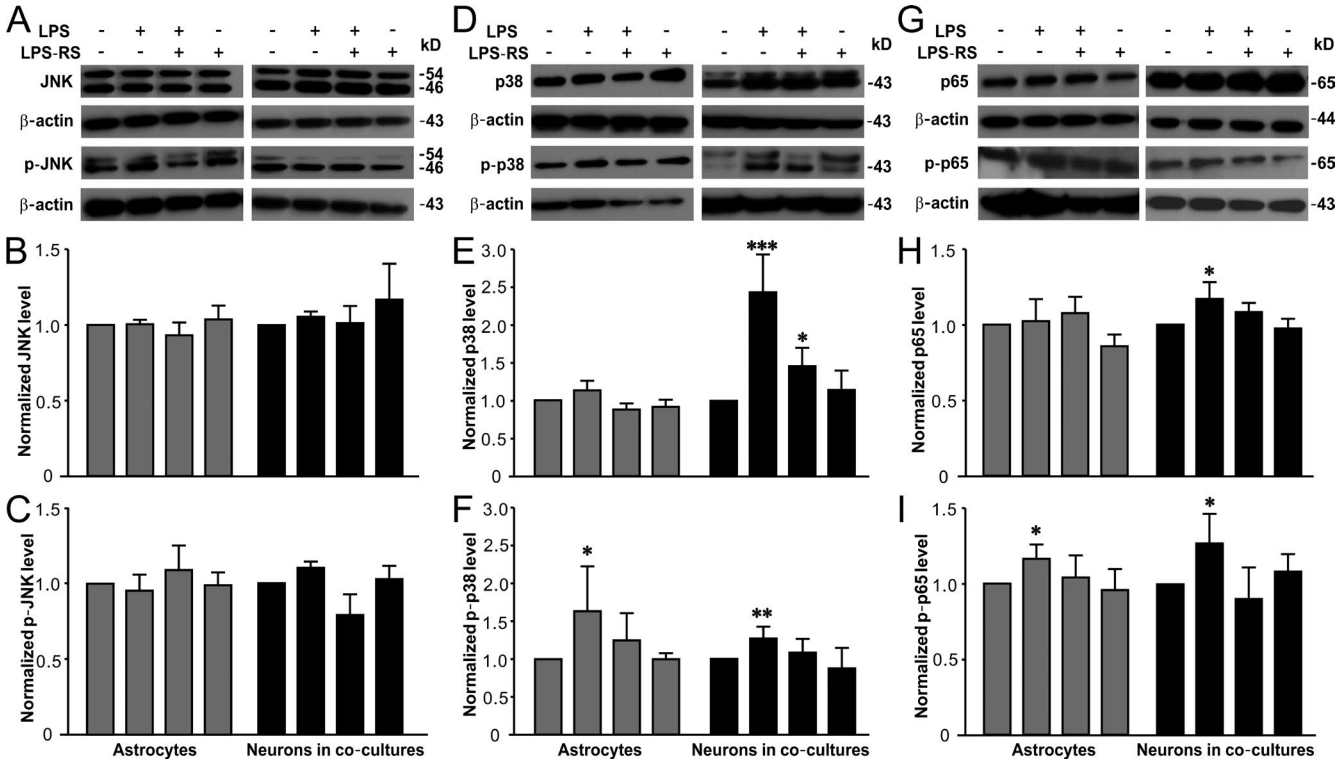


Figure S4. **Effect of LPS on MAPK and NF-κB expressions in cultured astrocytes and neurons co-cultured with astrocytes.** Representative Western blots of JNK/p-JNK (A) and quantification of relative expression of JNK (B) and p-JNK (C) showing LPS did not alter JNK and p-JNK levels in astrocytes and neurons. *n* = 3 for each group. Representative Western blots of p38/p-p38 (D) and quantification of relative expression of p38 (E) and p-p38 (F) showing LPS increased p-p38 but not p38 expression in astrocytes and increased both p38 and p-p38 in neurons. *n* = 5 for each group. Representative Western blots of p65/p-p65 (G) and quantification of relative expression of p65 (H) and p-p65 (I) showing LPS increased p-p65 but not p65 expression in astrocytes and increased both p65 and p-p65 in neurons (H and I). *n* = 4 for each group. *, P < 0.05; **, P < 0.01; ***, P < 0.001 (one-way ANOVA). Data are mean ± SEM.

Table S1. **Effect of LPS on synaptogenesis and dendritic branching in WT astrocyte co-cultured hippocampal neurons (DIV15) derived from *TLR4*$^{-/-}$ and their WT and *TLR4*$^{+/-}$ littermate mice**

| Mouse | PSD95 puncta | PSD95/synaptophysin colocalized puncta | Total length of dendrites | Total number of branching |
|---|---|---|---|---|
| | | | *μm* | |
| WT neuron-C | 59.4 ± 3.5 | 23.8 ± 2.4 | 1,066.5 ± 66.3 | 19.4 ± 1.2 |
| WT neuron-L | 75.2 ± 5.8[a] | 33.1 ± 2.9[a] | 1,467.1 ± 97.5[b] | 23.3 ± 1.1[a] |
| *TLR4*$^{+/-}$ neuron-C | 55.9 ± 4.2 | 26.7 ± 3.7 | 1,107.5 ± 76.3 | 18.3 ± 1.3 |
| *TLR4*$^{+/-}$ neuron-L | 68.4 ± 3.7[a] | 38.3 ± 3.8[a] | 1,505.6 ± 132.7[a] | 23.1 ± 1.2[b] |
| *TLR4*$^{-/-}$ neuron-C | 56.7 ± 2.8 | 23.4 ± 2.4 | 1,044.5 ± 109.0 | 18.0 ± 1.6 |
| *TLR4*$^{-/-}$ neuron-L | 65.7 ± 2.9[a] | 33.7 ± 3.2[a] | 1,073.7 ± 102.3[b] | 23.5 ± 1.6[a] |

The number of puncta was counted per 100-μm length of dendrites. *n* = 30 for each group. Data are mean ± SEM. C, control; L, LPS.
[a]P < 0.05 (*t* test).
[b]P < 0.01 (*t* test).