Supplementary material

"Ambiguous contribution of glucocorticosteroids to acute neuroinflammation in the hippocampus of rat" by Liya V. Tret'yakova, Alexey A. Kvichansky, Ekaterina S. Barkovskaya, Anna O. Manolova, Alexey P. Bolshakov, and Natalia V. Gulyaeva

Table S1. Nucleotide sequences of primers used

Gene	Forward primer	Reverse primer
Hprt NM_012583.2	CGT CGT GAT TAG TGA TGATGA AC	CAA GTC TTT CAG TCC TGT CCA TA
Ywhaz NM_013011.4	TTG AGC AGA AGA CGG AAG GT	GAA GCA TTG GGG ATC AAG AA
Il1b NM_031512.2	TCT GTG ACT CGT GGG ATG AT	CAC TTG TTG GCT TAT GTT CTG TC
Il6 NM_012589.2	GCC ACT GCC TTC CCT ACT TCA C	GAC AGT GCA TCA TCG CTG TTC ATA C
Tnf NM_012675.3	GTCCAACTCCGGGCTCAGAAT	ACT CCC CCG ATC CAC TCA G
Cx3cl1 NM_134455.2	ATC ACC ACC ATC ACC ACC AAC	GAG GAA CAC TTT AAA CCC TCA CAG A
Cx3cr1 NM_133534.2	GGA CCT CAC CAT GCC TAC CT	CAC CAA CAG ATT CCC CAC CAG
Ccl2 NM_031530.1	GTC GGC TGG AGA ACT ACA AGA G	GGG TCA AGT TCA CAT TCA AAG G
Tgfb1 NM_021578.2	GCG CCT GCA GAG ATT CAA GTC AAC	TCA GGC GTA TCA GTG GGG GTC A
Ncf1 NM_053734.2	CTG CAG CAA AGG ACA GGA CTG	GGG TCA TGG CCA ACA GGT T

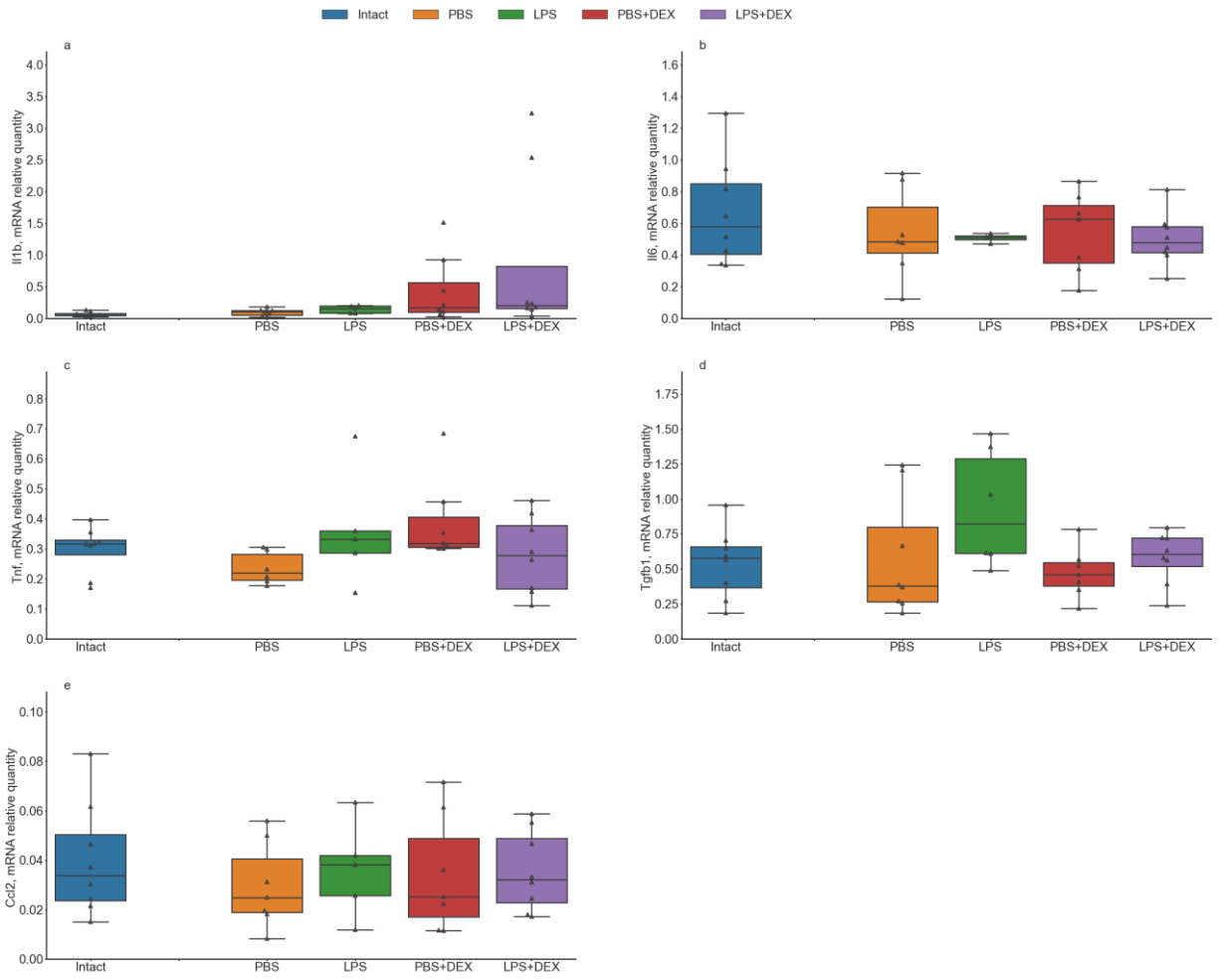


Figure S1. mRNA expression levels of neuroinflammation-associated genes *Il1b* (a), *Il6* (b), *Tnf* (c), *Tgfb1* (d), and *Ccl2* (e) in VH after intrahippocampal injection of PBS, LPS, DEX, or DEX + LPS. n=8.

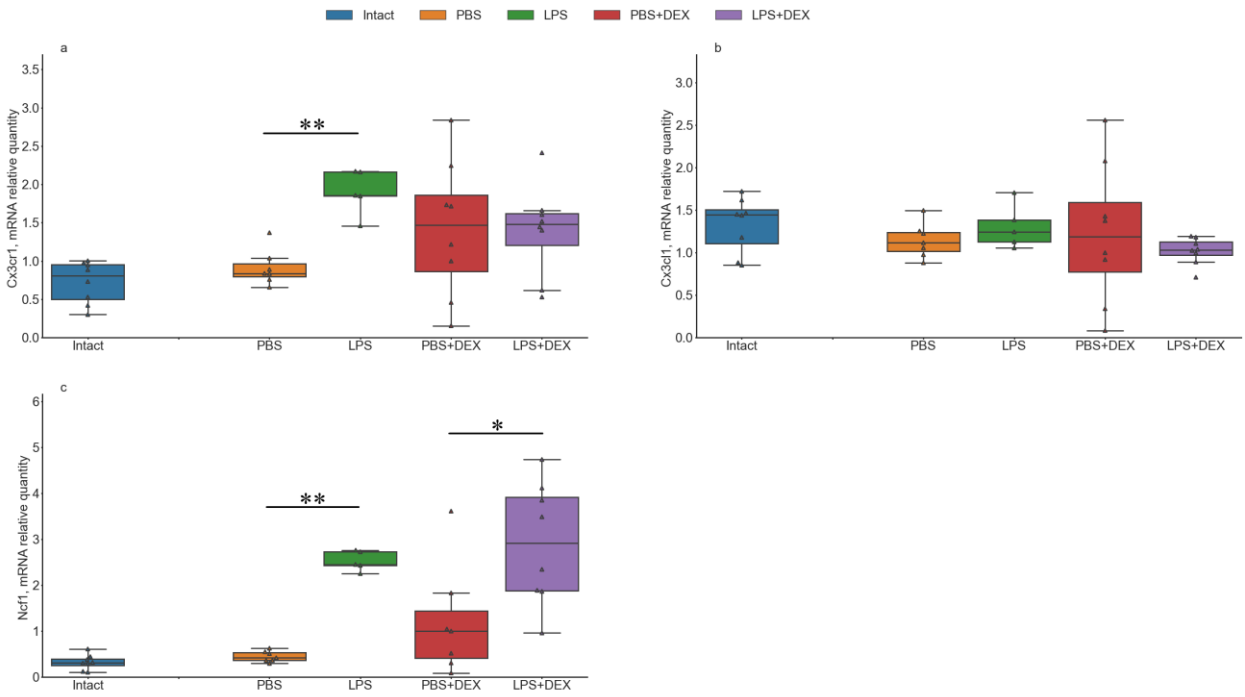


Figure S2. mRNA expression levels of microglial markers genes *Cx3cr1* (a), *Cx3cl1* (b), and *Ncf1* (c) in DH after intrahippocampal injection of PBS, LPS, DEX, or DEX + LPS. n=8. *Cx3cr1,* KW: H=8.0011, p=0.046; *Ncf1,* KW: H=15.4927, p=0.0014. * and **, a trend (0.01≤ p≤0.05) and significant (p<0.01) differences, respectively, according to Mann-Whitney U-test with multiple comparison correction.

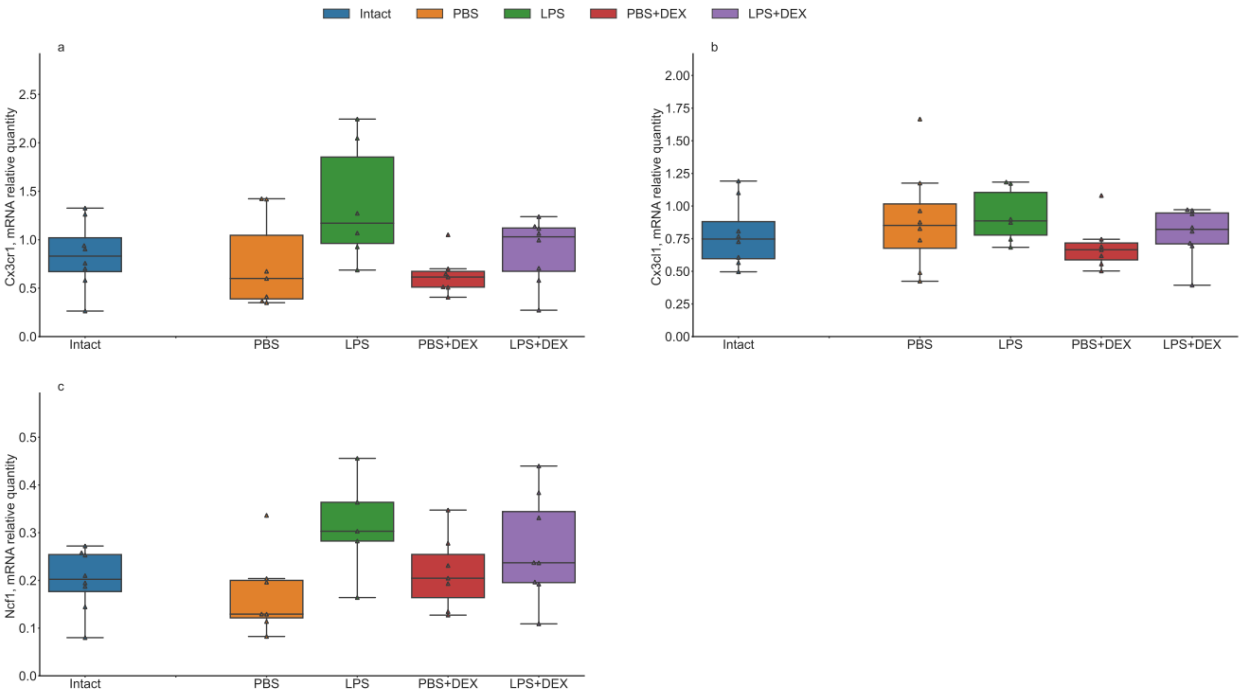


Figure S3. mRNA expression levels of microglial markers genes *Cx3cr1* (a), *Cx3cl1* (b), and *Ncf1* (c) in VH after intrahippocampal injection of PBS, LPS, DEX, or DEX + LPS. n=8.

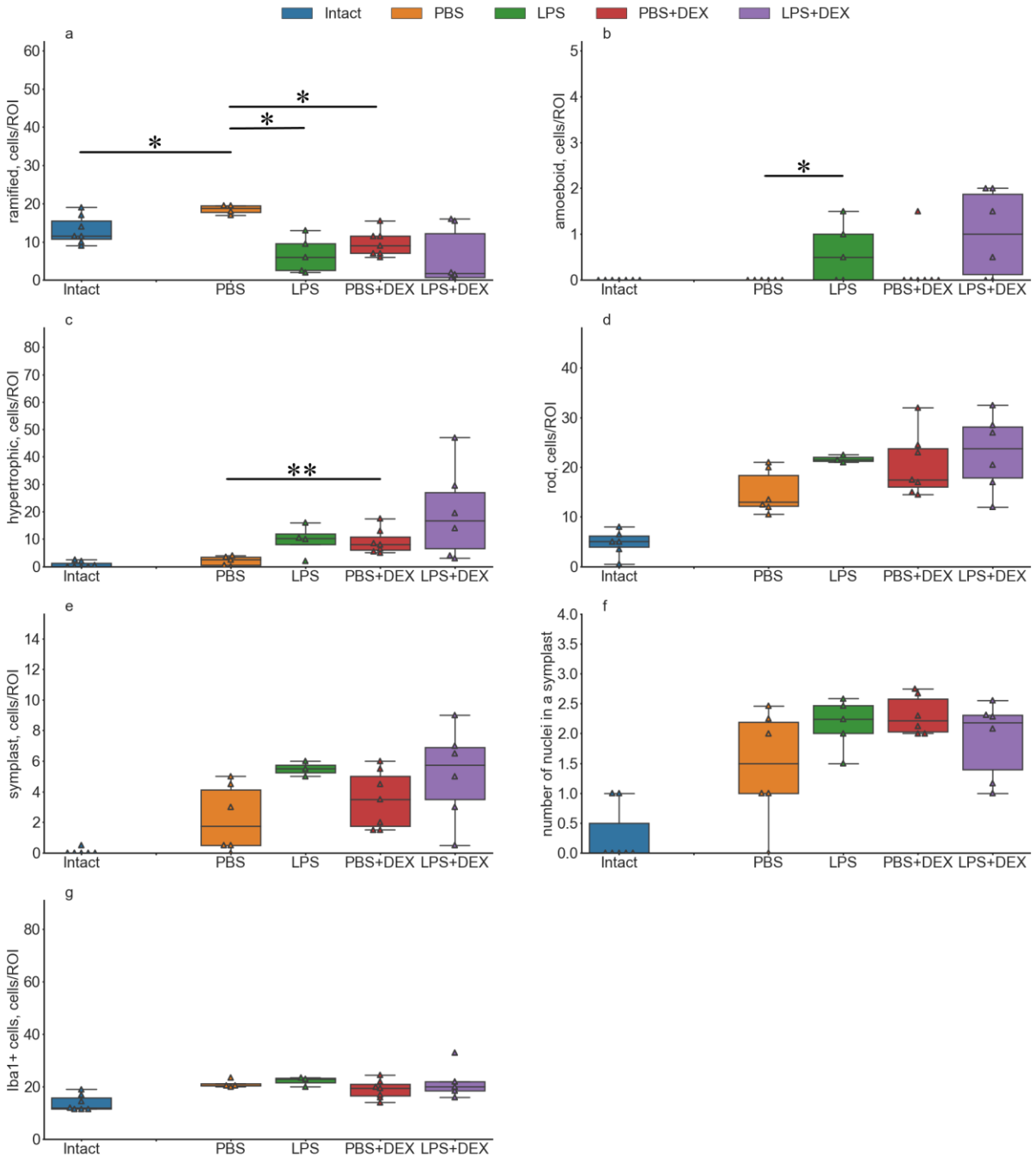


Figure S4. The number of Iba1+ cells of different subtypes in DH after intrahippocampal injection of PBS, LPS, DEX, or DEX + LPS. a, ramified cells, KW: H=10.7388, p=0.0132; b, amoeboid cells, KW: H=8.1273, p=0.0435; c, hypertrophic cells, KW: H=9.0818, p=0.0282; d, rod cells; e, symplasts; f, mean number of nuclei in a symplast; g, number of Iba1+ cells in ROI (region of interest). n=7. * and **, a trend ($0.01 \leq p \leq 0.05$) and significant ($p<0.01$) differences, respectively, according to Mann-Whitney U-test with multiple comparison correction.

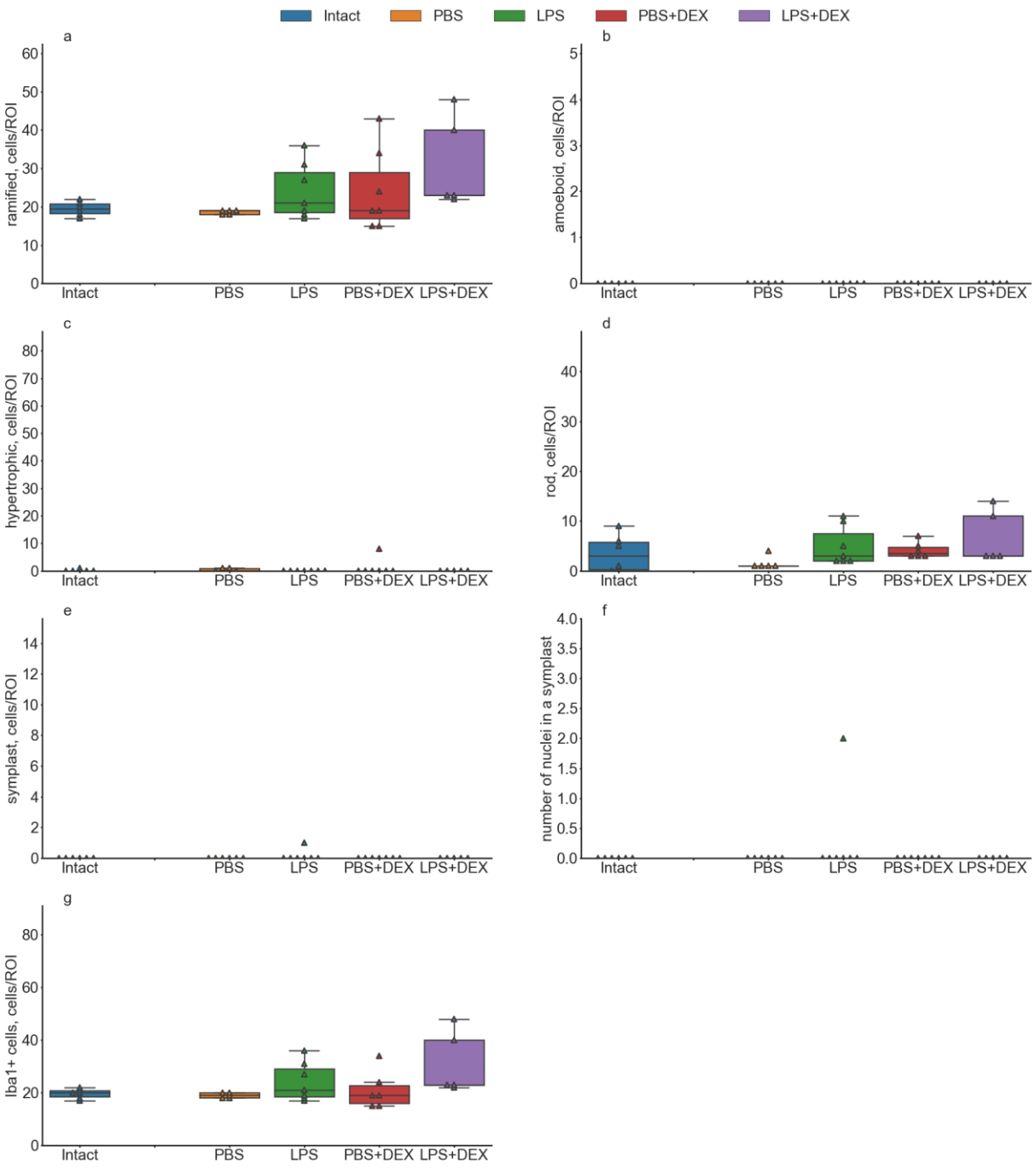


Figure S5. The number of Iba1+ cells of different subtypes in VH after intrahippocampal injection of PBS, LPS, DEX, or DEX + LPS. a, ramified cells; b, amoeboid cells; c, hypertrophic cells; d, rod cells; e symplasts; f, mean number of nuclei in a symplast; g, number of Iba1+ cells in ROI (region of interest). n=7.

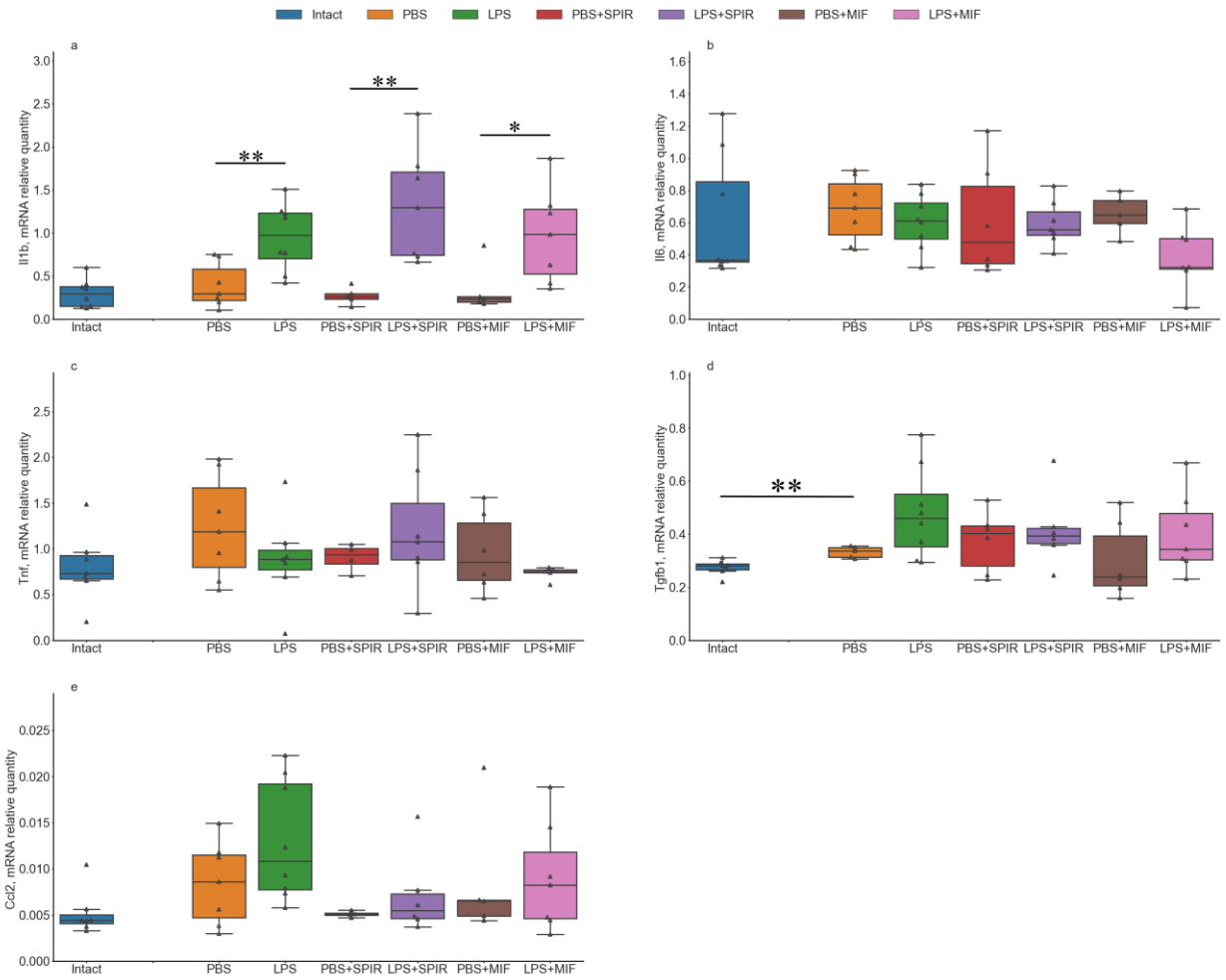


Figure S6. mRNA expression levels of neuroinflammation-associated genes *Il1b* (a), *Il6* (b), *Tnf* (c), *Tgfb1* (d), and *Ccl2* (e) in VH after intrahippocampal injection of PBS, LPS, SPIR, SPIR + LPS, MIF, or MIF + LPS. Intact and LPS groups, n=8; PBS, SPIR + LPS, and MIF + LPS groups, n=7; SPIR and MIF groups, n=6. *Il1b*, KW: H=21.2515, p=0.0007. * and **, a trend (0.00625≤ p≤0.05) and significant (p<0.00625) differences, respectively, according to Mann-Whitney U-test with multiple comparison correction.

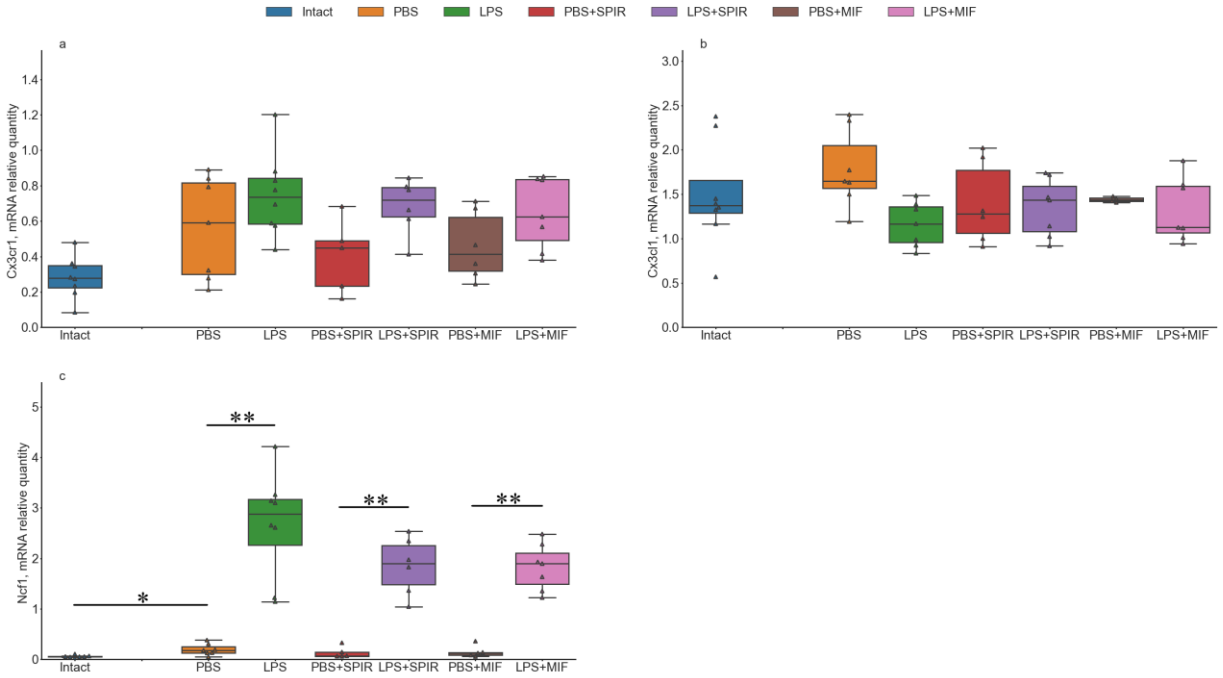


Figure S7. mRNA expression levels of microglial markers genes *Cx3cr1* (a), *Cx3cl1* (b), and *Ncf1* (c) in DH after intrahippocampal injection of PBS, LPS, SPIR, SPIR + LPS, MIF, or MIF + LPS. Intact and LPS groups, n=8; PBS, SPIR + LPS, and MIF + LPS groups, n=7; SPIR and MIF groups, n=6. *Ncf1*, KW: H=28.9908, p=0.00002. * and **, a trend (0.00625≤ p≤0.05) and significant (p<0.00625) differences, respectively, according to Mann-Whitney U-test with multiple comparison correction.

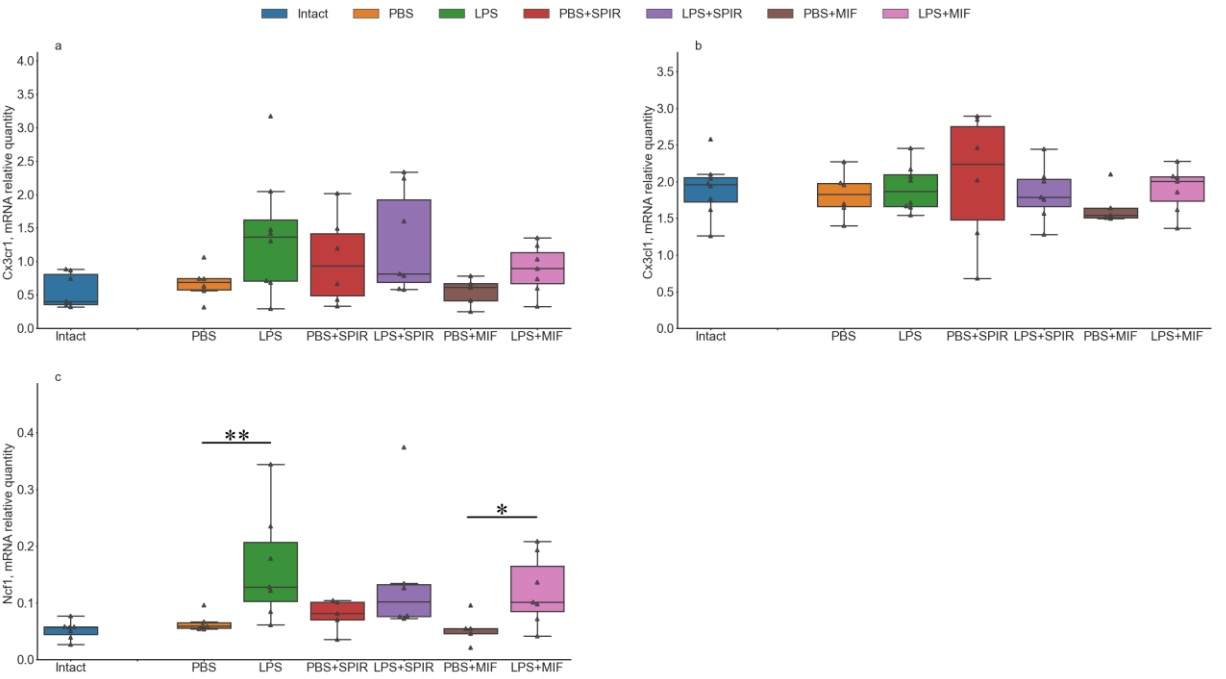


Figure S8. mRNA expression levels of microglial markers genes *Cx3cr1* (a), *Cx3cl1* (b), and *Ncf1* (c) in VH after intrahippocampal injection of PBS, LPS, SPIR, SPIR + LPS, MIF, or MIF + LPS. Intact and LPS groups, n=8; PBS, SPIR + LPS, and MIF + LPS groups, n=7; SPIR and MIF groups, n=6. *Ncf1*, KW: H=14.7948, p=0.0113. * and **, a trend (0.00625≤ p≤0.05) and significant (p<0.00625) differences, respectively, according to Mann-Whitney U-test with multiple comparison correction.

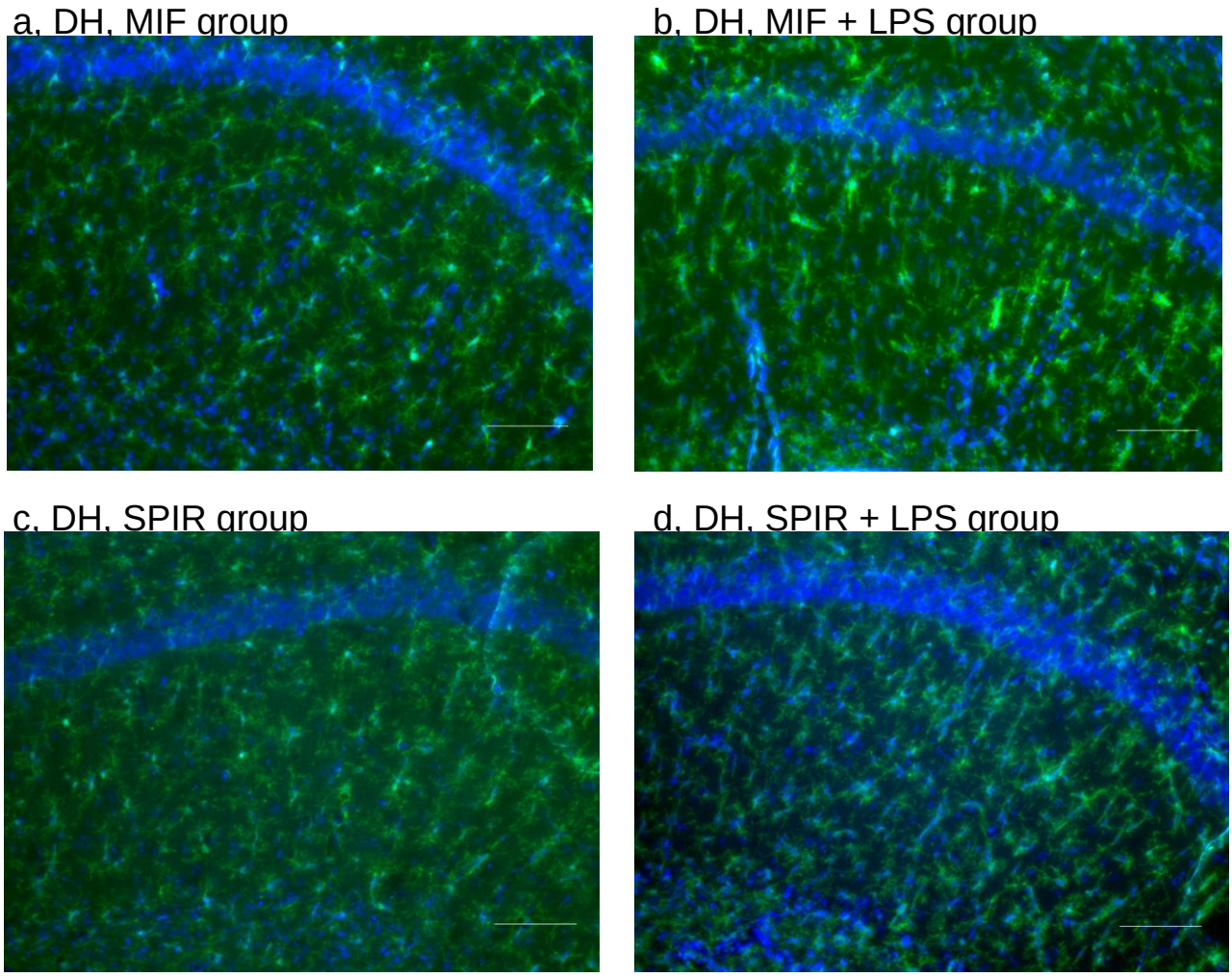


Figure S9. Microglial activation in the DH, representative microphotographs. a, DH MIF group; b, DH MIF + LPS group; c, DH SPIR group; d, DH SPIR + LPS group. Staining with anti-Iba1 and DAPI, x20. Scale bar 100 μm.

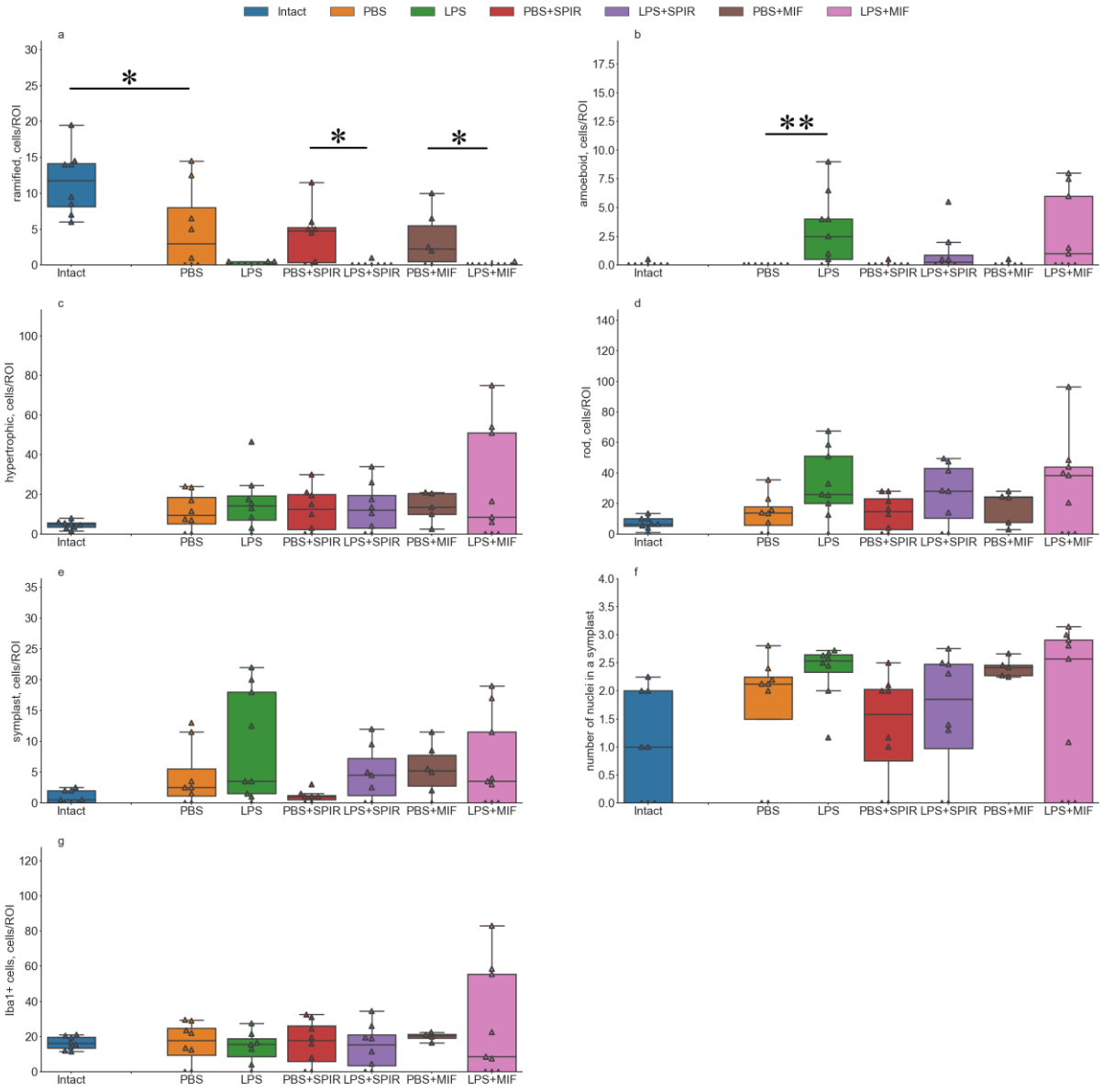


Figure S10. The number of Iba1+ cells of different subtypes in DH after intrahippocampal injection of PBS, LPS, SPIR, SPIR + LPS, MIF, or MIF + LPS. a, ramified cells, KW: H=16.6904, p=0.0051; b, amoeboid cells, KW: H=17.587, p=0.0035; c, hypertrophic cells; d, rod cells; e, symplasts; f, mean number of nuclei in a symplast; g, number of Iba1+ cells in ROI (region of interest). Intact and LPS groups, n=8; PBS, SPIR + LPS, and MIF + LPS groups, n=7; SPIR and MIF groups, n=6. * and **, a trend ($0.00625 \leq p \leq 0.05$) and significant ($p<0.00625$) differences, respectively, according to Mann-Whitney U-test with multiple comparison correction.

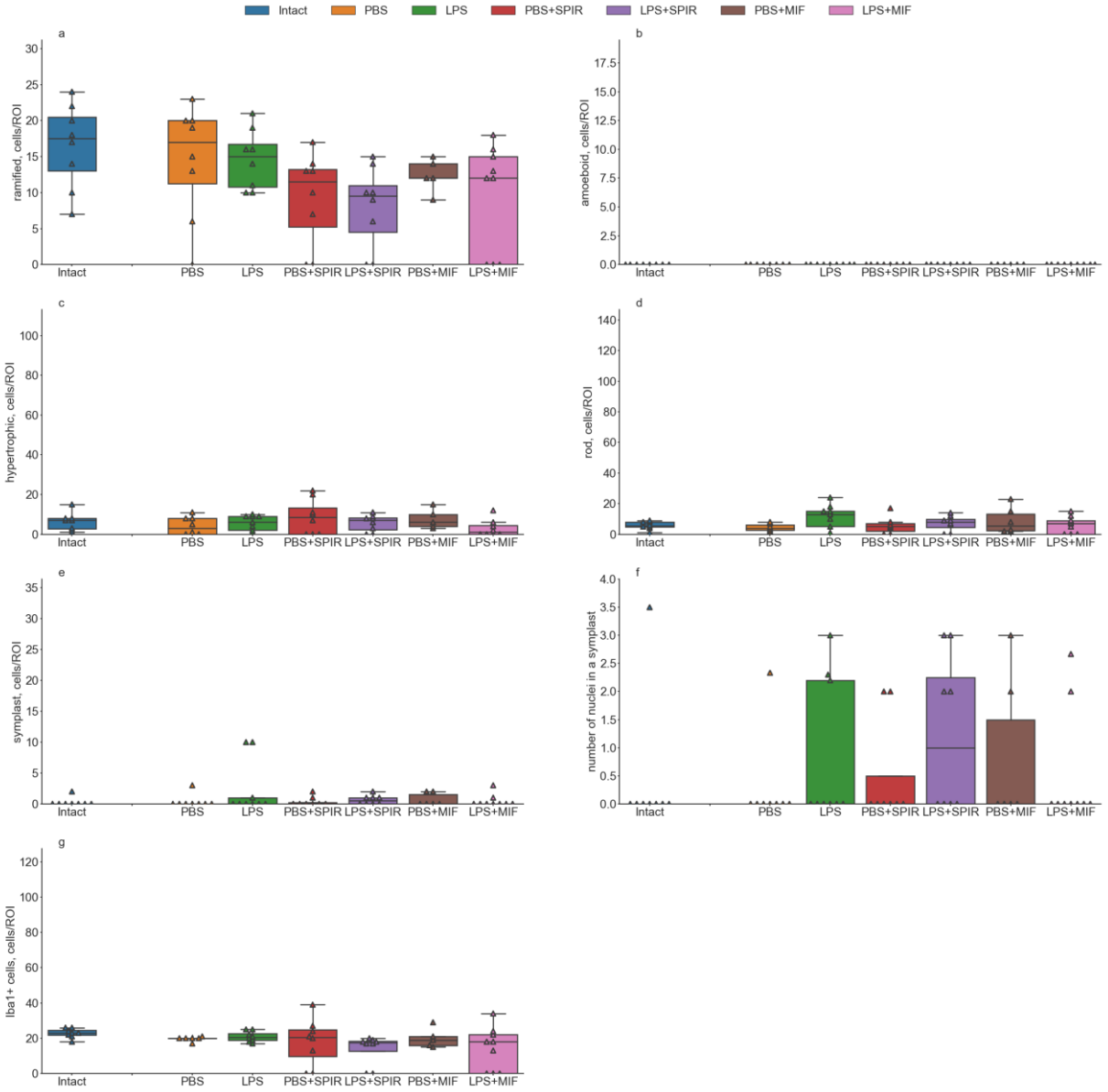


Figure S11. The number of Iba1+ cells of different subtypes in VH after intrahippocampal injection of PBS, LPS, SPIR, SPIR + LPS, MIF, or MIF + LPS. a, ramified cells; b, amoeboid cells; c, hypertrophic cells; d, rod cells; e symplasts; f, mean number of nuclei in a symplast; g, number of Iba1+ cells in ROI (region of interest). Intact and LPS groups, n=8; PBS, SPIR + LPS, and MIF + LPS groups, n=7; SPIR and MIF groups, n=6.

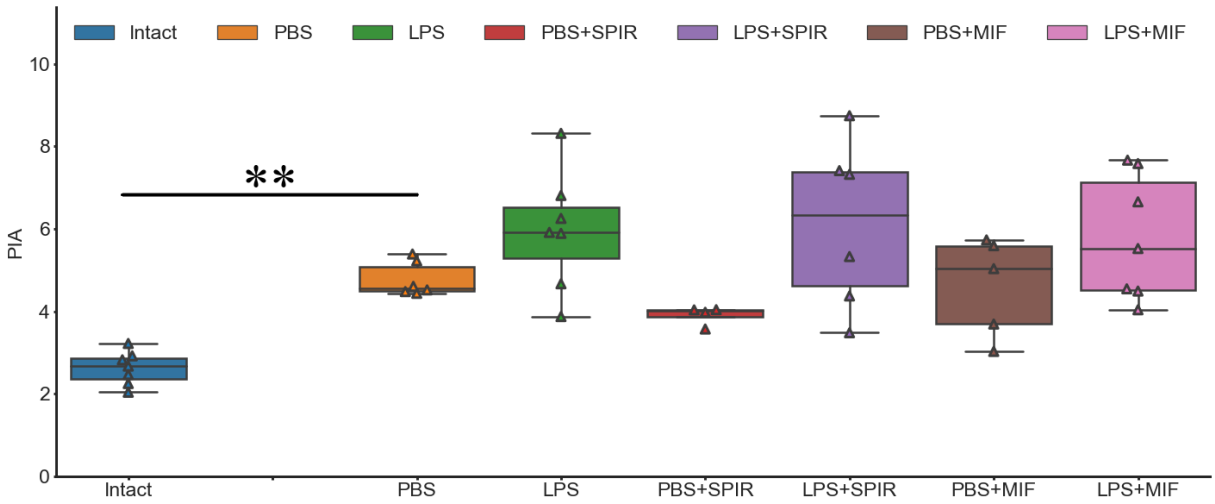


Figure S12. The percentage of Iba1-positively immunostained area (PIA) in DH after intrahippocampal injection of PBS, LPS, SPIR, SPIR+LPS, MIF, or MIF+LPS. Intact and LPS groups, n=8; PBS, SPIR+LPS, and MIF+LPS groups, n=7; SPIR and MIF groups, n=6. **, significant ($p<0.00625$) differences, respectively, according to Mann-Whitney U-test with multiple comparison correction.

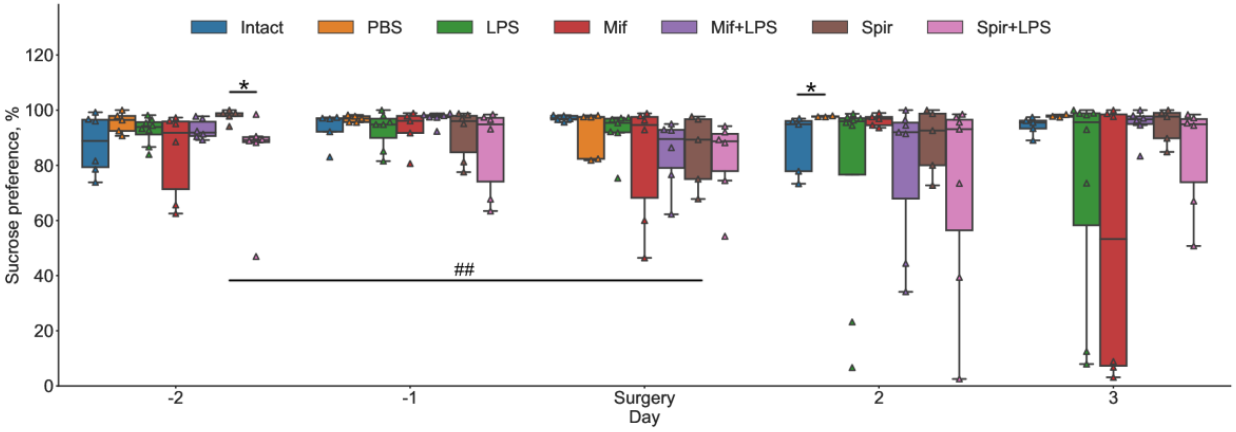


Figure S13. Changes in sucrose consumption of individual animals before and after intrahippocampal injection of PBS, LPS, SPIR, SPIR + LPS, MIF, or MIF + LPS. Intact and LPS groups, n=8; PBS, SPIR + LPS, and MIF + LPS groups, n=7; SPIR and MIF groups, n=6. *, differences at the trend level ($0.00625 \leq p \leq 0.05$) between groups in one day, according to Mann-Whitney U-test with multiple comparison correction. ##, significant ($p<0.00625$) differences between animals within one group in the certain day, according to the Friedman test with Nemenyi post hoc test.