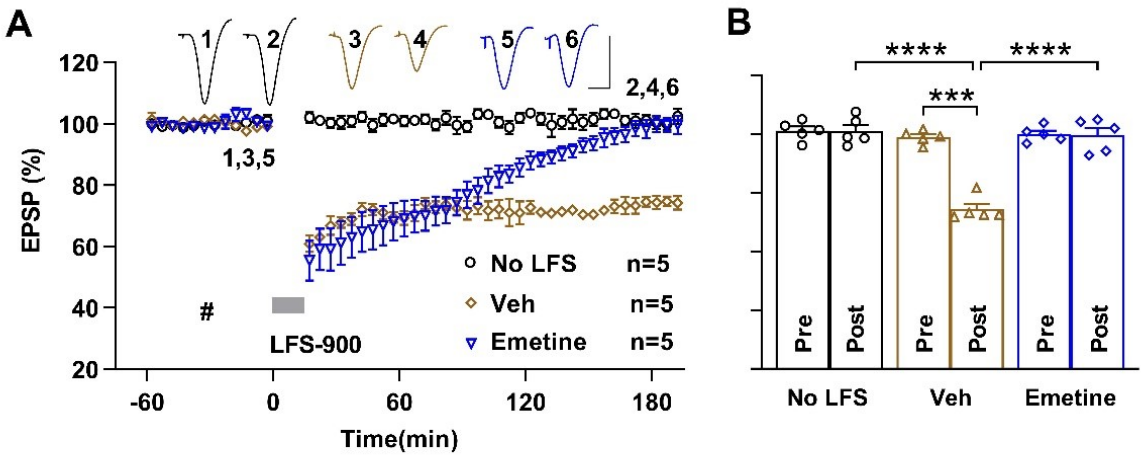
Supplementary Material

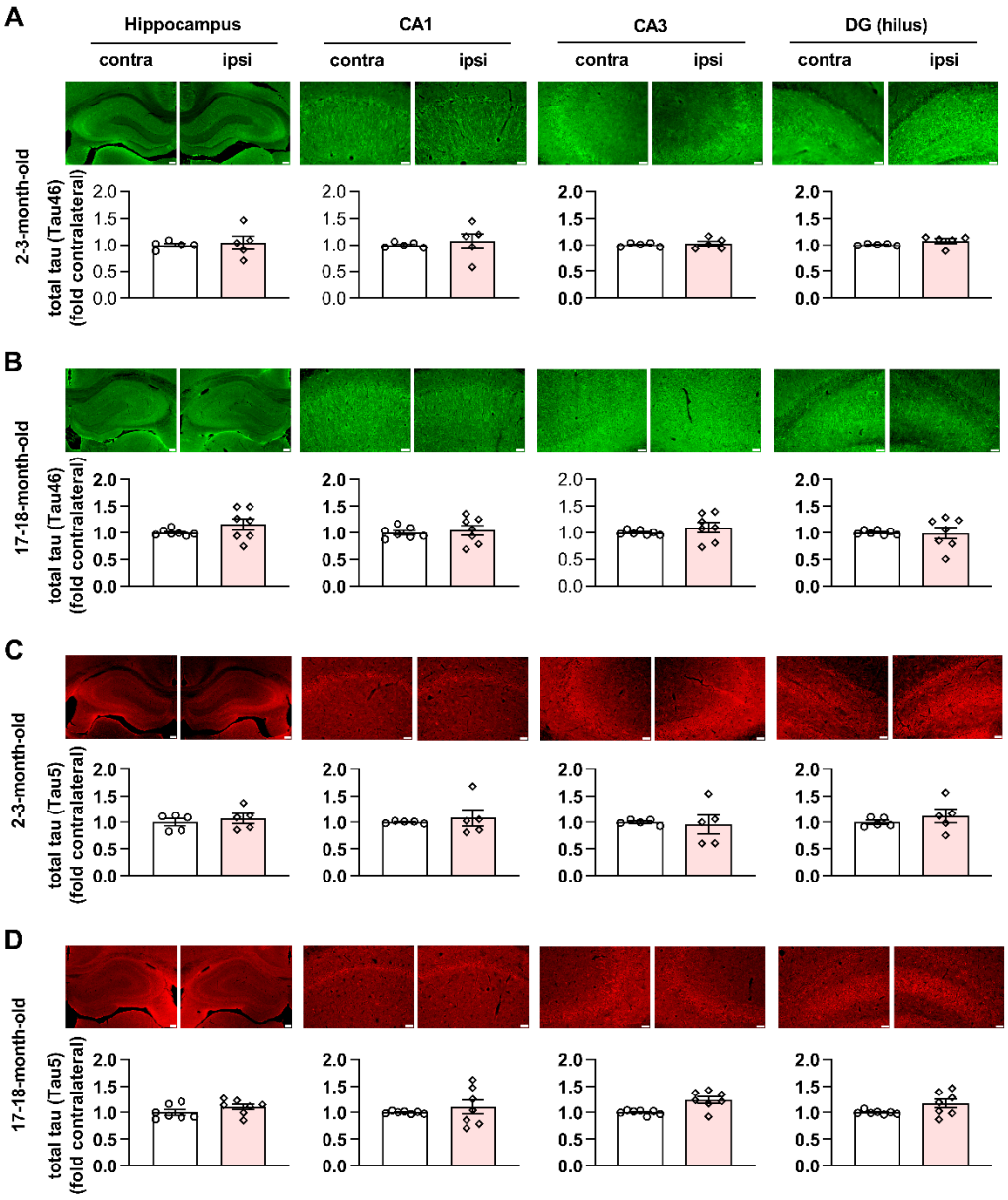
Long-Term Depression-Inducing Low Frequency Stimulation Enhances p-Tau181 and p-Tau217 in an Age-Dependent Manner in Live Rats

Supplementary Table 1. Antibodies used in this study

Antibody	Source	Cat.No	Dilution
Phospho-Tau (Thr181) (D9F4G)	Cell Signaling Technology	#12885	1:200 IF 1:500 WB
Phospho-Tau (Thr217)	ThermoFisher	#44-744	1:200 IF 1:500 WB
Phospho-Tau (Thr231) (EPR2488)	Abcam	ab151559	1:200 IF 1:5000 WB
Phospho-Tau (Ser202, Thr205) (AT8)	ThermoFisher	MN1020	1:200 IF 1:1000 WB
Phospho-Tau (Ser396)	Affinity Biosciences	AF3148	1:200 IF 1:1000 WB
Tau (D1M9X) XP® Rabbit mAb	Cell Signaling Technology	#46687	1:200 IF 1:500 WB
Tau (Tau46) Mouse mAb	Cell Signaling Technology	#4019	1:200 IF
Alexa Fluor® 488	Abcam	ab150113	1:500 IF
Alexa Fluor® 568	Abcam	ab175471	1:500 IF
β-actin Rabbit mAb	ABclonal	AC026	1:100000 WB
Goat anti-rabbit IgG	ZSGB-BIO	ZB-2301	1:25000 WB
Goat anti-mouse IgG	ZSGB-BIO	ZB-2305	1:25000 WB

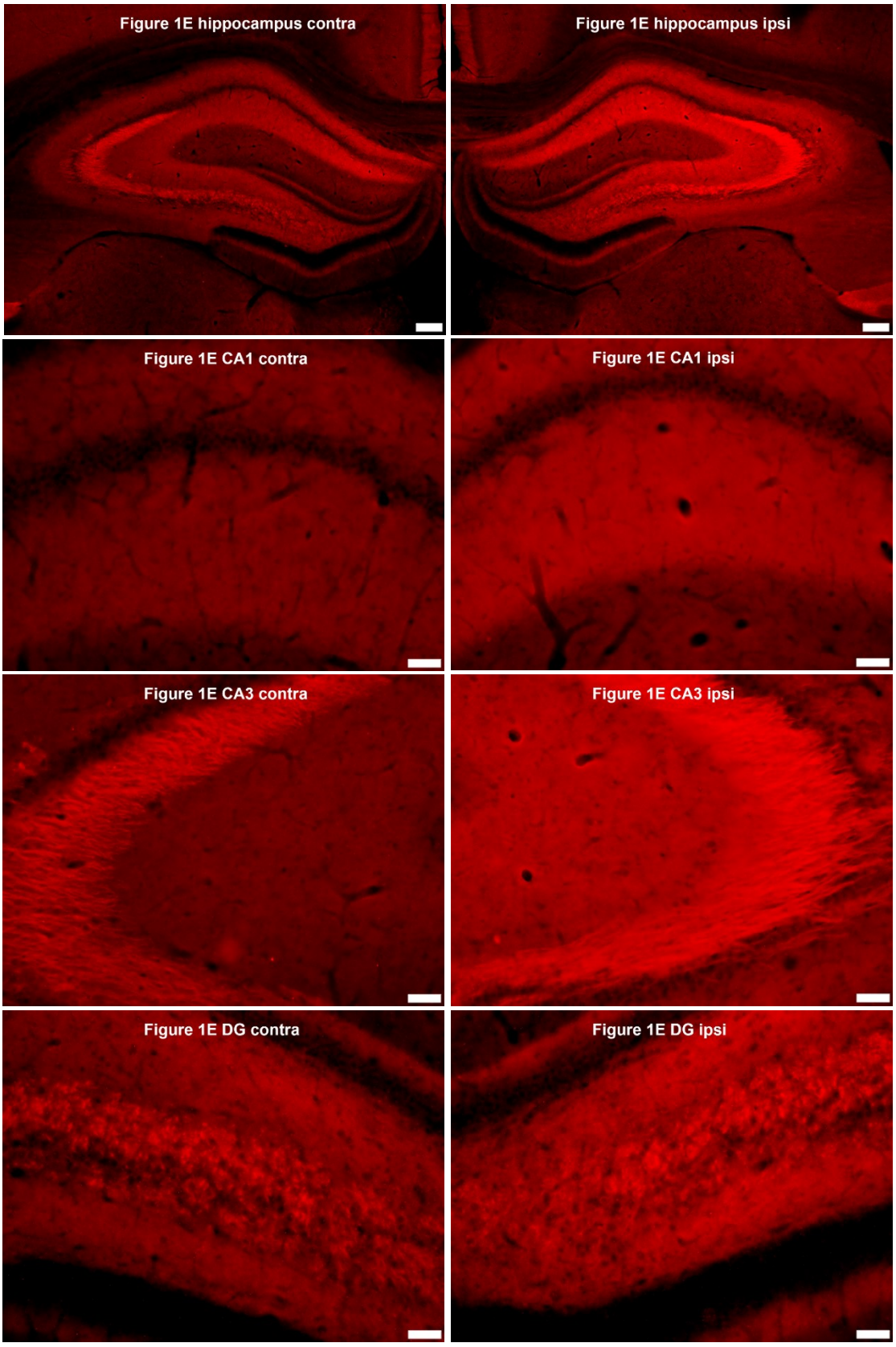


Supplementary Figure 1. Protein synthesis inhibitor emetine completely blocked the maintenance of LTD. A) Evoked EPSPs were recorded at CA3-CA1 synapses in anaesthetized rats at the age of 2-3 months. Application of LFS (horizontal bar, LFS-900; 900 pulses at 1 Hz) induced robust and persistent LTD 30 min after acute intracerebroventricular (i.c.v.) injection of vehicle (PBS, 5 µL). In contrast, i.c.v. administration of the protein synthesis inhibitor emetine (240 µg in 5 µL) did not significantly affect LTD induction but completely blocked the maintenance of LTD 3 h post LFS. B) Summarized EPSP amplitude before (pre) and 3 h after (post) the application of LFS. The EPSP decreased to 74.4 ± 1.8% in the vehicle control group ($n = 5$, $p = 0.0002$ compared with Pre and $p < 0.0001$ compared with No LFS group; paired *t* and one-way ANOVA-Bonferroni) and 99.6 ± 2.5% in the emetine treated group ($n = 5$, $p = 0.8972$ compared with Pre; $p > 0.9999$ compared with No LFS group; $p < 0.0001$ compared with Veh group). Values are mean ± s.e.m. Calibration bars: vertical, 2 mV; horizontal, 10 ms.

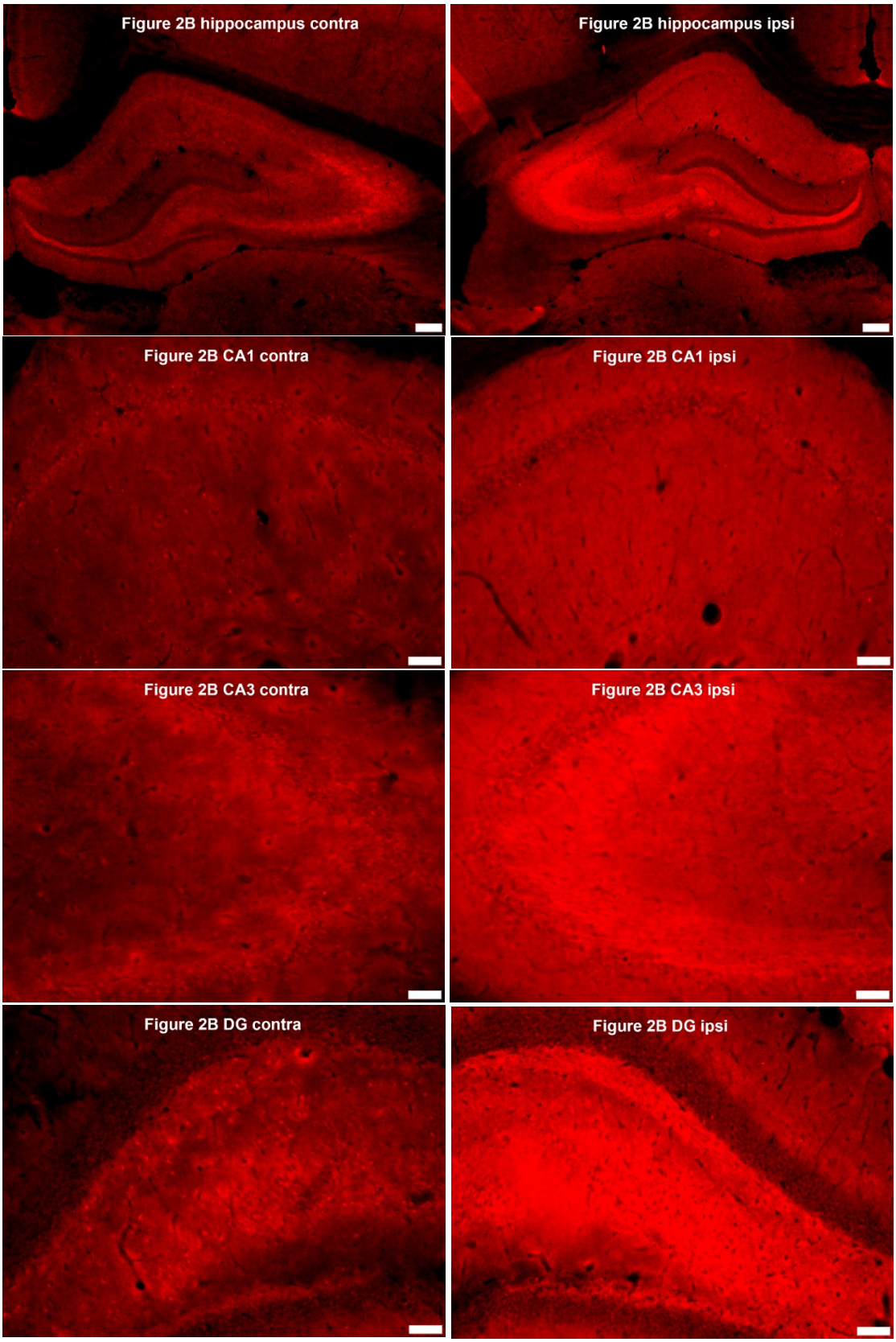


Supplementary Figure 2. LFS did not affect total tau expression level in either young or aged rats. A) Total tau expression level in 2-3-month-old rats. The upper panel shows the fluorescent images of Tau46 labeling (green) in the hippocampus. The corresponding statistical results are displayed in the lower panel. There was no significant difference, compared with contralateral side, in mean fluorescence intensity of Tau46 in the dorsal hippocampus ($p = 0.6800$), CA1 ($p = 0.6163$), CA3 ($p = 0.5821$) or DG ($p = 0.2119$); $n = 5$, paired *t* test. B) Total tau expression in 17-18-month-old rats. The upper panel shows the fluorescent images of Tau46 labeling (green) and the corresponding statistical results are displayed in the lower panel. There was no significant difference, compared with contralateral side, in mean fluorescence intensity of Tau46 in the dorsal hippocampus ($p = 0.1461$), CA1 ($p = 0.7280$), CA3 ($p = 0.3952$) or DG ($p = 0.9309$); $n = 7$, paired

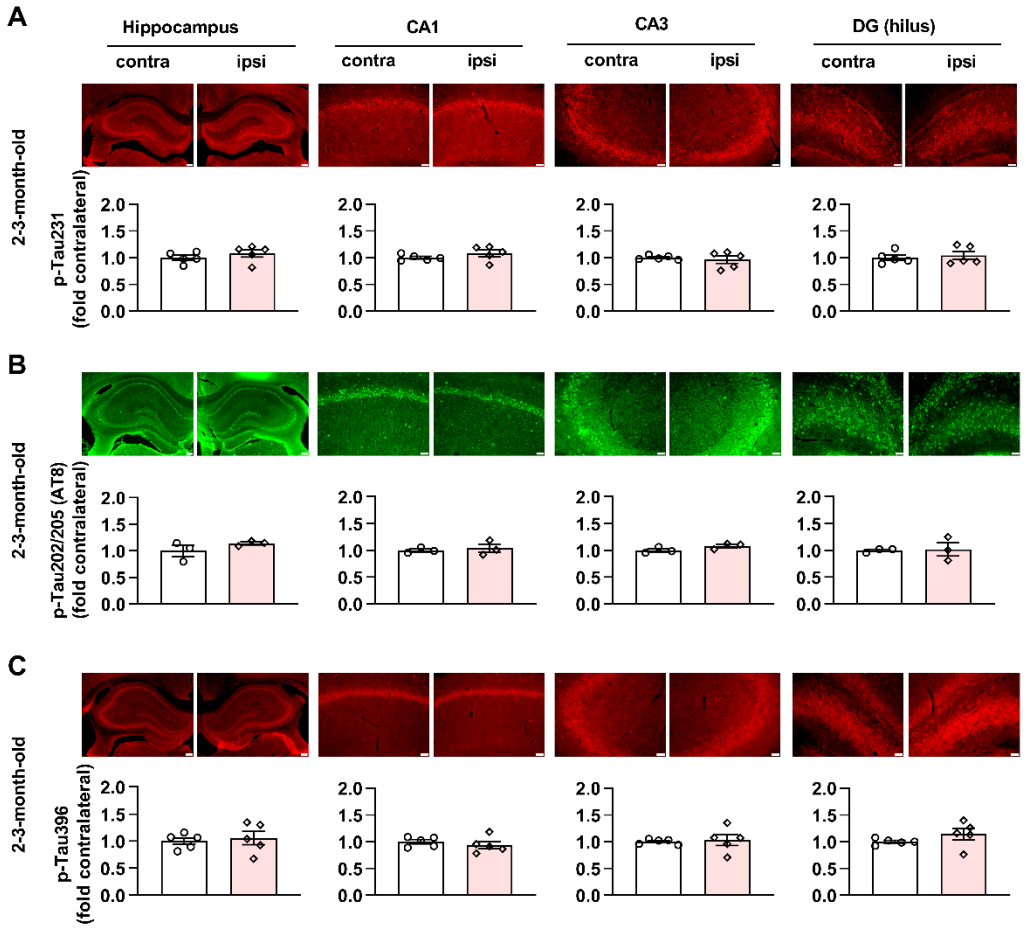
t test. C) Total tau expression level in 2-3-month-old rats. The upper panel shows the fluorescent images of Tau5 labeling (red) in the hippocampus. The corresponding statistical results are displayed in the lower panel. There was no significant difference, compared with contralateral side, in mean fluorescence intensity of Tau5 in the dorsal hippocampus ($p = 0.6074$), CA1 ($p = 0.6071$), CA3 ($p = 0.8486$) or DG ($p = 0.4827$); $n = 5$, paired *t* test. D) Total tau expression in 17-18-month-old rats. The upper panel shows the fluorescent images of Tau5 labeling (red) and the corresponding statistical results are displayed in the lower panel. There was no significant difference, compared with contralateral side, in mean fluorescence intensity of Tau5 in the dorsal hippocampus ($p = 0.3632$), CA1 ($p = 0.5303$), CA3 ($p = 0.0985$) or DG ($p = 0.0919$); $n = 5$, paired *t* test. Scale bar = 200 μm in hippocampus; scale bar = 50 μm in CA1, CA3 and DG regions. Values are mean ± s.e.m.



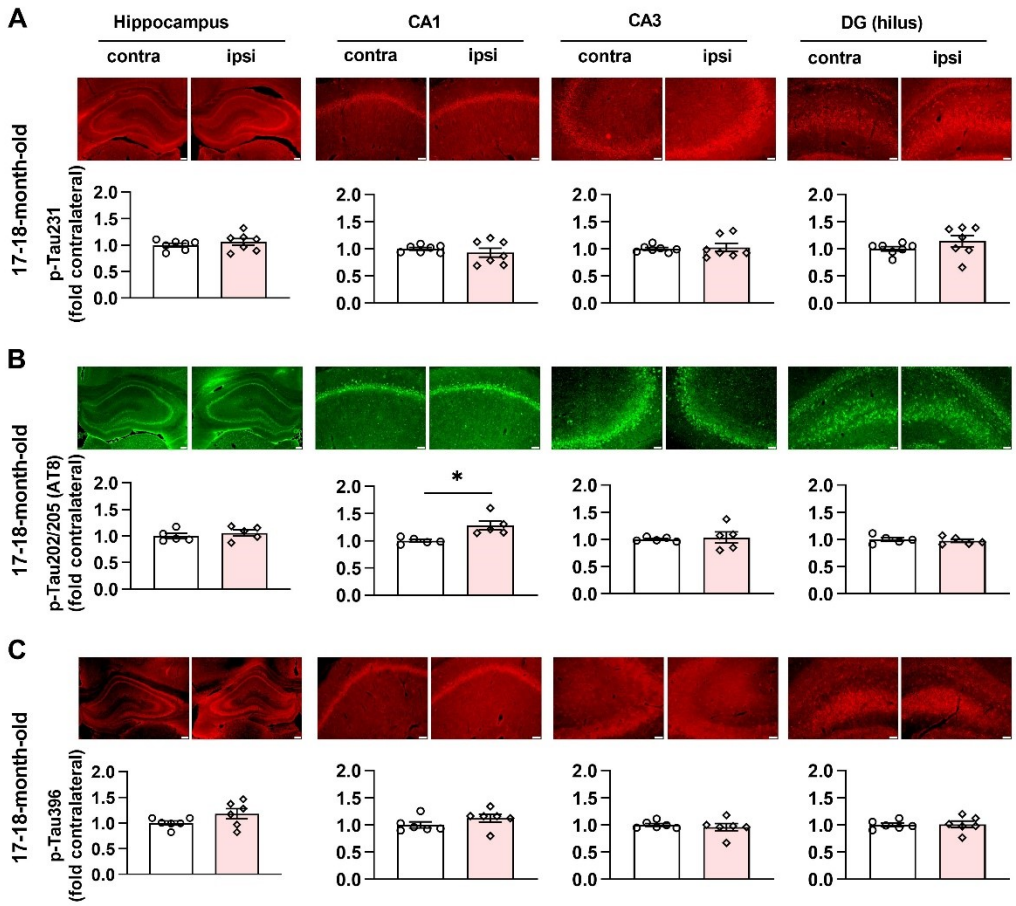
Supplementary Figure 3. High resolution images used to generate Figure 1E.



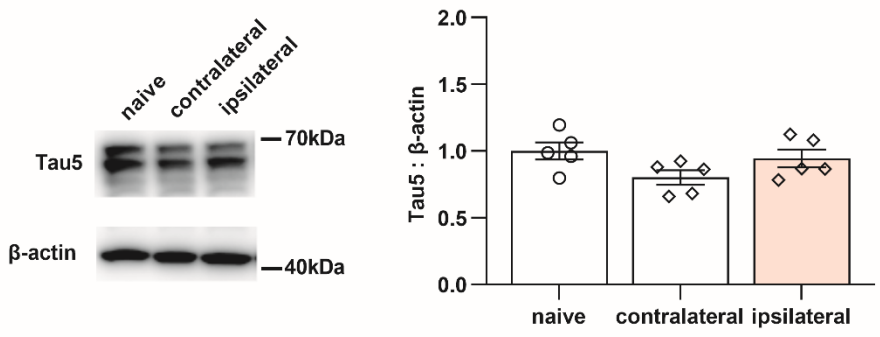
Supplementary Figure 4. High resolution images used to generate Figure 2B.



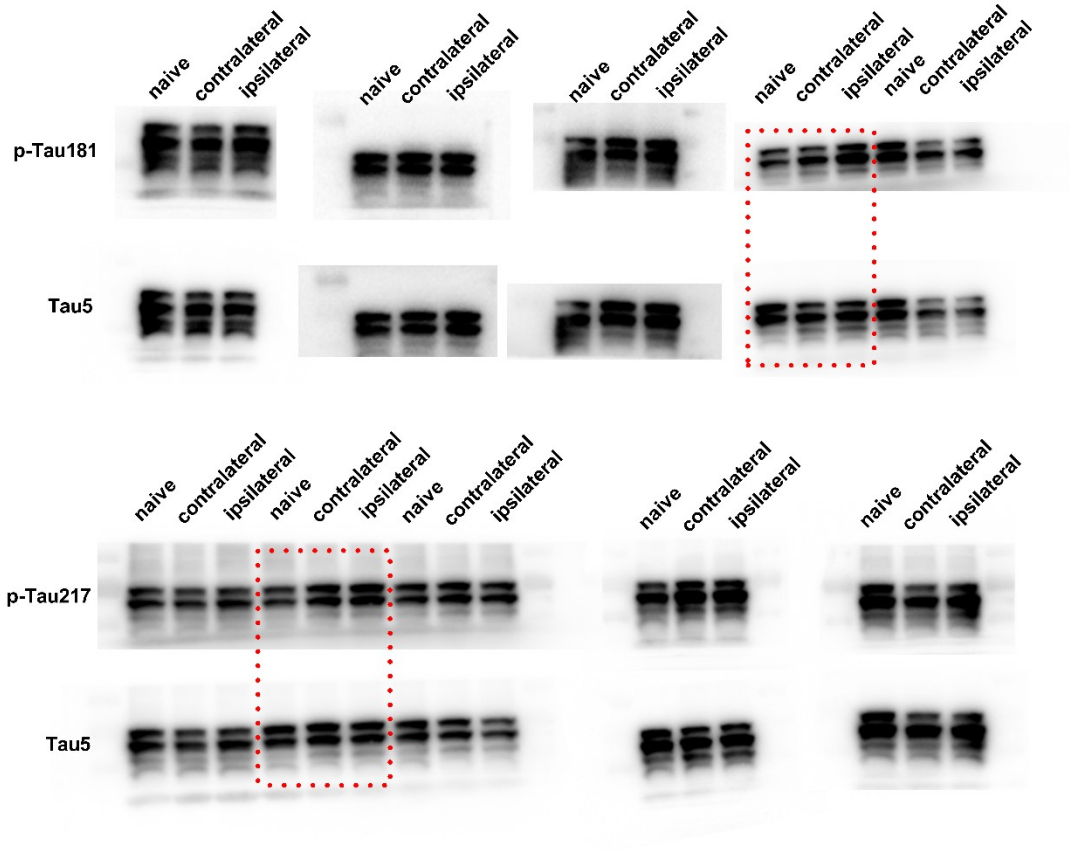
Supplementary Figure 5. LFS did not enhance p-Tau231, p-Tau202/205 or p-Tau396 in young rats. A) The upper panel shows p-Tau231 (red) immunofluorescent staining in dorsal hippocampus (Scale bar: 200 μm), CA1, CA3 and hilus of DG (scale bars: 50 μm) from 2-3-month-old rats. The corresponding statistical results compared with contralateral side are displayed in the lower panel. The expression level of p-Tau231 was not affected by LFS in dorsal hippocampus ($p = 0.1313$), CA1 ($p = 0.2606$), CA3 ($p = 0.6745$) or DG ($p = 0.4386$); paired *t* test. B) Immunofluorescent staining of p-Tau202/205 (AT8, green) in dorsal hippocampus, CA1, CA3 or DG from young rats. LFS did not affect the level of p-Tau202/205 in dorsal hippocampus ($p = 0.2379$), CA1 ($p = 0.5118$), CA3 ($p = 0.1989$) or DG ($p = 0.8594$); paired *t* test. C) Immunofluorescent staining of p-Tau396 (red) in dorsal hippocampus, CA1, CA3 and DG from young rats. LFS did not enhance the level of p-Tau396 in dorsal hippocampus ($p = 0.5272$), CA1 ($p = 0.3132$), CA3 ($p = 0.7471$) or DG ($p = 0.2619$); paired *t* test. Values are mean ± s.e.m.



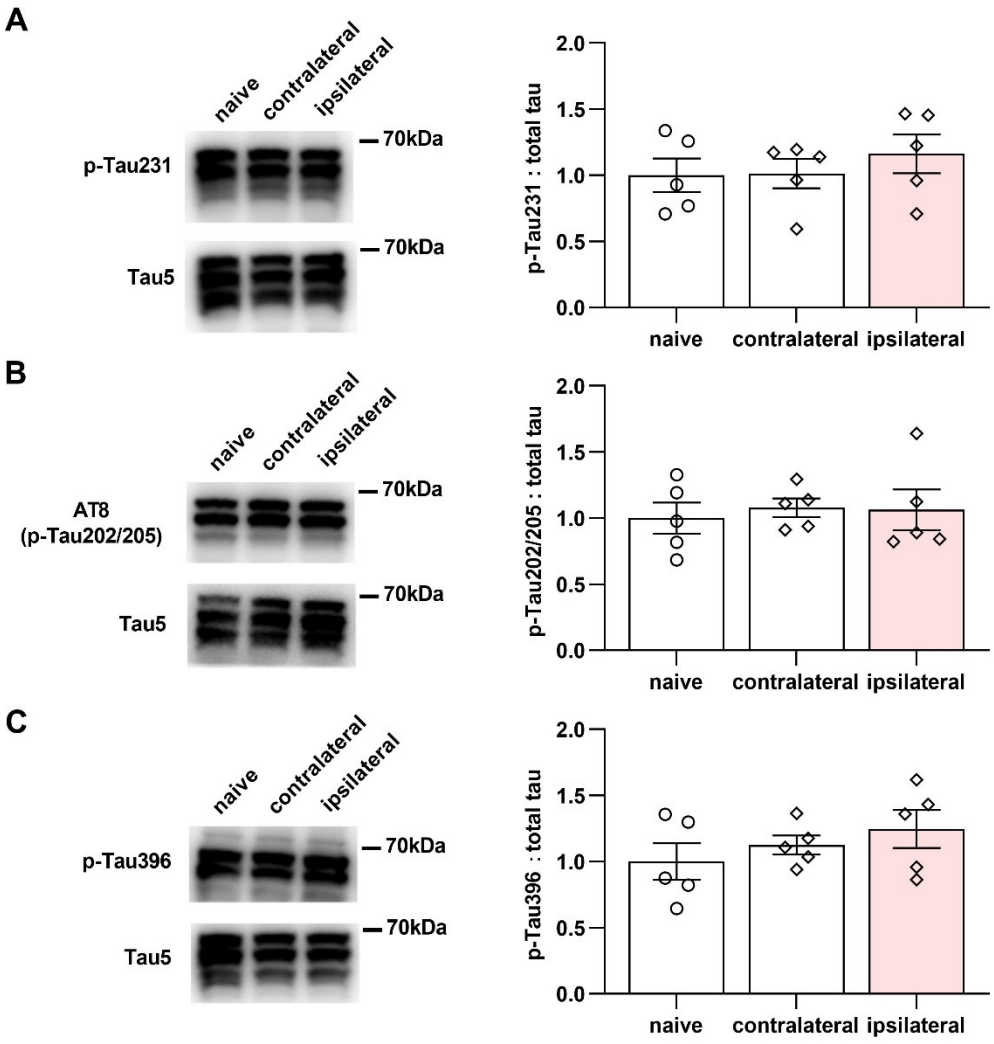
Supplementary Figure 6. Effects of LFS on p-Tau231, p-Tau202/205 and p-Tau396 in aged rats. A) The upper panel shows p-Tau231 (red) immunofluorescent staining in dorsal hippocampus (Scale bar: 200 μm), CA1, CA3 and hilus of DG (scale bars: 50 μm) from 17-18-month-old rats. The corresponding statistical results compared with contralateral side are displayed in the lower panel. LFS did not affect the level of p-Tau231 in dorsal hippocampus ($p = 0.2512$), CA1 ($p = 0.4233$), CA3 ($p = 0.7684$) or DG ($p = 0.2300$); paired *t* test. B) Immunofluorescent staining of p-Tau202/205 (AT8, green) in dorsal hippocampus, CA1, CA3 or DG from aged rats. LFS did not change the level of p-Tau202/205 in dorsal hippocampus ($p = 0.1846$), CA3 ($p = 0.7099$) or DG ($p = 0.6323$). However, the difference was significant in CA1 area ($p = 0.0264$); paired *t* test. C) Immunofluorescent staining of p-Tau396 (red) in dorsal hippocampus, CA1, CA3 or DG from aged rats. LFS did not enhance the level of p-Tau396 in dorsal hippocampus ($p = 0.1661$), CA1 ($p = 0.1886$), CA3 ($p = 0.6253$) or DG ($p = 0.8995$); paired *t* test. Values are mean ± s.e.m.



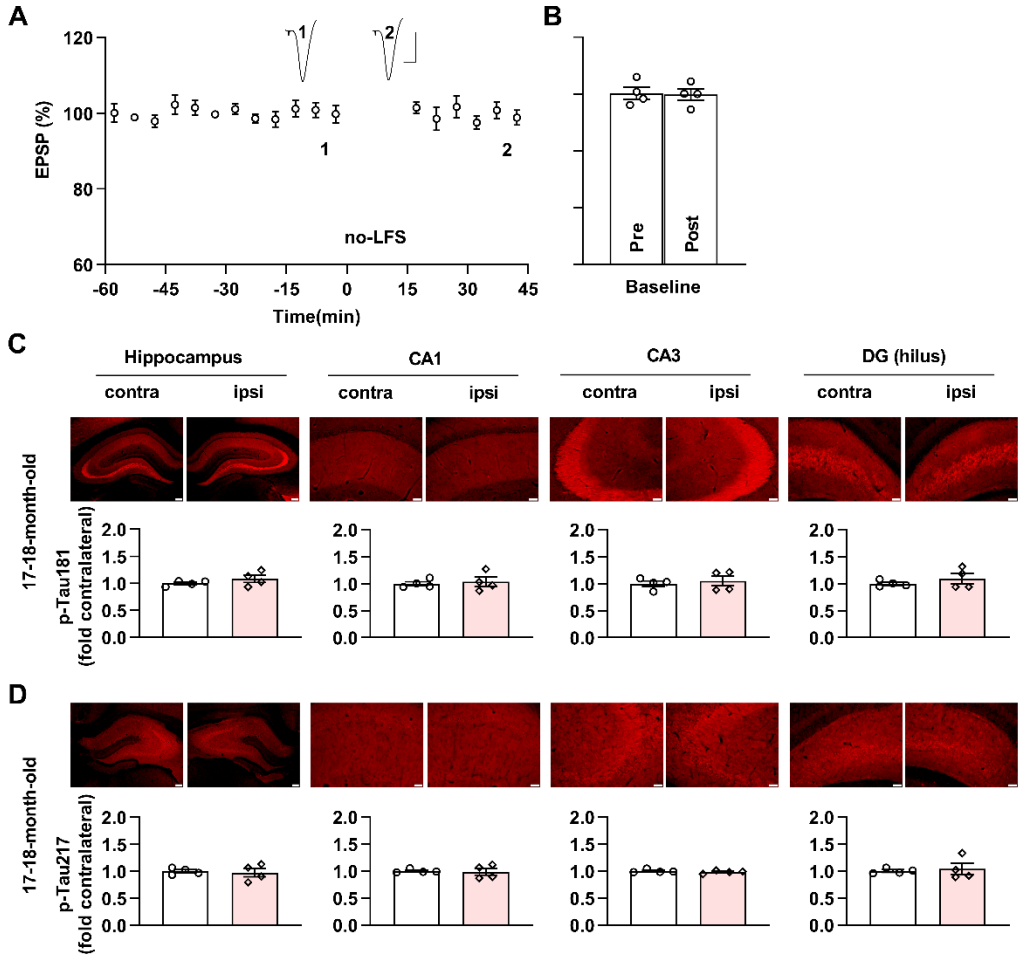
Supplementary Figure 7. LFS did not affect total tau expression level in aged rats. Left panels show representative blotting band of total tau (Tau5) and β-actin in age-matched naïve control group, contralateral hippocampus and ipsilaterally stimulated hippocampus. Right panel shows the ratio of Tau5 over β-actin. The expression level of total tau was comparable in all groups (n = 5 per group, one-way ANOVA, $F_{(2,12)} = 2.691$, p = 0.1082). Values are mean ± s.e.m.



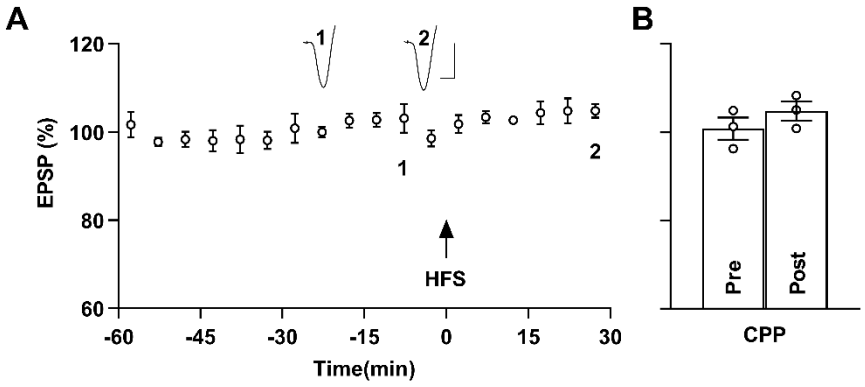
Supplementary Figure 8. Full western blots of p-Tau181 and p-Tau217 obtained in this study. Lanes shown in Figure 3 are boxed in red.



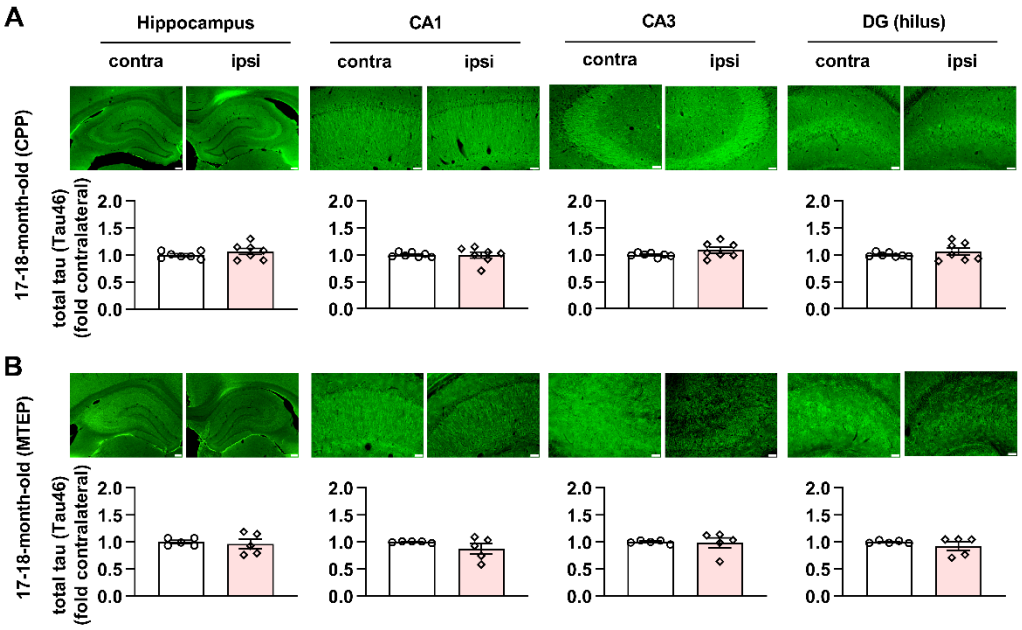
Supplementary Figure 9. LFS did not enhance p-Tau231, p-Tau202/205 or p-Tau396 in aged rats. A) Left panels show representative blotting band of p-Tau231 and total tau (tau5) in age-matched naïve control group, contralateral hippocampus and ipsilaterally stimulated hippocampus. Right panel, statistical results of p-Tau231 over total tau are quantified and normalized to naïve control (n = 5 per group, one-way ANOVA, $F_{(2,12)}$ = 0.4882, p = 0.6254). B) Left panels show representative blotting band of p-Tau202/205 (AT8) and total tau (tau5). Right panel, statistical results of p-Tau202/205 over total tau are quantified and normalized to naïve control (n = 5 per group, one-way ANOVA, $F_{(2,12)}$ = 0.1229, p = 0.8854). C) Left panels show representative blotting band of p-Tau396 and total tau (tau5). Right panel, statistical results of p-Tau396 over total tau are quantified and normalized to naïve control (n = 5 per group, one-way ANOVA, $F_{(2,12)}$ = 1.004, p = 0.3951).



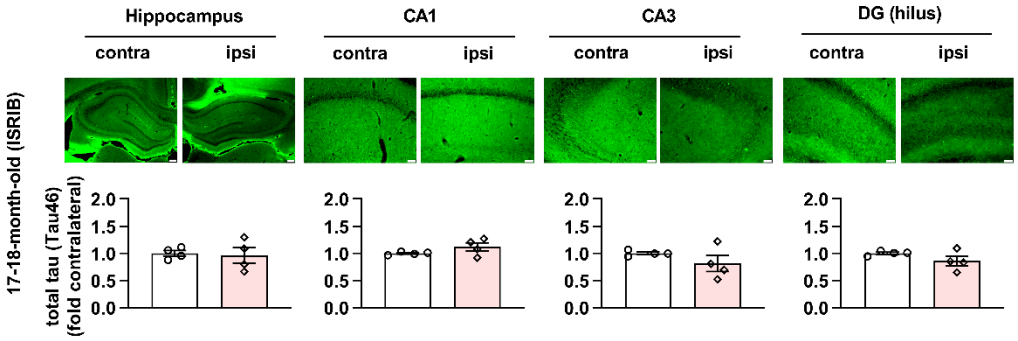
Supplementary Figure 10. Electrode implantation and baseline recording did not enhance p-Tau181 and p-Tau217 in aged rats. A) Stimulating electrode and recording electrode were implanted as used in the LFS delivery groups. The timeline of baseline recording was also the same as that used for LTD induction by LFS. Representative EPSP traces are shown at the times indicated. Calibration bars: vertical, 2 mV; horizontal, 10 ms. B) The amplitude of EPSP at -10 min (Pre) and 30 min (Post) are summarized (n = 4, 100.3 ± 2.1% versus 99.8 ± 2.0%, p = 0.8327, paired *t* test). C) Electrode implantation and baseline recording did not affect the level of p-Tau181 in aged rats. The upper panel shows the fluorescent images of p-Tau181 labeling (red) and the corresponding statistical results are displayed in the lower panel. There was no significant difference in the expression level of p-Tau181, compared with contralateral side, in the dorsal hippocampus (p = 0.3299), CA1 (p = 0.7910), CA3 (p = 0.5110) or DG (p = 0.4433); n = 4, paired *t* test. D) Electrode implantation and baseline recording did not affect the level of phospho-Tau217 in aged rats. The upper panel shows the fluorescent images of p-Tau217 labeling (red) and the corresponding statistical results are displayed in the lower panel. Compared with contralateral side, there was no significant difference in the expression level of p-Tau217 in the dorsal hippocampus (p = 0.7526), CA1 (p = 0.8234), CA3 (p = 0.4321) or DG (p = 0.7395); n = 4, paired *t* test. Scale bar = 200 μm in hippocampus; scale bar = 50 μm in CA1, CA3 and DG regions. Values are mean ± s.e.m.



Supplementary Figure 11. The NMDA receptor antagonist CPP blocked LTP induction in aged rats. A) The competitive NMDAR antagonist CPP (10 mg/kg, i.p.), injected alone 1 h prior to the application of high frequency stimulation (HFS), completely blocked LTP induction. B) Summary of the mean EPSP amplitude data before (pre) and 30 min after (post) the application of HFS (n = 3, 100.8 ± 2.5% versus 104.8 ± 2.2%, p = 0.1888, paired *t* test). Values are mean ± s.e.m. Calibration bar: vertical, 2 mV; horizontal, 10 ms.



Supplementary Figure 12. LFS did not affect total tau expression in CPP or MTEP -treated aged rats. A) Total tau expression in CPP-injected aged rats. The upper panel shows the fluorescent images of Tau46 labeling (green) and the corresponding statistical results are displayed in the lower panel. There was no significant difference in mean fluorescence intensity of Tau46 in LTD induction side compared with contralateral side including the dorsal hippocampus ($p = 0.3046$), CA1 ($p = 0.8944$), CA3 ($p = 0.1508$) or DG ($p = 0.3163$); $n = 7$, paired *t* test. B) Total tau expression in MTEP-injected aged rats. The upper panel shows the fluorescent images of Tau46 labeling (green) and the lower panel displays the corresponding statistical results. Compared with contralateral side, there was no significant difference in mean fluorescence intensity of Tau46 in the dorsal hippocampus ($p = 0.6855$), CA1 ($p = 0.2741$), CA3 ($p = 0.8719$) or DG ($p = 0.3840$); $n = 5$, paired *t* test. Scale bar = 200 μm in hippocampus; scale bar = 50 μm in CA1, CA3 or DG regions. Values are mean ± s.e.m.



Supplementary Figure 13. LFS did not affect total tau expression in ISRIB-treated aged rats. Total tau expression in ISRIB-injected 17-18-month-old rats. The upper panel shows the fluorescent images of Tau46 labeling (green) and the corresponding statistical results are displayed in the lower panel. There was no significant difference, compared with contralateral side, in mean fluorescence intensity of Tau46 in the dorsal hippocampus ($p = 0.7535$), CA1 ($p = 0.2133$), CA3 ($p = 0.3503$) or DG ($p = 0.1900$); $n = 4$, paired *t* test. Scale bar = 200 μm in hippocampus; scale bar = 50 μm in CA1, CA3 and DG regions. Values are mean ± s.e.m.