Supplementary data

Reduced BDNF expression in the auditory cortex

contributed to neonatal pain-induced hearing impairment and dendritic pruning deficiency in mice

Running Head: Neonatal pain-induced hearing impairment

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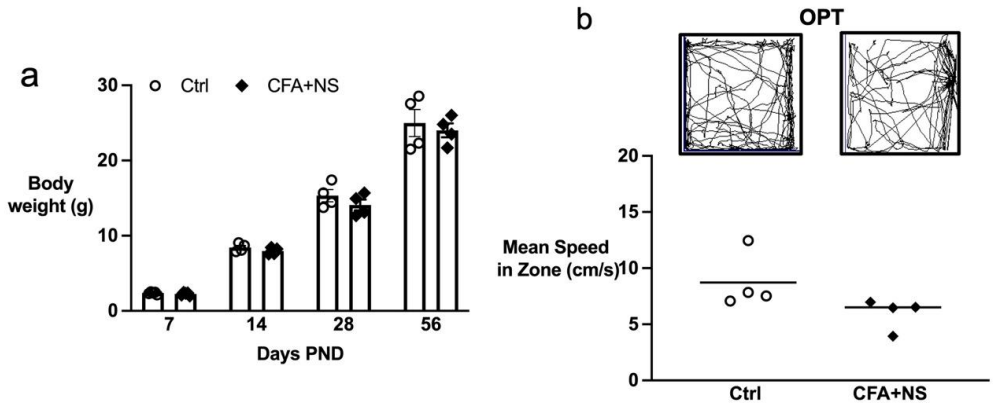
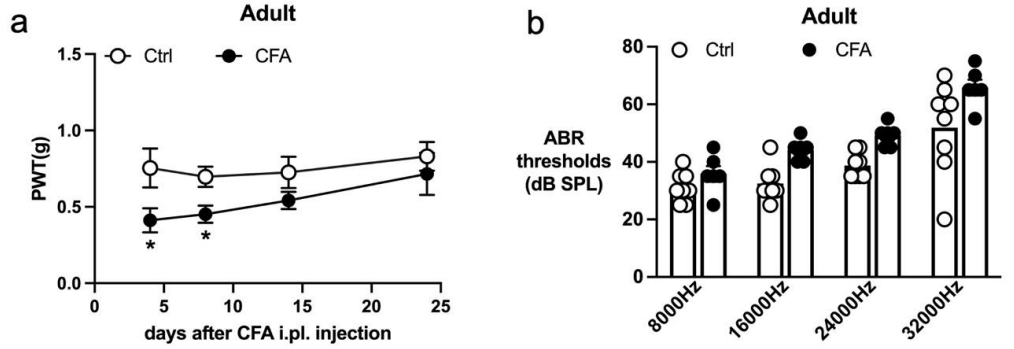
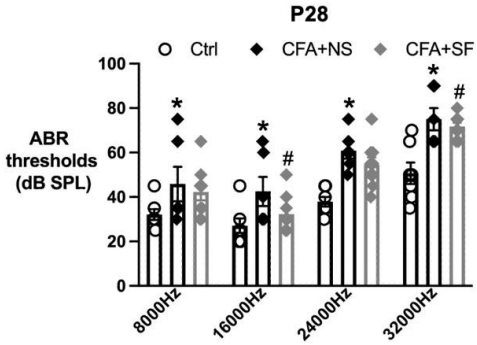
Supplementary figures



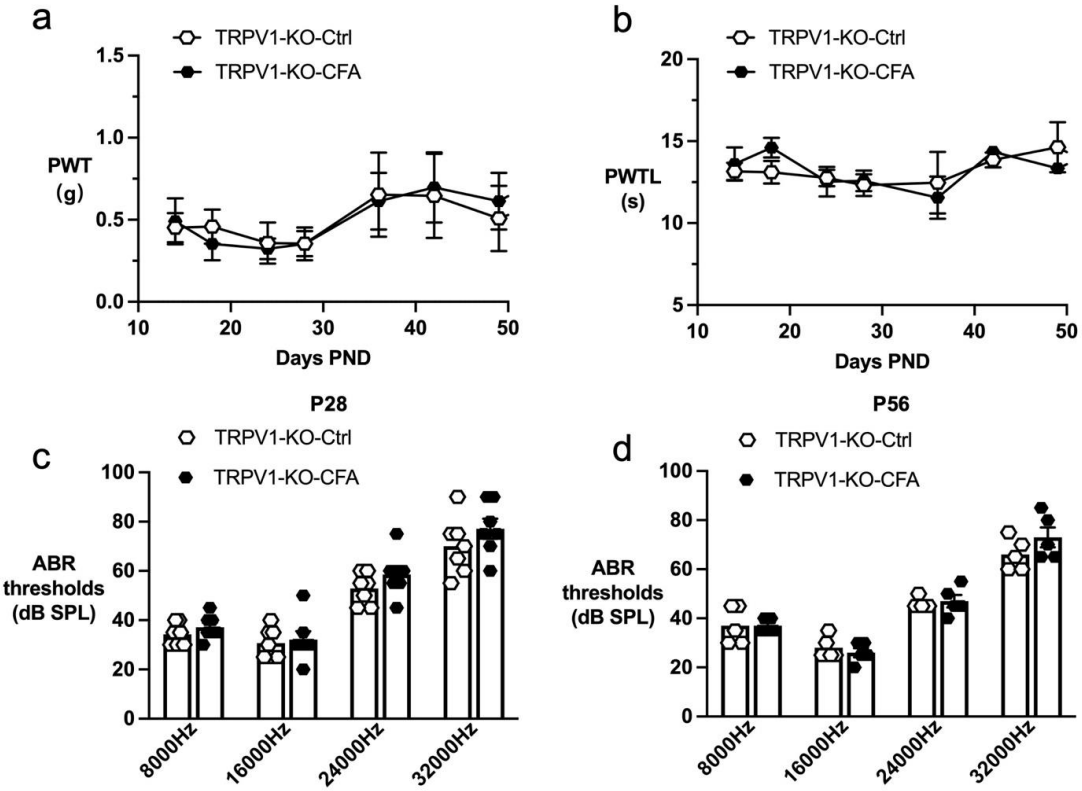
SFig. 1 CFA administration did not affect the growth and motor function of mice. (a) The weight changes among Ctrl, CFA + NS showed no significant difference at 7, 14, 28, and 56 days post-natal. (b) The open-field test showed that CFA + NS did not affect the motor function of mice. n= 4, One-way ANOVA followed by Dunnett's post hoc test. Abbreviations: Ctrl, Control; CFA, Complete Freund Adjuvant injection; NS, Normal Saline; PND, Postnatal Day.



SFig. 2 Intra-plantar CFA administration in adult mice did not impair hearing. n=8, *P< 0.05, compared to the Ctrl group. One-way ANOVA followed by Dunnett's post hoc test. Abbreviations: Ctrl, Control; CFA, Complete Freund Adjuvant injection

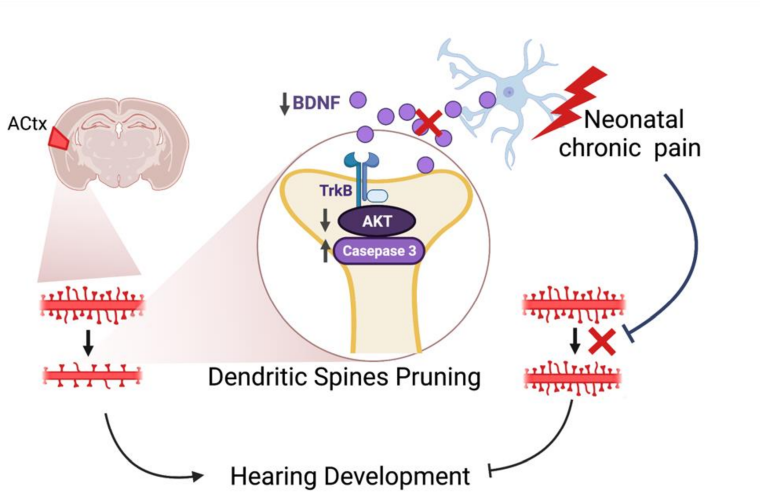


SFig. 3 Sufentanil administration partially rescued the hearing loss of CFA-induced ABR thresholds increase <u>at P28</u>. n =4~5, **P* < 0.05 versus Ctrl group; #*P* < 0.05 versus CFA+NS group; One-way ANOVA followed by Dunnett's post hoc test. Abbreviations: Ctrl, Control; CFA, Complete Freund Adjuvant injection; SF, Sufentanil; ABR, Auditory Brainstem Response.



SFig. 4 <u>Intraplantar CFA injection at P7</u> did not induce persistent neonatal pain <u>and hearing loss in adults</u> in TRPV1-KO mice. (a) Time course of the mechanical threshold changes in TRPV1-KO-Ctrl and TRPV1-KO-CFA mice. (b) Time course of the paw withdrawal

thermal latency (PWTL) in TRPV1-KO-Ctrl and TRPV1-KO-CFA mice. (c) ABR thresholds of TRPV1-KO-Ctrl and TRPV1-KO-CFA mice at 8000, 16000, 24000, and 32000 Hz frequencies at P28. (d) ABR thresholds of TRPV1-KO-Ctrl and TRPV1-KO-CFA mice at 8000, 16000, 24000, and 32000 Hz frequencies at P56. n =5~7. One-way ANOVA was followed by Dunnett's post hoc test. Abbreviations: Ctrl, Control; CFA, Complete Freund Adjuvant injection; KO, knockout, TRPV1, Transient receptor potential cation channel subfamily V member 1; PND, Post-natal day; ABR, Auditory Brainstem Response.



SFig. 5 The mechanism underlying the neonatal persistent pain-induced hearing impairment. AC, auditory cortex; BNDF, Brain-Derived Neurotrophic Factor; AKT, Protein kinase B; TrkB, Tropomyosin receptor kinase B. Created with BioRender.com