

Supplementary Materials for  
**Reversal of hyperactive higher-order thalamus attenuates defensiveness in a mouse model of PTSD**

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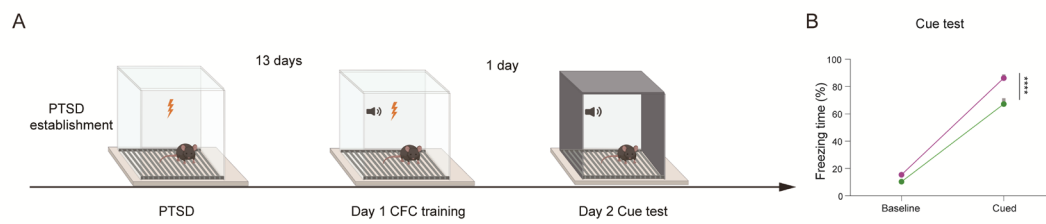
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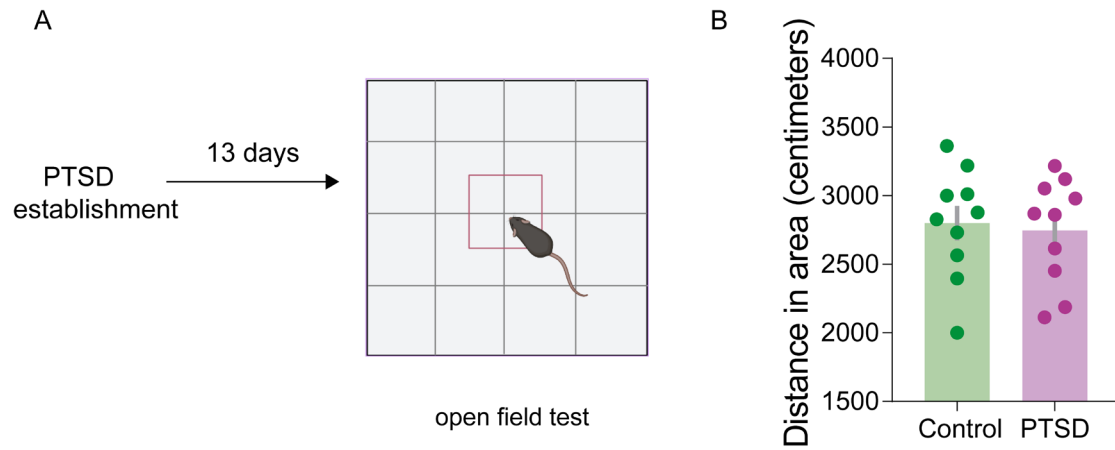
Figs. S1 to S18  
Tables S1 to S3

**Other Supplementary Material for this manuscript includes the following:**

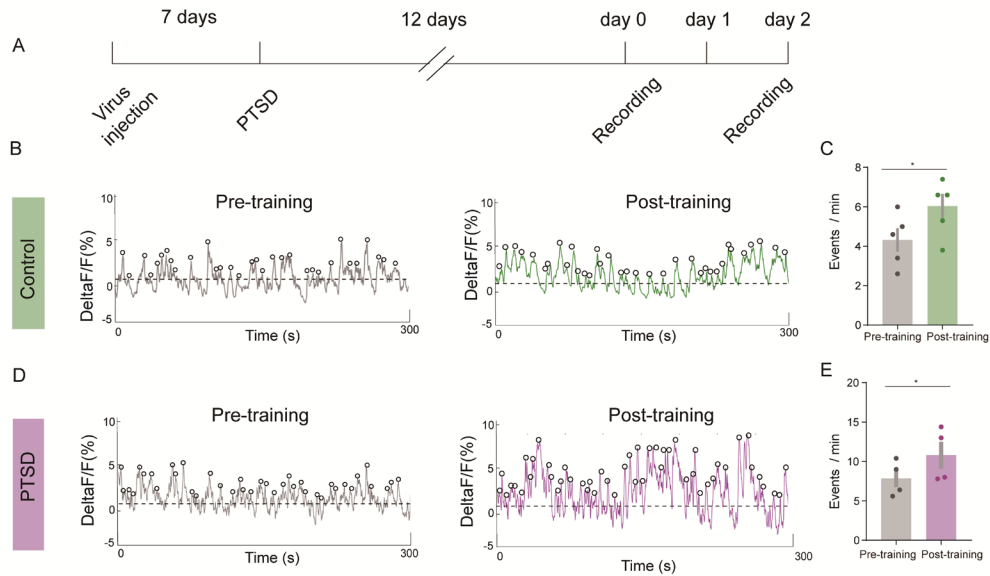
Movie S1



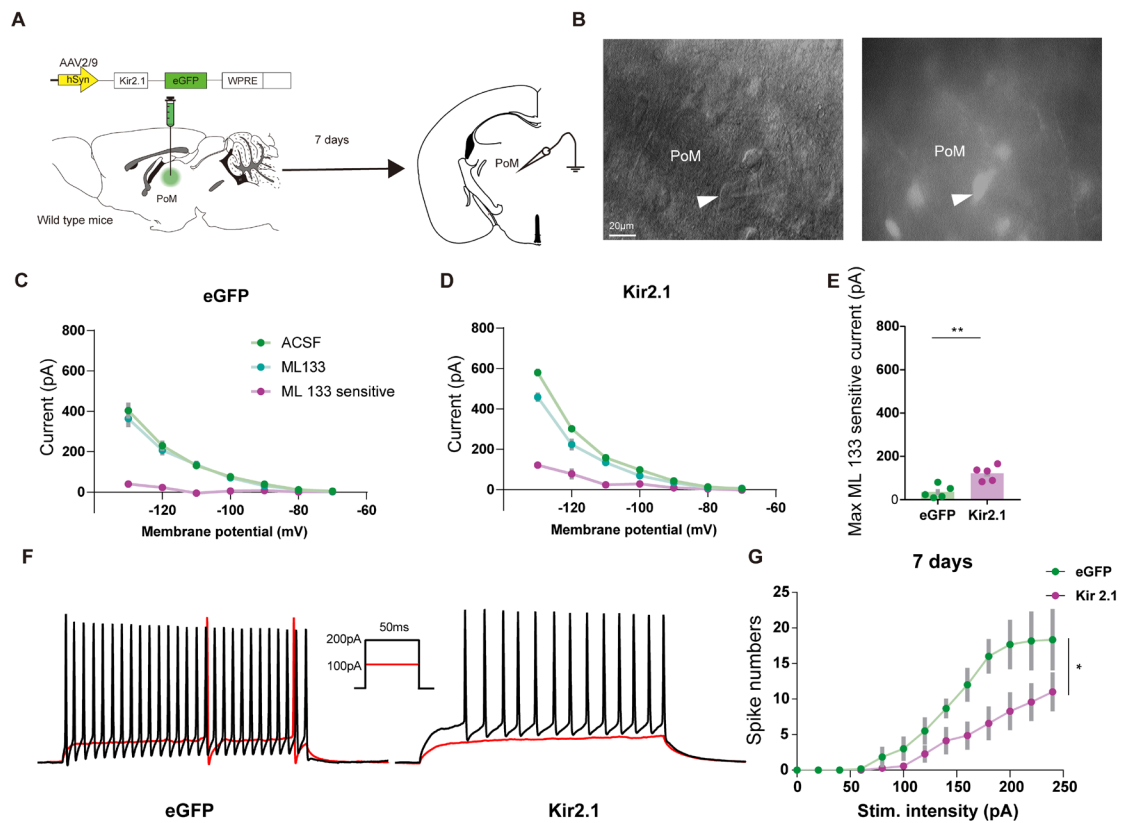
**Fig. S1. Excessive defensive behavior in PTSD mice caused by auditory stimulation. (A)** Diagram of Day 2 cue test. **(B)** Elevated freezing time on Day 2 cue test in PTSD mice (n = 10 mice for each group). \*\*\*\* $P < 0.0001$ , by Two-way ANOVA. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



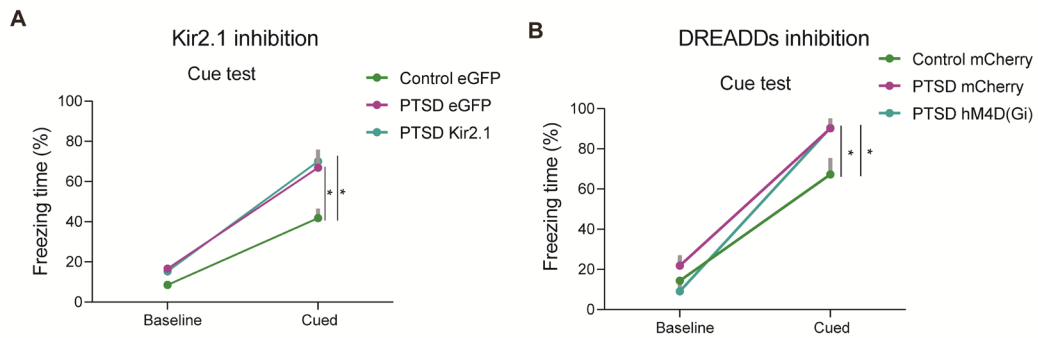
**Fig. S2. PTSD stress did not influence the locomotor activity.** (A) Diagram of the open field test 13 days after the PTSD establishment. (B) Histogram of the distance in the open field between control and PTSD mice (n = 10 mice for each group). By Two-tailed unpaired t-test. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



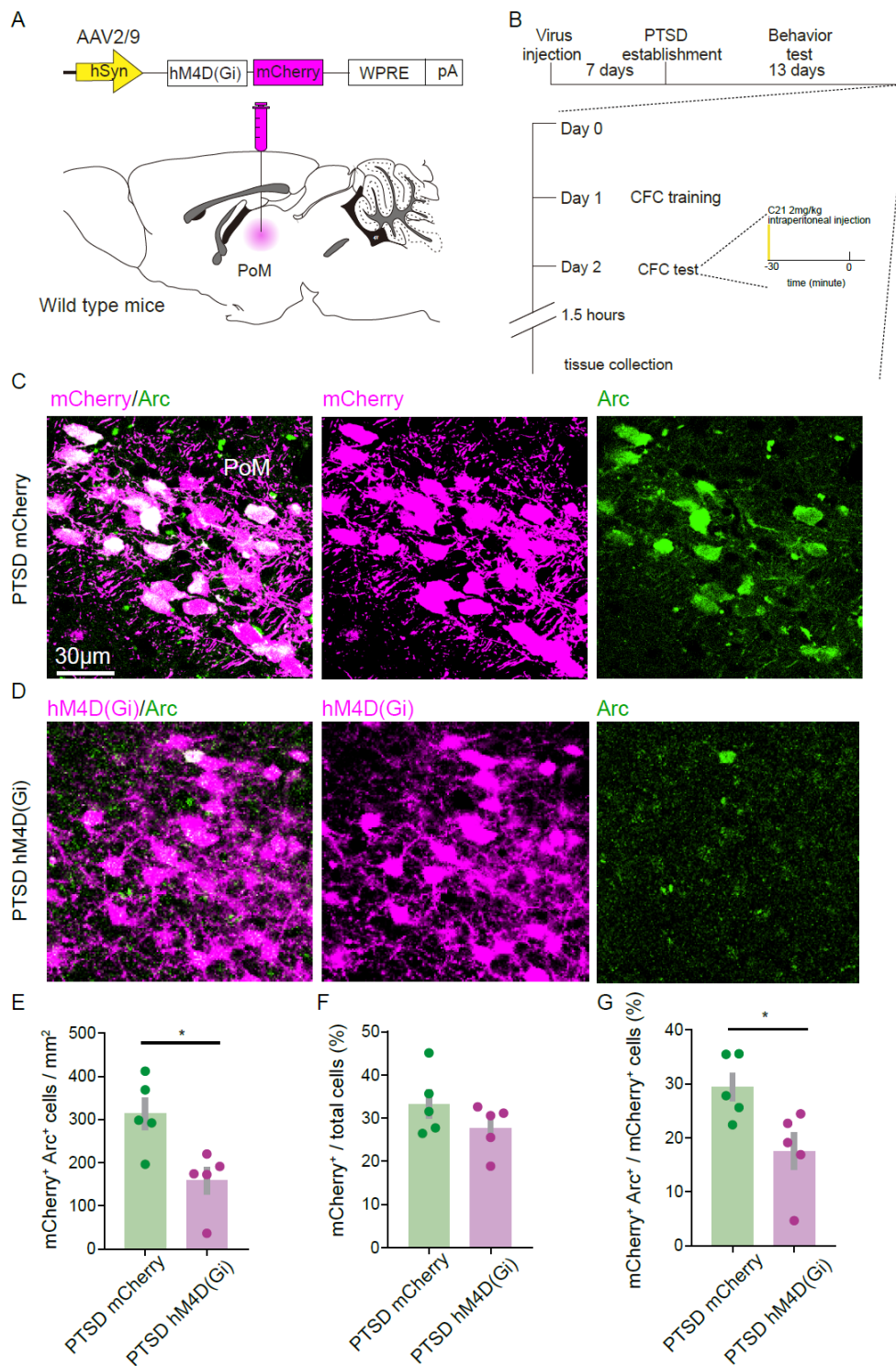
**Fig. S3. Increased neuronal activity of PoM following CFC training.** (A) Schematic of fiber photometry. (B) Schematic and representative traces of tests of fiber photometry in PoM of control mice were subjected to environmental stimulation on the day before training and 2<sup>nd</sup> day after training. (C) Frequency of spontaneous calcium events in PoM of control mice on the day before and 2<sup>nd</sup> day after training (n = 5 mice for control group). \**P* < 0.05, by Two-tailed paired t-test. (D) Schematic and representative traces of tests of fiber photometry in PoM when PTSD mice were subjected to environmental stimulation on the day before and 2<sup>nd</sup> day after training. (E) Frequency of spontaneous calcium events in PoM of PTSD mice on the day before training and 2<sup>nd</sup> day after training (n = 4 mice for PTSD group). \**P* < 0.05, by Two-tailed paired t-test. Data are presented as the mean ± S.E.M. See Table S1 for detailed statistical information.



**Fig. S4. Kir2.1 overexpression reduced the excitability of PoM neurons.** (A) Schematic of virus injection and patch clamp recordings. (B) Representative images of patch clamp recordings of PoM neurons with Kir2.1 overexpression. Scale bar: 20µm. Left: Patch clamp recording of the PoM neuron in DIC microscope. Right: Fluorescence of neurons. (C-D) ML 133 sensitive currents in eGFP-expressing (C) and Kir2.1-expressing mice (D) 7 days after virus injection. (E) Max ML133 sensitive current in eGFP-expressing and Kir2.1-expressing mice after 7 days virus expression. (n = 5 neurons for eGFP group, n = 5 neurons for Kir2.1 group). \*\* $P < 0.01$ , by Two-tailed unpaired t-test. (F) Representative action potentials of PoM neurons in eGFP-expressing and Kir2.1-expressing mice after 7-day virus expression. (G) Numbers of spikes of eGFP-expressing and Kir2.1-expressing neurons after 7-day virus expression. (n = 6 neurons for eGFP group, n = 7 neurons for Kir2.1 group). \* $P < 0.05$ , by Two-way ANOVA. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



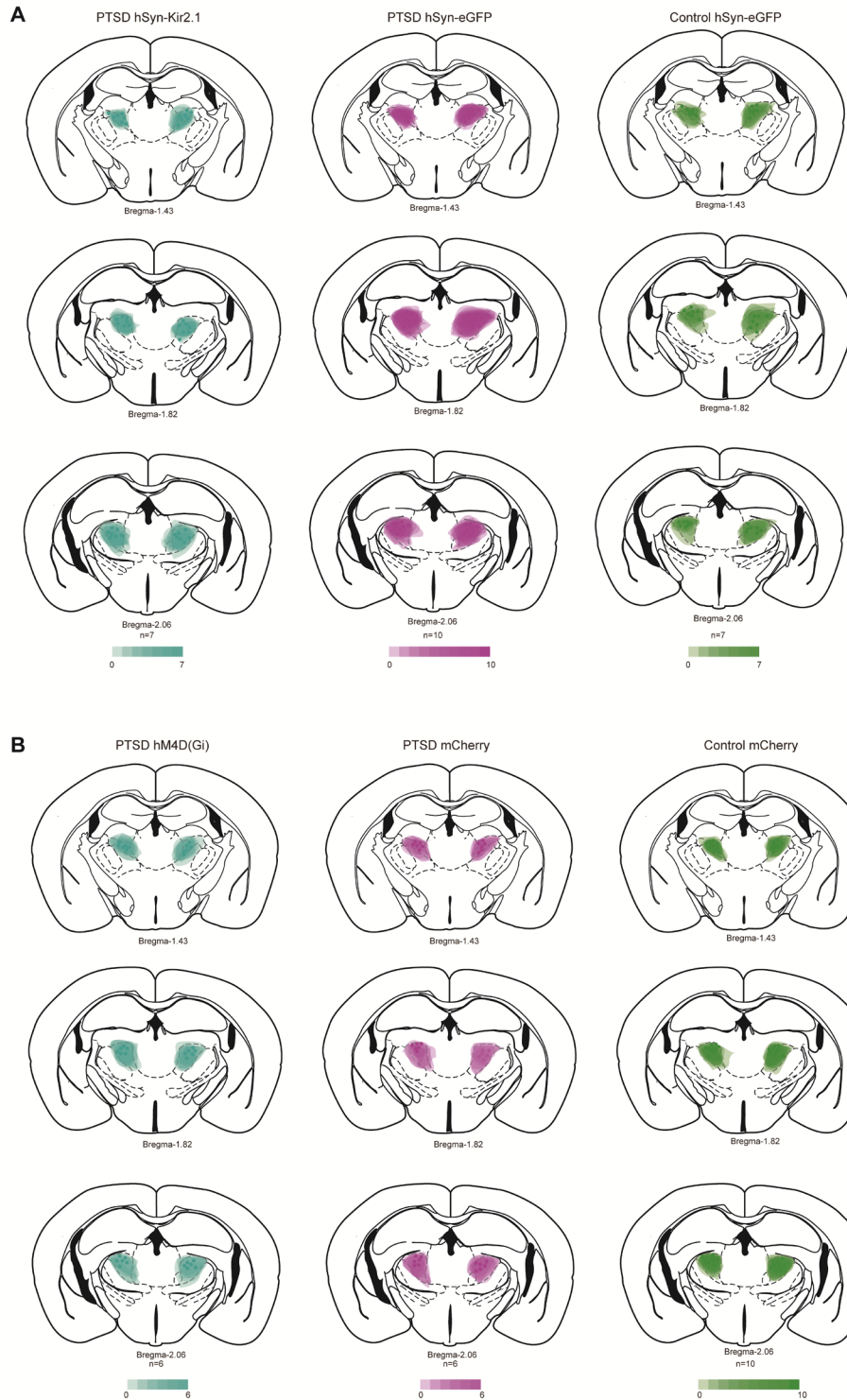
**Fig. S5. Failure of PoM inhibition to correct auditory-related defensive response in PTSD mice.** (A) Freezing time in day 2 cue test between eGFP-expressing control mice, eGFP-expressing PTSD mice and Kir2.1-expressing PTSD mice (n = 7 mice for control eGFP group, n = 6 mice for PTSD eGFP group and n = 7 mice for PTSD Kir2.1 group). \* $P < 0.05$ , by Two-way ANOVA. (B) Freezing time in day 2 cue test between mCherry-expressing control mice, mCherry-expressing PTSD mice and hM4D(Gi)-expressing PTSD mice (n = 9 mice for control mCherry group, n = 5 mice for PTSD mCherry group and n = 5 mice for PTSD hM4D(Gi) group). \* $P < 0.05$ , by Two-way ANOVA. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



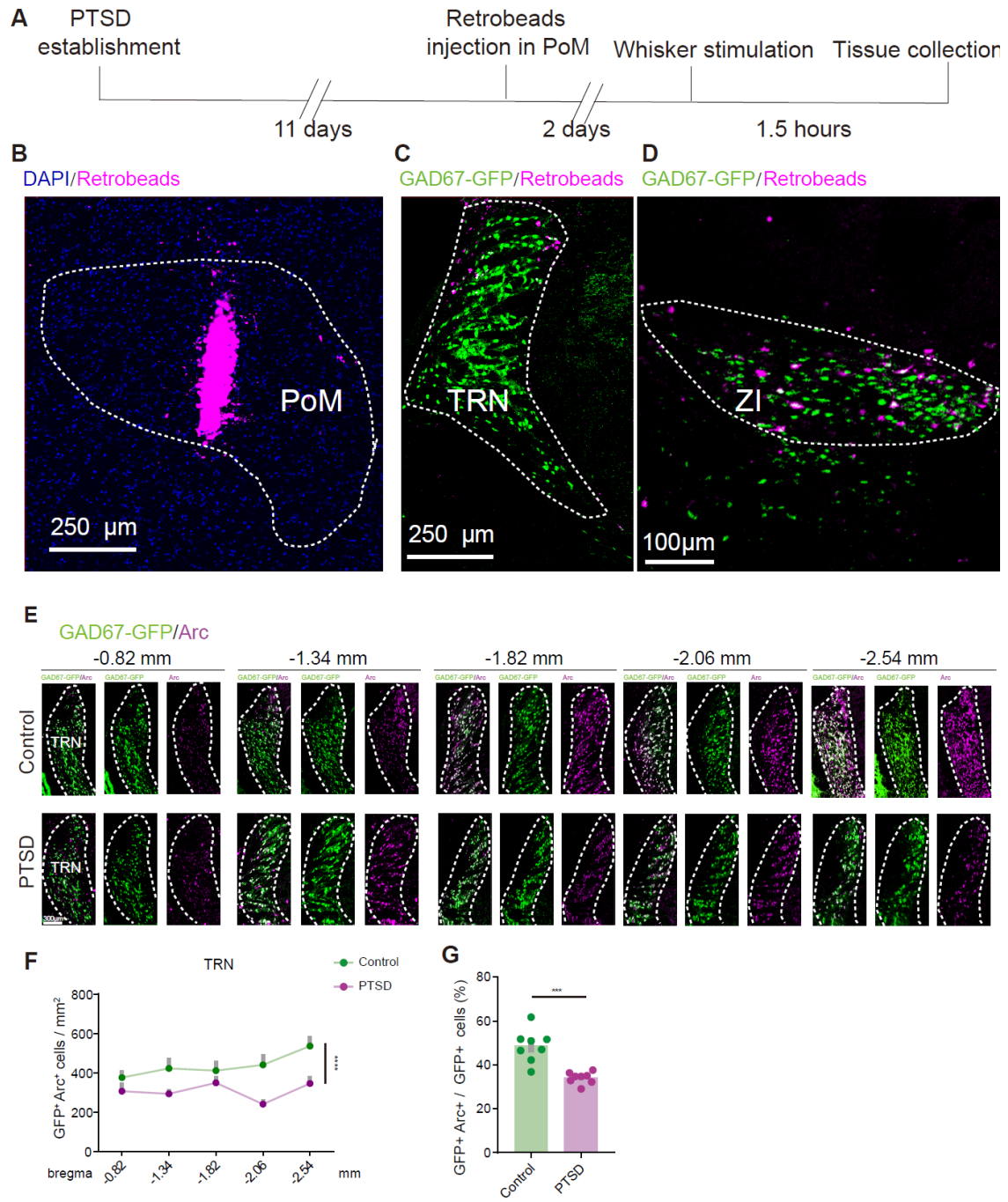
**Fig. S6. hM4D(Gi) suppressed Arc expression in PoM neurons in PTSD mice.** (A) Schematic of the viral strategy to chemo-genetically inhibit PoM neurons. (B) Schematic of behavioral tests and immunohistochemical fluorescence staining. (C) Representative images showing mCherry expression and Arc expression in PoM. Scale bar: 30µm. (D) Representative images showing

hM4D(Gi)-mCherry expression and Arc expression in PoM. Scale bar: 30 $\mu$ m. **(E)** Quantification of mCherry<sup>+</sup> Arc<sup>+</sup> cells in PoM (n = 5 mice for each group). \**P* < 0.05, by Two-tailed unpaired t-test. **(F)** Percentage of mCherry<sup>+</sup> cells in total cells of PoM (n = 5 mice for each group), by Two-tailed unpaired t-test. **(G)** Percentage of mCherry<sup>+</sup> Arc<sup>+</sup> cells in mCherry<sup>+</sup> cells (n = 5 mice for each group). \**P* < 0.05, by Two-tailed unpaired t-test. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



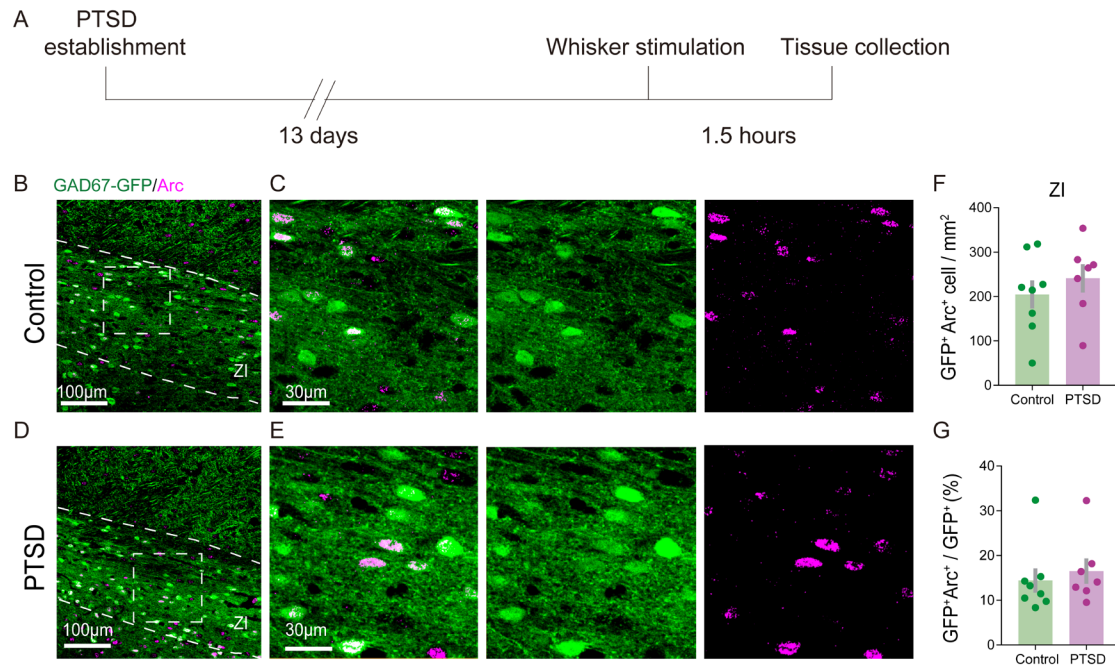


**Fig. S7. Verification of the region showing virus infection for PoM inhibition. (A) Schematic showing areas expressing Kir2.1 or eGFP in PoM. (B) Schematic showing areas expressing hM4D(Gi) or mCherry in PoM.**

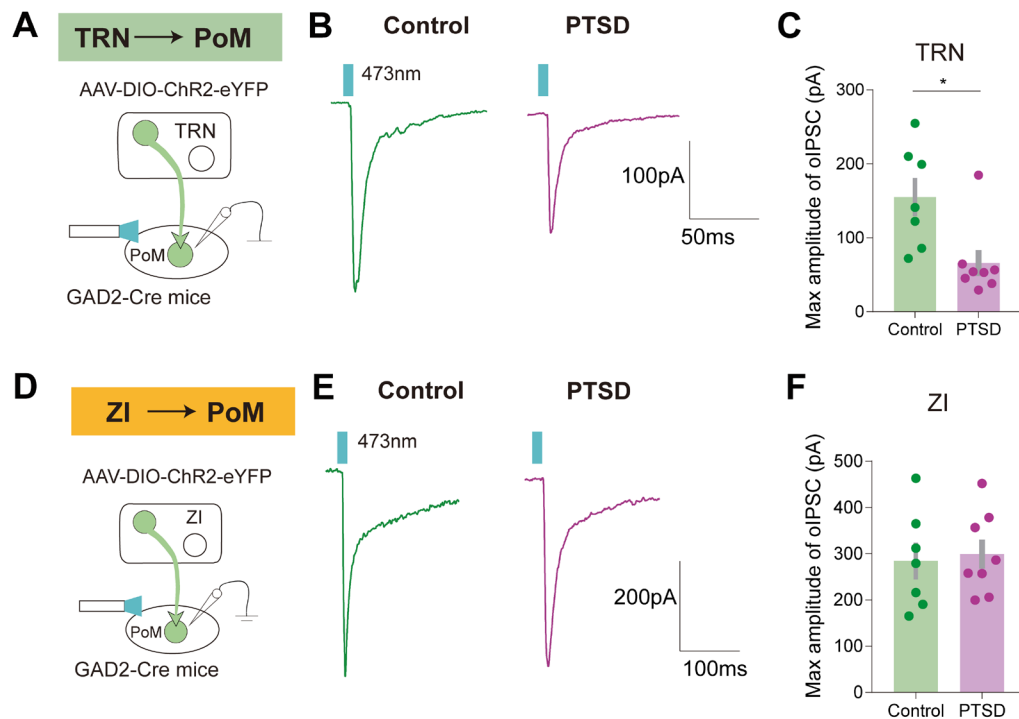


**Fig. S8. Reduced Arc expression in TRN in PTSD mice.** (A) Schematic for retrobeads injection in PoM and tissue collection strategy. (B) Representative images of injection sites in PoM. Scale bar: 250 $\mu$ m. (C) Representative images of GAD67-GFP and retrobeads in TRN. Scale bar: 250 $\mu$ m. (D) Representative images of GAD67-GFP and retrobeads in ZI. Scale bar: 100 $\mu$ m. (E) Representative images of GAD67 and Arc in TRN along the anterior (A) to posterior (P) axis between control and PTSD mice. Scale bar: 300 $\mu$ m. (F) The number of GFP<sup>+</sup> Arc<sup>+</sup> cells in TRN along the anterior (A) to posterior (P) axis between control and PTSD mice (n = 8 mice for each

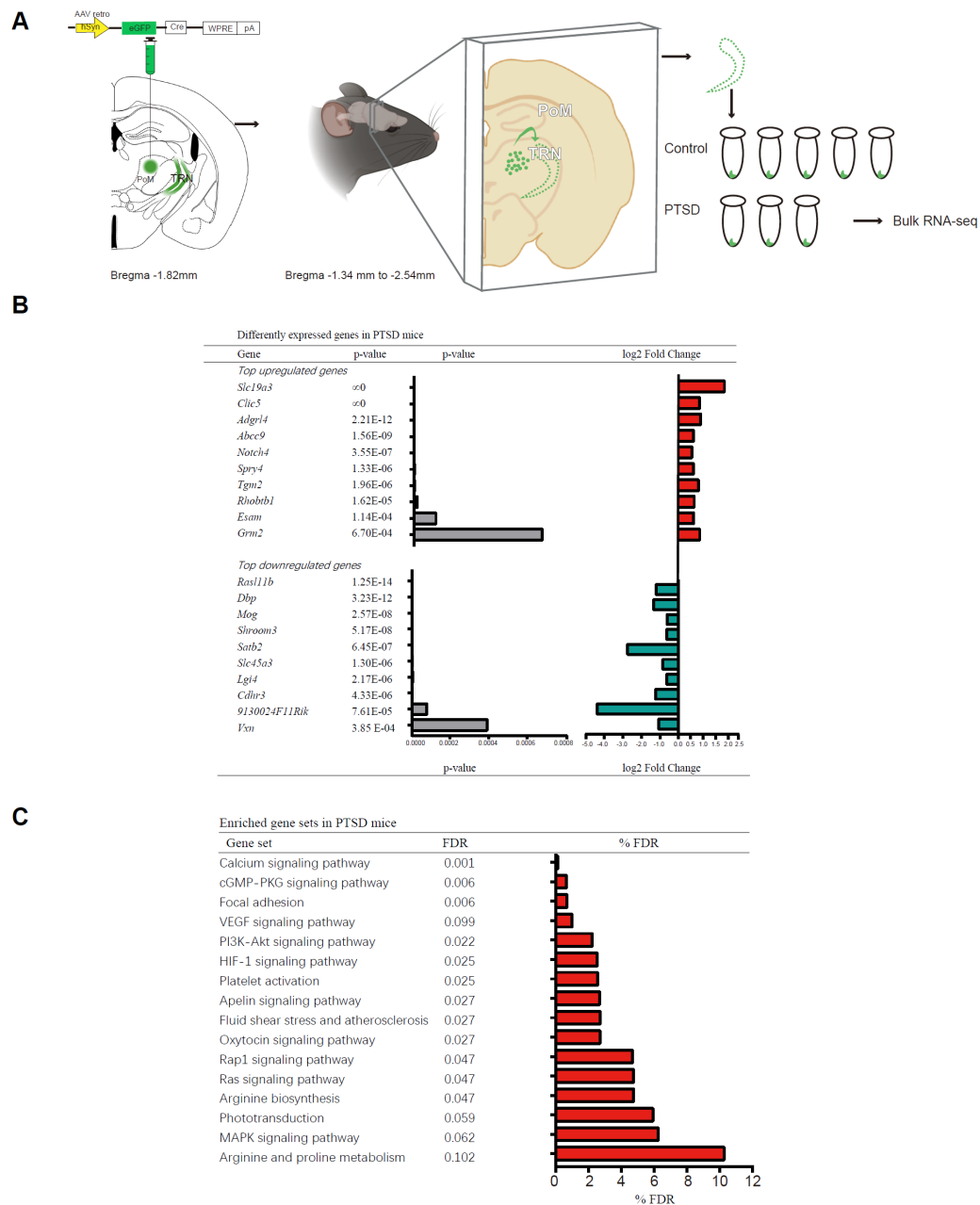
group). \*\*\*\* $P < 0.0001$ , by Friedman's M test. (G) Percentage of GFP<sup>+</sup> Arc<sup>+</sup> cells in GFP<sup>+</sup> cells in TRN in control and PTSD mice (n=8 mice for each group). \*\*\* $P < 0.001$ , by Two-tailed unpaired t-test. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



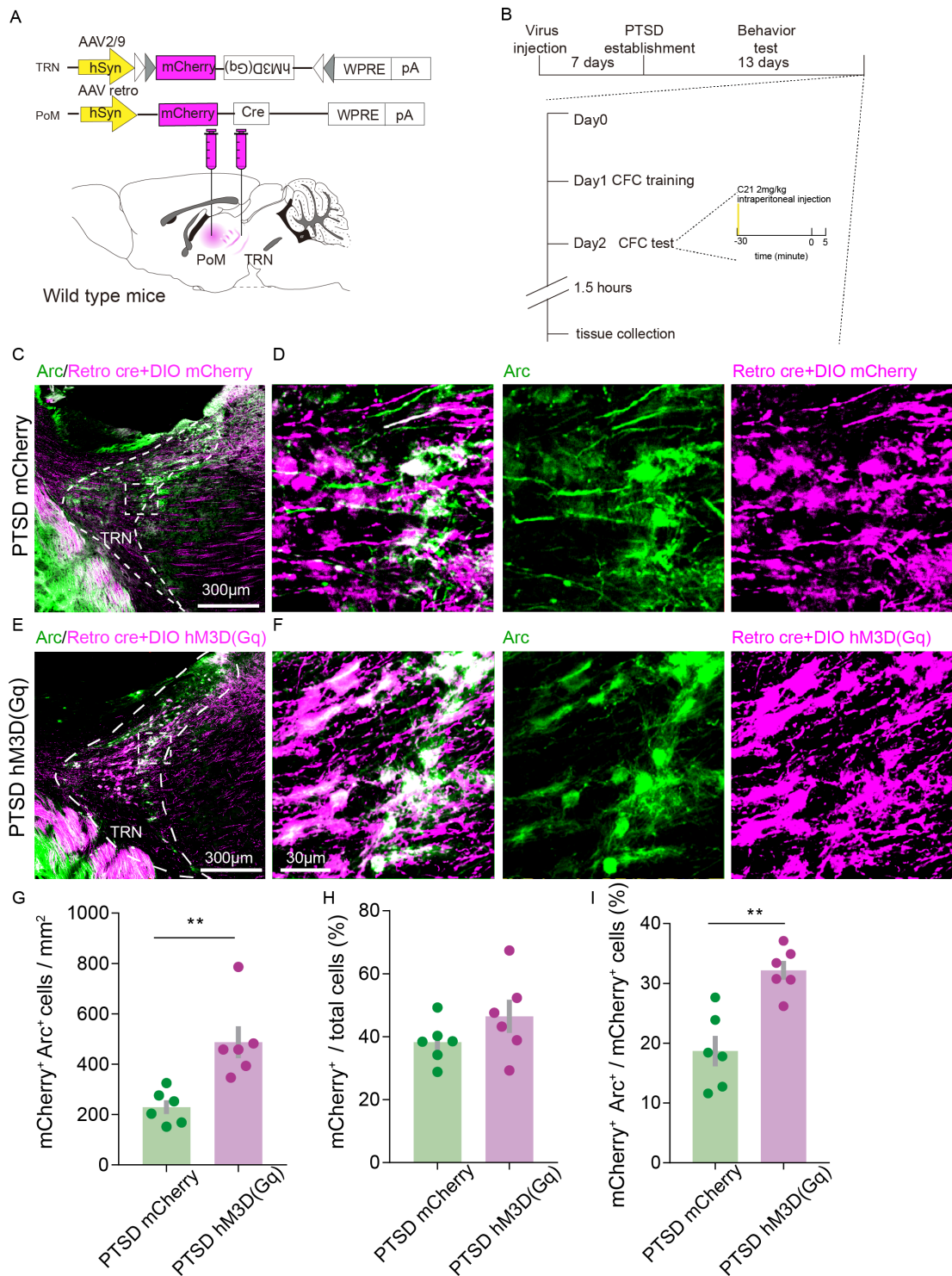
**Fig. S9. Intact Arc expression in ZI in PTSD mice.** (A) Schematic for tissue collection. (B-C) Representative images of GAD67 and Arc in ZI in control mice. Scale bar: 100  $\mu\text{m}$  and 30  $\mu\text{m}$ . (D-E) Representative images of GAD67 and Arc in ZI in PTSD mice. Scale bar: 100  $\mu\text{m}$  and 30  $\mu\text{m}$ . (F) The number of GFP<sup>+</sup> Arc<sup>+</sup> cells in ZI between control and PTSD mice (n = 8 mice for control group and n = 7 mice for PTSD group). Statistical significance was determined by Two-tailed unpaired t-test. (G) Percentage of GFP<sup>+</sup> Arc<sup>+</sup> cells in GFP<sup>+</sup> cells in control and PTSD mice (n=7-8). Statistical significance was determined by Two-tailed unpaired t-test. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



**Fig. S10. Impaired TRN inhibitory inputs to PoM in PTSD mice.** (A) Schematic of the viral strategy and slice recordings in PoM evoked by photoactivation of TRN terminals. (B) Representative oIPSC evoked by 473nm light stimulation in PoM neurons received inputs from TRN in control and PTSD mice. (C) The maximal amplitude of oIPSC in control and PTSD mice ( $n = 7$  neurons for control group and  $n = 8$  neurons for PTSD group).  $*P < 0.05$ , by Two-tailed unpaired t-test. (D) Schematic of the viral strategy and slice recordings in PoM evoked by photoactivation of ZI terminals. (E) Representative oIPSC evoked by 473nm light stimulation in PoM neurons received inputs from ZI in control and PTSD mice. (F) The maximal amplitude of oIPSC in control and PTSD mice ( $n = 7$  neurons for control group and  $n = 8$  neurons for PTSD group). Statistical significance was determined by Two-tailed unpaired t-test. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



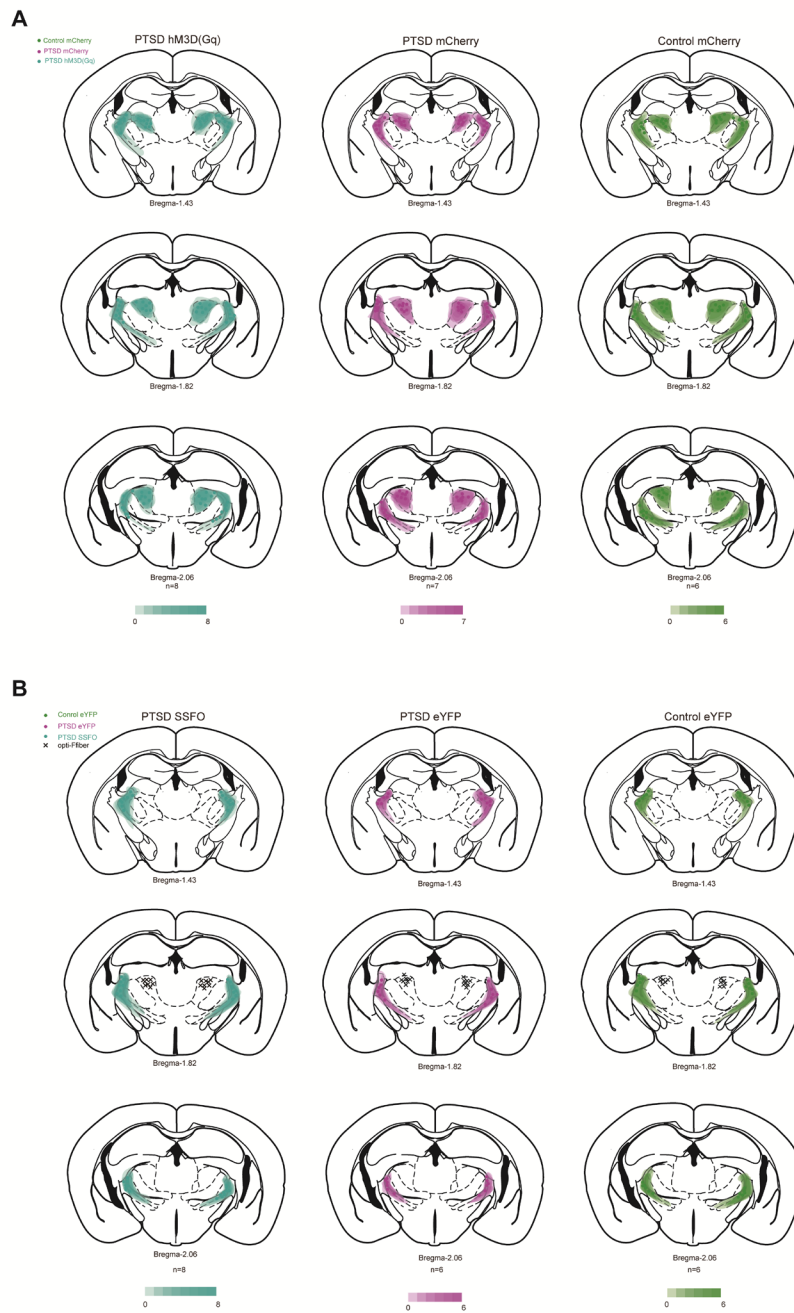
**Fig. S11. Dysregulated genes and pathways of somatosensory TRN in PTSD mice.** (A) Schematic of the viral strategy and tissue collection for bulk RNA seq from 6 control mice and 3 PTSD mice. (B) Table of the top ten upregulated (top) and downregulated (bottom) differentially expressed genes. Differential expression analysis using DEseq2 was performed on a TPM expression matrix from RNA sequencing libraries generated from TRN when comparing control and PTSD mice. (C) Enriched gene sets in PTSD mice. Gene set enrichment analysis was performed on RNA sequencing libraries generated from TRN when comparing control and PTSD mice. Table of enriched gene sets with FDR below 12%.



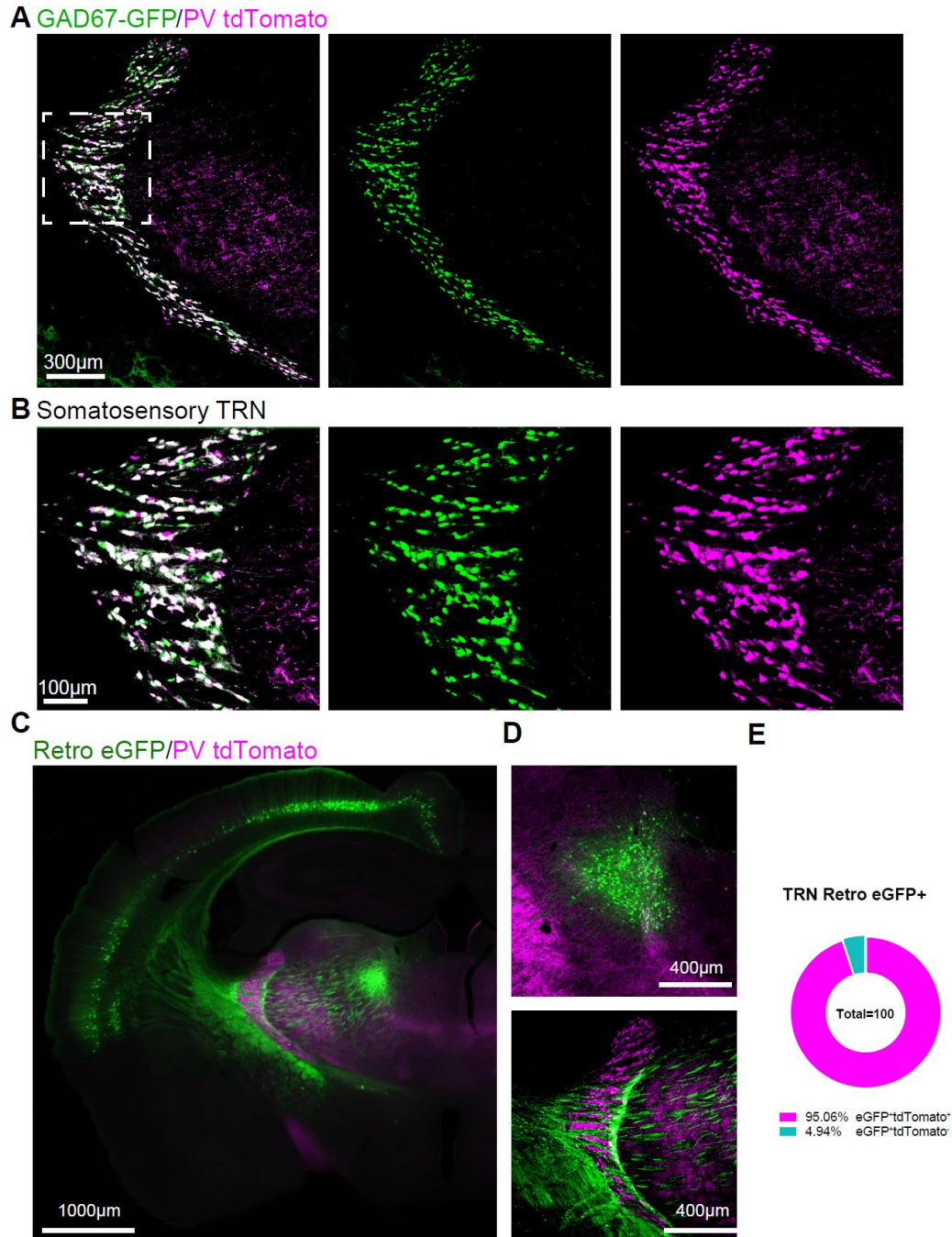
**Fig. S12. hM3D(Gq) increased Arc expression in TRN neurons in PTSD mice.** (A) Schematic of the viral strategy to chemo-genetically activate TRN neurons projecting to PoM. (B) Schematic of behavioral tests and immunohistochemical fluorescence staining. (C-D) Representative images showing mCherry expression and Arc expression in TRN. Scale bar: 300 µm (left) and 30 µm (right). (E) Representative images showing hM3D(Gq)-mCherry and Arc expression in TRN. Scale bar: 300 µm (left) and 30 µm (right). (G) The number of mCherry<sup>+</sup> Arc<sup>+</sup> cells (n = 6 mice for each group). (H) Percentage of mCherry<sup>+</sup> cells in TRN cells (n = 6 mice for each group). (I)

Percentage of mCherry<sup>+</sup> Arc<sup>+</sup> cells in mCherry<sup>+</sup> cells (n = 6 mice for each group). \*\**P* < 0.01, by Two-tailed unpaired t-test. Data are presented as the mean ± S.E.M. See Table S1 for detailed statistical information.

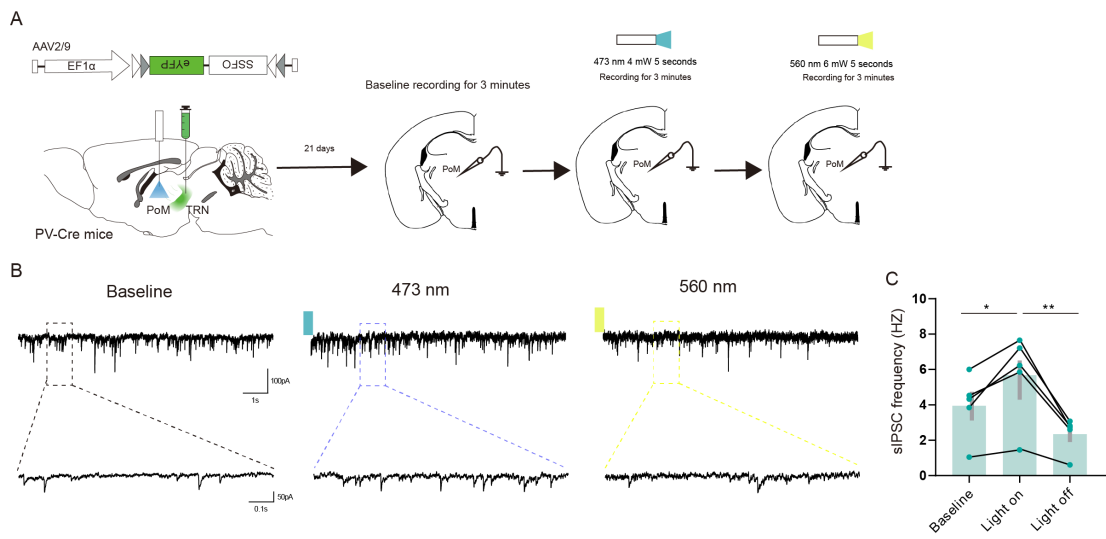




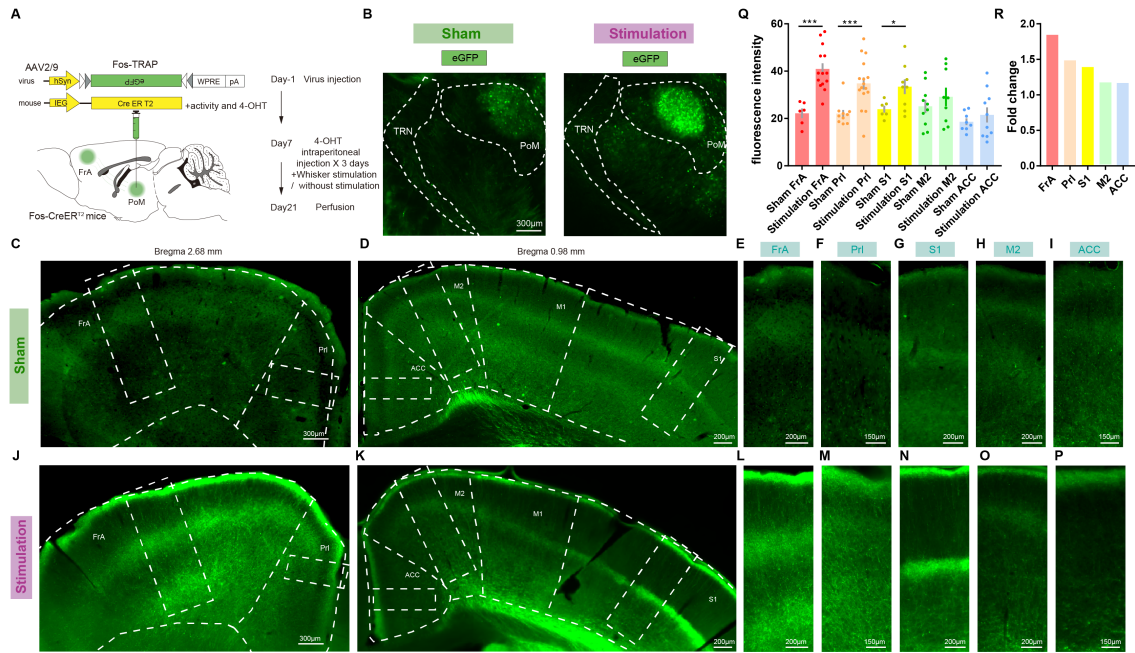
**Fig. S13. Verification of the region showing virus infection for optogenetics and DREADDs activation. (A) Schematic showing areas expressing hM3D(Gq) or mCherry in TRN or PoM. (B) Schematic showing areas expressing SSFO or eYFP in TRN.**



**Fig. S14. Verification of PV expression in TRN and PoM projecting cells.** (A) Representative images of TRN neurons in GAD67-GFP<sup>+</sup> and PV tdTomato<sup>+</sup> mice. Scale bar: 300 µm. (B) Representative images of neurons in somatosensory TRN in GAD67-GFP<sup>+</sup> and PV tdTomato<sup>+</sup> mice. Scale bar: 100 µm. (C) A low-magnification image showing eGFP expression in PV tdTomato<sup>+</sup> mice. Scale bar: 1000 µm. (D) Representative images of Retro-eGFP virus expression in PoM (up) and TRN (bottom). Scale bar: 400 µm. (E) Percentage of eGFP<sup>+</sup> tdTomato<sup>+</sup> cells in eGFP<sup>+</sup> cells in TRN.

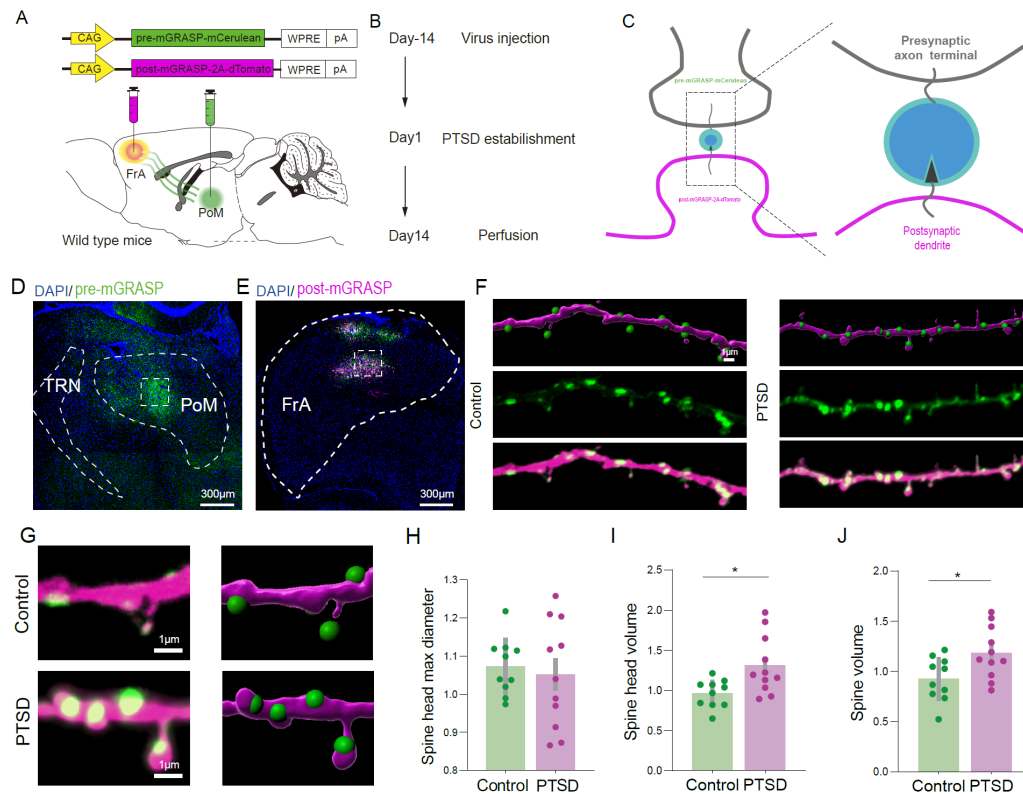


**Fig. S15. SSFO enhanced the inhibitory input to PoM.** (A) Schematic of the viral strategy and slice recordings. (B) Representative sIPSCs of PoM neurons received illuminating of 473nm and 560nm light. Left: Baseline of sIPSCs. Middle: sIPSCs with 473nm light stimulation. Right: sIPSCs with 560nm light stimulation. (C) Summary of the frequency of sIPSCs ( $n = 5$  neurons from 3 experimental mice).  $*P < 0.05$ ,  $**P < 0.01$ , by Two-tailed paired t-test. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.

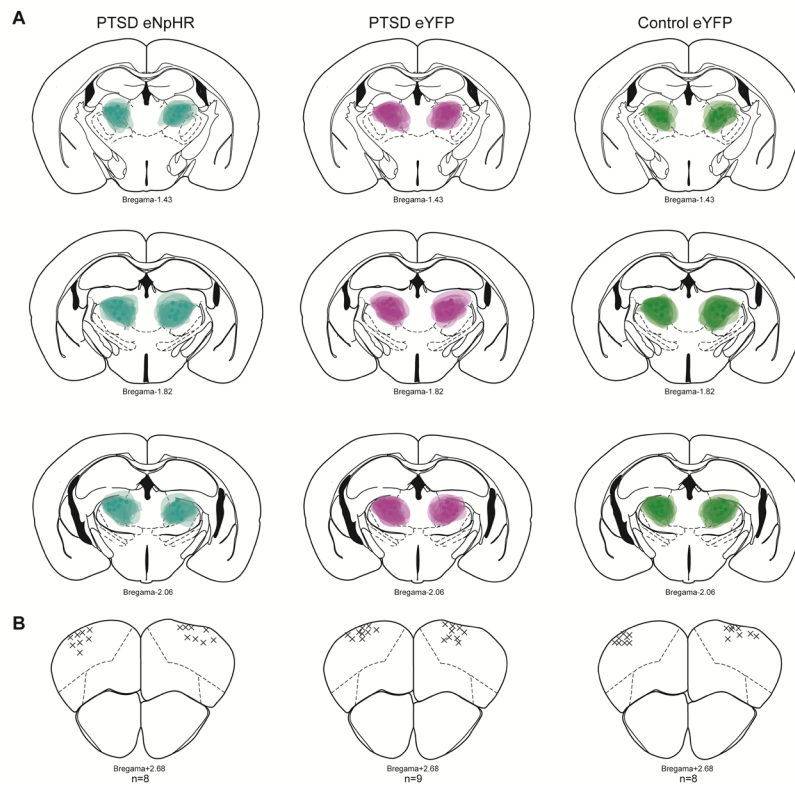


**Fig. S16. TRAPing axons of PoM cells responding to tactile threats in the cortex.** (A) Schematic of Fos-TRAP strategy for anterograde tracing of the downstream regions of PoM. (B) Representative images showing eGFP expression in PoM under low magnification in a mouse with sham stimulation and whisker stimulation. Scale bar: 300 $\mu$ m. (C) Representative images of FrA and Pr1 under low magnification in a mouse with sham stimulation. Scale bar: 300 $\mu$ m. (D) Representative images of M2, ACC and S1 under low magnification in a mouse with sham stimulation. Scale bar: 200 $\mu$ m. (E) Representative images of FrA under high magnification in a mouse with sham stimulation. Scale bar: 200 $\mu$ m. (F) Representative images of Pr1 under high magnification in a mouse with sham stimulation. Scale bar: 150 $\mu$ m. (G) Representative images of S1 under high magnification in a mouse with sham stimulation. Scale bar: 200 $\mu$ m. (H) Representative images of M2 under high magnification in a mouse with sham stimulation. Scale bar: 200 $\mu$ m. (I) Representative images of ACC under high magnification in a mouse with sham stimulation. Scale bar: 150 $\mu$ m. (J) Representative images of FrA and Pr1 under low magnification in a mouse with whisker stimulation. Scale bar: 300 $\mu$ m. (K) Representative images of M2, ACC and S1 under low magnification in a mouse with whisker stimulation. Scale bar: 200 $\mu$ m. (L) Representative images of FrA under high magnification in a mouse with whisker stimulation. Scale bar: 200 $\mu$ m. (M) Representative images of Pr1 under high magnification in a mouse with whisker stimulation. Scale bar: 150 $\mu$ m. (N) Representative images of S1 under high magnification

in a mouse with whisker stimulation. Scale bar: 200 $\mu$ m. **(O)** Representative images of M2 under high magnification in a mouse with whisker stimulation. Scale bar: 200 $\mu$ m. **(P)** Representative images of ACC under high magnification in a mouse with whisker stimulation. Scale bar: 150 $\mu$ m. **(Q)** Fluorescence intensity of different cortex in the downstream of PoM using Fos-TRAP strategy (n = 6 slices from 3 sham mice and 14 slices from 3 stimulation mice for FrA statistics; n = 10 slices from 3 sham mice and 16 slices from 3 stimulation mice for Prl statistics; n = 6 slices from 3 sham mice and 9 slices from 3 stimulation mice for S1 statistics; n = 10 slices from 3 sham mice and 9 slices from 3 stimulation mice for M2 statistics; n = 8 slices from 3 sham mice and 10 slices from 3 stimulation mice for ACC statistics.). **(R)** Fold change of fluorescence intensity of different cortex in the downstream of PoM using Fos-TRAP strategy. \* $P < 0.05$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ , by Two-tailed paired t-test. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



**Fig. S17. Altered spine morphology in the PoM-FrA pathway in PTSD mice.** (A) Schematic of viral strategy to selectively label synapses in FrA originating from PoM. (B) Schematic of sample preparation. (C) Schematic of dual-enhanced green fluorescent protein reconstitution across synaptic partners (dual-eGRASP). (D) Representative images showing pre-mGRASP expression in PoM. Scale bar: 300 $\mu$ m. (E) Representative images showing post-mGRASP expression in FrA. Scale bar: 300 $\mu$ m. (F) Representative images of synaptic inputs from PoM in a single FrA neuron under low magnification in control and PTSD mice. (G) Representative images of synaptic inputs from PoM in a single FrA neuron under low magnification in control and PTSD mice. (H) Max diameter of spine head in control and PTSD mice (n = 10 slices from 3 mice for control group and n = 11 slices from 3 mice for PTSD group). By Two-tailed unpaired t-test. (I) Head volume of spine in control and PTSD mice (n = 10-11 slices from 3 mice each). \* $P < 0.05$ , by Two-tailed unpaired t-test. (J) Spine volume in control and PTSD mice (n = 10-11 slices from 3 mice each). \* $P < 0.05$ , by Two-tailed unpaired t-test. Data are presented as the mean  $\pm$  S.E.M. See Table S1 for detailed statistical information.



**Fig. S18. Verification of the region showing virus infection and fiber placements for optogenetic inhibition. (A) Schematic showing areas expressing eNpHR in PoM. (B) Schematic showing optical fiber site in FrA**

**Table S1. Statistical table**

Statistical table										
Data	Response variable	Groups	n define as	Normality Test (Shapiro-Wilk)	Homogeneity of variance test (Levene's test)	Hypothesis test	Test value	P value	summary	
Figure 1C	Response score (Total)	control n=7	mouse	W=0.9151 df=7 p=0.4324	F=2 df1=1 df2=11 p=0.4196	Mann-Whitney U test	U=0	p=0.0006	***	
		PTSD n=7		W=0.6644 df=7 p=0.0015						
Figure 1E	Freezing time (%) in the Day 2 CFC test	control n=10	mouse	W=0.9222 df=10 p=0.3755	F=5.089 df1=1 df2=19 p=0.0238	Two-tailed unpaired t-test	t=2.399 df=18	p=0.0275	*	
		PTSD n=10		W=0.899 df=10 p=0.2138						
Figure 1F	Freezing time (%) in the Day 7 CFC test	control n=10	mouse	W=0.9356 df=10 p=0.5056	F=17.36 df1=1 df2=19 p=0.0002	Two-tailed unpaired t-test	t=2.118 df=18	p=0.0484	*	
		PTSD n=10		W=0.8484 df=10 p=0.0556						
Figure 2F	PoM Arc	control n=10 PTSD n=9	mouse	W=0.8957 df=10 p=0.1963 W=0.8664 df=9 p=0.1123	F=11.33 df1=1 df2=17 p=0.0014	Two-tailed unpaired t-test	t=3.122 df=17	p=0.0062	**	
Figure 2G	VPM Arc	control n=10 PTSD n=9	mouse	W=0.9648 df=10 p=0.8389 W=0.958 df=9 p=0.7767	F=1.976 df1=1 df2=17 p=0.3303	Two-tailed unpaired t-test	t=0.469 df=17	p=0.6450	ns	
Figure 2J	Area under curve in fiber recording	control n=7 PTSD n=5	mouse	W=0.9625 df=7 p=0.1594 W=0.8784 df=5 p=0.3022	F=1.849 df1=1 df2=10 p=0.3303	Two-tailed unpaired t-test	t=2.538 df=10	p=0.0294	*	
Figure 2N	Event frequency in the Day 2 CFC test in fiber recording	control n=5 PTSD n=4	mouse	W=0.8853 df=5 p=0.3342 W=0.8317 df=4 p=0.1724	F=6.116 df1=1 df2=7 p=0.1127	Two-tailed unpaired t-test	t=2.89 df=7	p=0.0233	*	
Figure 2P	Event frequency in the Day 7 CFC test in fiber recording	control n=5 PTSD n=4	mouse	W=0.9385 df=5 p=0.6557 W=0.8844 df=4 p=0.3577	F=41.39 df1=1 df2=7 p=0.0036	Two-tailed unpaired t-test	t=2.629 df=7	p=0.0339	*	
Figure 3D	Response score (Total)	Control eGFP n=10	mouse	W=0.9674 df=10 p=0.8654	F=0.3346 df1=2 df2=21 p=0.7194	One-way ANOVA	F=25.35 df1=1 df2=21 p<0.0001	p Control eGFP vs. PTSD eGFP<0.0001	****	
		PTSD eGFP n=7		W=0.9671 df=7 p=0.8766						
		PTSD Kir2.1 n=7		W=0.9654 df=7 p=0.8632						
Figure 3E	Freezing time (%) in the Day 2 CFC test	Control eGFP n=10	mouse	W=0.8827 df=10 p=0.1402	F=0.04811 df1=2 df2=21 p=0.9531	One-way ANOVA	F=10.03 df1=1 df2=21 p=0.0009	p Control eGFP vs. PTSD Kir2.1=0.4084	***	
		PTSD eGFP n=7		W=0.941 df=7 p=0.6475						
		PTSD Kir2.1 n=7		W=0.9468 df=7 p=0.7008						
Figure 3F	Freezing time (%) in the Day 7 CFC test	Control eGFP n=10	mouse	W=0.9557 df=10 p=0.7360	F=1.974 df1=2 df2=21 p=0.1639	One-way ANOVA	F=5.265 df1=1 df2=21 p=0.0140	p Control eGFP vs. PTSD Kir2.1=0.9749	*	
		PTSD eGFP n=7		W=0.971 df=7 p=0.9056						
		PTSD Kir2.1 n=7		W=0.9613 df=7 p=0.8297						
Figure 3J	Response score (Total)	Control mCherry n=10	mouse	W=0.8752 df=10 p=0.1149	F=0.2484 df1=2 df2=19 p=0.7825	One-way ANOVA	F=11.09 df1=1 df2=21 p=0.0006	p Control mCherry vs. PTSD mCherry=0.0016	***	
		PTSD mCherry n=6		W=0.9076 df=6 p=0.4207						
		PTSD hM4D (Gi) n=6		W=0.9713 df=6 p=0.9009						
Figure 3K	Freezing time (%) in the Day 2 CFC test	Control mCherry n=10	mouse	W=0.9558 df=10 p=0.7374	F=1.834 df1=2 df2=19 p=0.1870	One-way ANOVA	F=12.8 df1=1 df2=21 p=0.0003	p Control mCherry vs. PTSD mCherry=0.0002	***	
		PTSD mCherry n=6		W=0.9063 df=6 p=0.4128						
		PTSD hM4D (Gi) n=6		W=0.9776 df=6 p=0.9390						
Figure 3L	Freezing time (%) in the Day 7 CFC test	Control mCherry n=10	mouse	W=0.8667 df=10 p=0.0915	F=0.1991 df1=2 df2=19 p=0.8212	One-way ANOVA	F=11.93 df1=1 df2=21 p=0.0004	p Control mCherry vs. PTSD hM4D (Gi)=0.0114	***	
		PTSD mCherry n=6		W=0.9172 df=6 p=0.4854						
		PTSD hM4D (Gi) n=6		W=0.9225 df=6 p=0.5232						
Figure 4E	Density of TRN GFP+ Arc+	control n=12 PTSD n=12	mouse	W=0.9548 df=12 p=0.7077 W=0.9344 df=12 p=0.4292	F=8.621 df1=1 df2=22 p=0.0012	Two-tailed unpaired t-test	t=5.271 df=22	p<0.0001	****	
Figure 4F	Percentage of GFP+ Arc+ / GFP+ cells in TRN	control n=12 PTSD n=12	mouse	W=0.9637 df=12 p=0.8354 W=0.9531 df=12 p=0.6824	F=8.134 df1=1 df2=22 p=0.0016	Two-tailed unpaired t-test	t=5.258 df=22	p<0.0001	****	
Figure 4I	TRN-PoM PPR	50ms control n=7 50ms PTSD n=8	neuron	W=0.908 df=7 p=0.3825 W=0.7552 df=8 p=0.0093	F=8.127 df1=1 df2=13 p=0.0208	Mann-Whitney U test	U=6	p=0.0093	**	
		100ms control n=7 100ms PTSD n=8	neuron	W=0.9489 df=7 p=0.7195 W=0.8858 df=8 p=0.2138	F=15 df1=1 df2=13 p=0.0040	Two-tailed unpaired t-test	t=3.21 df=13	p=0.0068	**	
		200ms control n=7 200ms PTSD n=8	neuron	W=0.9087 df=7 p=0.3868 W=0.8987 df=8 p=0.2810	F=4.801 df1=1 df2=13 p=0.0744	Two-tailed unpaired t-test	t=5.293 df=13	p=0.0001	***	
		control n=7 PTSD n=8	neuron	Not all subgroups meets p>=0.05	Not all subgroups meets p>=0.05	Friedman's M test	X <sup>2</sup> =21.000 df=1		p < 0.0001	****



**Statistical table**

Data	Response variable	Groups	n define as	Normality Test (Shapiro-Wilk)	Homogeneity of variance test (Levene's test)	Hypothesis test	Test value	P value	summary
Figure 4L	ZI-PoM PPR	50ms control n=8	neuron	W=0.975 df=8 p=0.9340	F=1.726 df1=1 df2=13 p=0.4912	Mann-Whitney U test	U=14	p=0.1206	ns
		50ms PTSD n=7		W=0.7441 df=7 p=0.0110					
		100ms control n=8	neuron	W=0.9354 df=8 p=0.5668	F=1.041 df1=1 df2=13 p=0.9446				
		100ms PTSD n=7		W=0.8728 df=7 p=0.1963					
		200ms control n=8	neuron	W=0.9431 df=8 p=0.6420	F=1.599 df1=1 df2=13 p=0.5513				
		200ms PTSD n=7		W=0.8403 df=7 p=0.1000					
control n=8 PTSD n=7	neuron	Not all subgroups meets p > 0.05	Not all subgroups meets p > 0.05	Friedman's M test	$\chi^2=2.333$ df=1	p =0.2385	ns		
Figure 5D	Response score (Total)	Control mCherry n=6	mouse	W=0.8221 df=6 p=0.0921	F=0.5297 df1=2 df2=18 p=0.5977	One-way ANOVA	F=39.58 df1=1 df2=18 p<0.0001	p Control mCherry vs. PTSD mCherry <0.0001 p Control mCherry vs. PTSD hM3D (Gq) =0.9242 p PTSD mCherry vs. PTSD hM3D (Gq) <0.0001	****
		PTSD mCherry n=7		W=0.9666 df=7 p=0.8733					
		PTSD hM3D (Gq) n=8		W=0.8352 df=8 p=0.0672					
Figure 5E	Freezing time (%) in the Day 2 CFC test	Control mCherry n=6	mouse	W=0.9113 df=6 p=0.4449	F=0.183 df1=2 df2=18 p=0.8343	One-way ANOVA	F=44.58 df1=1 df2=18 p<0.0001	p Control mCherry vs. PTSD mCherry <0.0001 p Control mCherry vs. PTSD hM3D (Gq) =0.1324 p PTSD mCherry vs. PTSD hM3D (Gq) <0.0001	****
		PTSD mCherry n=7		W=0.8351 df=7 p=0.0895					
		PTSD hM3D (Gq) n=8		W=0.9689 df=8 p=0.8894					
Figure 5F	Freezing time (%) in the Day 7 CFC test	Control mCherry n=6	mouse	W=0.9076 df=6 p=0.4210	F=2.512 df1=2 df2=18 p=0.1091	Kruskal-Wallis H test	Kruskal-Wallis statistic=13.73 p<0.0001	p Control mCherry vs. PTSD mCherry =0.0022 p Control mCherry vs. PTSD hM3D (Gq) >0.9999 p PTSD mCherry vs. PTSD hM3D (Gq) =0.0082	****
		PTSD mCherry n=7		W=0.8048 df=7 p=0.0457					
		PTSD hM3D (Gq) n=8		W=0.8818 df=8 p=0.1959					
Figure 5J	Response score (total)	Control eYFP n=6	mouse	W=0.7511 df=6 p=0.0204	F=0.2371 df1=2 df2=17 p=0.7915	Kruskal-Wallis H test	Kruskal-Wallis statistic=11.76 p=0.0005	p Control eYFP vs. PTSD eYFP =0.0123 p Control eYFP vs. PTSD SSFO >0.9999 p PTSD eYFP vs. PTSD SSFO =0.0054	***
		PTSD eYFP n=6		W=0.7709 df=6 p=0.0317					
		PTSD SSFO n=8		W=0.935 df=8 p=0.5630					
Figure 5K	Freezing time (%) in the Day 2 CFC test PTSD SSFO	off n=8 on n=8	mouse	W=0.7526 df=8 p=0.0087 W=0.7872 df=8 p=0.0208	F=2.987 df1=1 df2=14 p=0.1721	Wilcoxon signed-rank test	rs=0.6667 p=0.0415	p=0.0078	**
Figure 5K	Freezing time (%) in the Day 2 CFC test Control eYFP	off n=6 on n=6	mouse	W=0.9564 df=6 p=0.7913 W=0.7895 df=6 p=0.0472	F=9.1114 df1=1 df2=10 p=0.0299	Wilcoxon signed-rank test	rs=0.2571 p=0.3292	p=0.4375	ns
Figure 5K	Freezing time (%) in the Day 2 CFC test PTSD eYFP	off n=6 on n=6	mouse	W=0.8184 df=6 p=0.70854 W=0.9537 df=6 p=0.7704	F=1.08 df1=1 df2=10 p=0.9351	Two-tailed paired t-test	t=0.02308 df=10	p=0.9825	ns
Figure 5L	Freezing time (%) in the Day 7 CFC test Control eYFP	off n=6 on n=6	mouse	W=0.7557 df=6 p=0.0226 W=0.7896 df=6 p=0.0472	F=1.657 df1=1 df2=10 p=0.5931	Wilcoxon signed-rank test	rs=0.6 p=0.1208	p=0.0625	ns
Figure 5L	Freezing time (%) in the Day 7 CFC test PTSD SSFO	off n=8 on n=8	mouse	W=0.9134 df=8 p=0.3786 W=0.8959 df=8 p=0.2651	F=3.225 df1=1 df2=14 p=0.1452	Two-tailed paired t-test	t=2.738 df=14	p=0.0160	*
Figure 5L	Freezing time (%) in the Day 7 CFC test PTSD eYFP	off n=6 on n=6	mouse	W=0.6638 df=6 p=0.0025 W=0.908 df=6 p=0.4233	F=1.03 df1=1 df2=10 p=0.9753	Wilcoxon signed-rank test	rs=0.7143 p=0.0681	p=0.2188	ns
Figure 6Q	CNQX in FrA	baseline n=5 neurons from 3 mice CNQX n=5 neurons from 3 mice	neuron	W=0.8666 df=6 p=0.2529 W=0.8601 df=6 p=0.2286	F=859.2 df1=1 df2=8 p<0.0001	Two-tailed paired t-test	t=5.607 df=4	p=0.0050	**
Figure 7D	Response score (Total)	Control eYFP n=8	mouse	W=0.8352 df=8 p=0.0672	F=2.092 df1=2 df2=22 p=0.1474	One-way ANOVA	F=36.44 df1=1 df2=22 p<0.0001	p Control eYFP vs. PTSD eYFP <0.0001 p Control eYFP vs. PTSD eNPHR =0.7749 p PTSD eYFP vs. PTSD eNPHR <0.0001	****
		PTSD eNPHR n=9		W=0.9258 df=9 p=0.4423					
		PTSD eYFP n=8		W=0.8774 df=8 p=0.1780					
Figure 7E	Freezing time (%) in the Day 2 CFC test control eYFP	off n=8 on n=8	mouse	W=0.8476 df=8 p=0.0901 W=0.734 df=8 p=0.0054	F=8.437 df1=1 df2=14 p=0.0116	Wilcoxon signed-rank test	rs=0.4524 p=0.1337	p=0.1094	ns
Figure 7E	Freezing time (%) in the Day 2 CFC test PTSD eYFP	off n=9 on n=9	mouse	W=0.9538 df=9 p=0.7316 W=0.9357 df=9 p=0.5375	F=1.621 df1=1 df2=16 p=0.0116	Two-tailed paired t-test	t=1.441 df=8	p=0.1876	ns
Figure 7E	Freezing time (%) in the Day 2 CFC test PTSD eNPHR	off n=8 on n=8	mouse	W=0.9525 df=8 p=0.7364 W=0.7295 df=8 p=0.1148	F=3.164 df1=1 df2=14 p=0.1628	Wilcoxon signed-rank test	rs=0.04762 p=0.4674	p=0.0156	*

**Statistical table**

Data	Response variable	Groups	n define as	Normality Test (Shapiro-Wilk)	Homogeneity of variance test (Levene's test)	Hypothesis test	Test value	P value	summary
Figure 7F	Freezing time (%) in the Day 7 CFC test control eYFP	off n=8	mouse	W=0.9361 df=8 p=0.5730	F=7.097 df1=1 df2=14 p=0.0192	Wilcoxon signed-rank test	rs=-0.381 p=0.1799	p=0.3828	ns
		on n=8		W=0.7307 df=8 p=0.0050					
Figure 7F	Freezing time (%) in the Day 7 CFC test PTSD eYFP	off n=9	mouse	W=0.8211 df=9 p=0.0354	F=1.509 df1=1 df2=16 p=0.5740	Wilcoxon signed-rank test	rs=0.4 p=0.1456	p=0.5703	ns
		on n=9		W=0.8039 df=9 p=0.0226					
Figure 7F	Freezing time (%) in the Day 7 CFC PTSD eNPHR	off n=8	mouse	W=0.6516 df=8 p=0.0006	F=1.699 df1=1 df2=14 p=0.5012	Wilcoxon signed-rank test	rs=0.2381 p=0.2911	p=0.0078	**
		on n=8		W=0.8149 df=8 p=0.0413					
Figure S1B	Freezing time (%) in Cue test baseline	control n=10	mouse	W=0.8779 df=10 p=0.1236	F=1.316 df1=1 df2=18 p=0.6895	Two-tailed paired t-test	t=2.3 df=18	p=0.0336	*
		PTSD n=10		W=0.9316 df=10 p=0.4637					
Figure S1B	Freezing time (%) in Cue test cue	control n=10	mouse	W=0.9502 df=10 p=0.6707	F=2.42 df1=1 df2=18 p=0.2042	Two-tailed paired t-test	t=3.774 df=18	p=0.0014	**
		PTSD n=10		W=0.8847 df=10 p=0.1479					
Figure S1B	Freezing time (%) in Cue test	control n=10	mouse			Two-way ANOVA		p<0.0001	****
		PTSD n=10							
Figure S2B	Distance in area (centimeters)	control n=10	mouse	W=0.9675 df=10 p=0.8663	F=1.068 df1=1 df2=18 p=0.9234	Two-tailed unpaired t-test	t=0.2966 df=18	p=0.7702	ns
		PTSD n=10		W=0.9161 df=10 p=0.3256					
Figure S3C	Event frequency in CFC test before and after training in fiber recording in control mice	pre n=5	mouse	W=0.9767 df=5 p=0.9163	F=1.053 df1=1 df2=8 p=0.9610	Two-tailed paired t-test	t=2.005 df=8	p=0.0325	*
		post n=5		W=0.8853 df=5 p=0.3342					
Figure S3E	Event frequency in CFC test before and after training in fiber recording in PTSD mice	pre n=4	mouse	W=0.9288 df=4 p=0.5873	F=2.311 df1=1 df2=6 p=0.5093	Two-tailed paired t-test	t=1.451 df=6	p=0.0325	*
		post n=4		W=0.8317 df=4 p=0.1724					
Figure S4E	Max ML133 sensitive current (pA) in control eGFP and Kir2.1 expressed mice	control eGFP n=5	neuron	W=0.8901 df=5 p=0.3575	F=1.366 df1=1 df2=8 p=0.7700	Two-tailed unpaired t-test	t=4.195 df=8	p=0.0030	**
		Kir2.1 n=5		W=0.9131 df=5 p=0.4866					
Figure S4G	Spike number of PoM neurons in control eGFP and Kir2.1 expressed mice	control n=6 PTSD n=7	neuron			Two-way ANOVA		p=0.0462	*
Figure S5A	Freezing time (%) in Cue test in Kir baseline	Control eGFP n=7	mouse	W=0.9794 df=7 p=0.9567	F=0.9389 df1=2 df2=17 p=0.4104	One-way ANOVA	F=6.929 df1=2 df2=17 p=0.0063	p Control eGFP vs. PTSD eGFP=0.0090	**
		PTSD eGFP n=6		W=0.9491 df=6 p=0.7329				p Control eGFP vs. PTSD Kir2.1=0.0228	
		PTSD Kir2.1 n=7		W=0.9376 df=7 p=0.6176				p PTSD eGFP vs. PTSD Kir2.1=0.8419	
Figure S5A	Freezing time (%) in Cue test in Kir cue	Control eGFP n=7	mouse	W=0.907 df=7 p=0.3754	F=0.7377 df1=2 df2=17 p=0.4929	One-way ANOVA	F=6.304 df1=2 df2=17 p=0.0090	p Control eGFP vs. PTSD eGFP=0.0324	**
		PTSD eGFP n=6		W=0.8482 df=6 p=0.1522				p Control eGFP vs. PTSD Kir2.1=0.0119	
		PTSD Kir2.1 n=7		W=0.9079 df=7 p=0.3813				p PTSD eGFP vs. PTSD Kir2.1=0.9320	
Figure S5A	Freezing time (%) in Cue test in Kir	Control eGFP n=7	mouse			Two-way ANOVA		p Control eGFP vs. PTSD eGFP=0.0324	**
		PTSD eGFP n=6						p Control eGFP vs. PTSD Kir2.1=0.0119	
		PTSD Kir2.1 n=7						p PTSD eGFP vs. PTSD Kir2.1=0.9320	
Figure S5B	Freezing time (%) in Cue test in Gi baseline	Control mCherry n=9	mouse	W=0.912 df=9 p=0.3301	F=0.8938 df1=2 df2=16 p=0.4286	One-way ANOVA	F=3.091 df1=1 df2=16 p=0.0733	p Control eGFP vs. PTSD mCherry=0.2520	ns
		PTSD mCherry n=5		W=0.8825 df=5 p=0.3206				p Control eGFP vs. PTSD hM4D(Gi)=0.5039	
		PTSD hM4D(Gi) n=5		W=0.8959 df=5 p=0.3876				p PTSD eGFP vs. PTSD hM4D(Gi)=0.0601	
Figure S5B	Freezing time (%) in Cue test in Gi cue	Control mCherry n=9	mouse	W=0.7722 df=9 p=0.0098	F=2.516 df1=2 df2=16 p=0.1122	Kruskal-Wallis H test	Kruskal-Wallis statistic=4.042 p=0.1332	p Control eGFP vs. PTSD mCherry=0.5221	ns
		PTSD mCherry n=5		W=0.8797 df=5 p=0.3079				p Control eGFP vs. PTSD hM4D(Gi)=0.1848	
		PTSD hM4D(Gi) n=5		W=0.7577 df=5 p=0.0350				p PTSD eGFP vs. PTSD hM4D(Gi)>0.9999	
Figure S5B	Freezing time (%) in Cue test in Gi	Control mCherry n=9	mouse			Two-way ANOVA		p Control eGFP vs. PTSD mCherry=0.0189	*
		PTSD mCherry n=5						p Control eGFP vs. PTSD hM4D(Gi)=0.0175	
		PTSD hM4D(Gi) n=5						p PTSD eGFP vs. PTSD hM4D(Gi)=0.9996	
Figure S6E	Density of mCherry+ Arc+ cells in PoM	PTSD mCherry n=5 PTSD hM4D(Gi) n=5	mouse	W=0.9646 df=5 p=0.8397 W=0.7932 df=5 p=0.0712	F=1.348 df1=1 df2=8 p=0.7793	Two-tailed unpaired t-test	t=3.182 df=8	p=0.0130	*

**Statistical table**

Data	Response variable	Groups	n define as	Normality Test (Shapiro-Wilk)	Homogeneity of variance test (Levene's test)	Hypothesis test	Test value	P value	summary																																																																																																																																																																																																		
Figure S6F	percentage of mCherry+ cells in PoM	PTSD mCherry n=5	mouse	W=0.9056 df=5 p=0.4417	F=1.781 df1=1 df2=8 p=0.5899	Two-tailed unpaired t-test	t=1.319 df=8	p=0.2236	ns																																																																																																																																																																																																		
		PTSD hM4D(Gi) n=5		W=0.8671 df=5 p=0.2547						Figure S6G	percentage of mCherry+ Arc+ in mCherry+ cells in PoM	PTSD mCherry n=5	mouse	W=0.8805 df=5 p=0.8708	F=1.731 df1=1 df2=8 p=0.6082	Two-tailed unpaired t-test	t=2.702 df=8	p=0.0270	*	PTSD hM4D(Gi) n=5	W=0.3117 df=5 p=0.2697	Figure S8F	density of TRN GFP+ Arc+ along the bregma	control n=8 PTSD n=8	mouse	Not all subgroups meets p > 0.05	Not all subgroups meets p > 0.05	Friedman's M test	χ <sup>2</sup> =74.387 df=1	p < 0.0001	****	Figure S8G	Percentage of GFP+ Arc+ in GFP+ cells in TRN	control n=8 PTSD n=8	mouse	W=0.9618 df=8 p=0.8268 W=0.9493 df=8 p=0.7040	F=7.689 df1=1 df2=14 p=0.0153	Two-tailed unpaired t-test	t=5.207 df=14	p=0.0001	***	Figure S9F	density of ZI GFP+ Arc+ along the bregma	control n=8 PTSD n=7	mouse	W=0.9722 df=8 p=0.9145 W=0.9512 df=7 p=0.7402	F=1.364 df1=1 df2=13 p=0.7213	Two-tailed unpaired t-test	t=0.6605 df=13	p=0.5205	ns	Figure S9G	Percentage of GFP+ Arc+ in GFP+ cells in ZI	control n=8 PTSD n=7	mouse	W=0.7143 df=8 p=0.0033 W=0.8056 df=7 p=0.0465	F=1.033 df1=1 df2=13 p=0.9850	Mann-Whitney U test	U=21	p=0.4634	ns	Figure S10C	Max amplitude of oIPSC in PoM neurons received inputs from TRN	control n=7 PTSD n=8	mouse	W=0.9455 df=7 p=0.6887 W=0.6383 df=8 p=0.0004	F=1.908 df1=1 df2=13 p=0.4176	Two-tailed unpaired t-test	t=2.933 df=13	p=0.0116	*	Figure S10F	Max amplitude of oIPSC in PoM neurons received inputs from ZI	control n=7 PTSD n=8	mouse	W=0.9493 df=7 p=0.7236 W=0.9277 df=8 p=0.4954	F=1.417 df1=1 df2=13 p=0.6546	Two-tailed unpaired t-test	t=2.938 df=13	p=0.7735	ns	Figure S12G	Density of mCherry+ Arc+ cells in TRN	control n=6 PTSD n=6	mouse	W=0.9546 df=6 p=0.7776 W=0.7919 df=6 p=0.0497	F=5.376 df1=1 df2=10 p=0.0886	Mann-Whitney U test	U=0	p=0.0022	**	Figure S12H	Percentage of mCherry+ cells in TRN	control n=6 PTSD n=6	mouse	W=0.9561 df=6 p=0.7894 W=0.982 df=6 p=0.9611	F=3.619 df1=1 df2=10 p=0.1844	Two-tailed unpaired t-test	t=1.376 df=10	p=1.1990	ns	Figure S12I	Percentage of mCherry+ Arc+ in mCherry+ cells in TRN	control n=6 PTSD n=6	mouse	W=0.9409 df=6 p=0.6661 W=0.9721 df=6 p=0.9062	F=2.625 df1=1 df2=10 p=0.3130	Two-tailed unpaired t-test	t=4.513 df=10	p=0.0011	**	Figure S15C	sIPSC frequency between baseline and light on	control n=5 PTSD n=5	neuron	W=0.9108 df=5 p=0.4727 W=0.8102 df=5 p=0.0978	F=1.86 df1=1 df2=18 p=0.5625	Two-tailed unpaired t-test	t=1.261 df=8	p=0.0229	*	Figure S15C	sIPSC frequency between light on and light off	control n=5 PTSD n=5	neuron	W=0.8102 df=5 p=0.0978 W=0.7183 df=5 p=0.0147	F=6.145 df1=1 df2=18 p=0.1066	Two-tailed unpaired t-test	t=2.831 df=8	p=0.0079	**	Figure S16Q	Fluorescence intensity in FrA between sham and stimulation	control n=6 slices from 3 mice PTSD n=14 slices from 3 mice	Slice	W=0.9098 df=6 p=0.4349 W=0.9395 df=14 p=0.4119	F=3.373 df1=1 df2=18 p=0.1874	Two-tailed unpaired t-test	t=4.713 df=18	p=0.0002	***	Figure S16Q	Fluorescence intensity in PrI between sham and stimulation	control n=10 slices from 3 mice PTSD n=16 slices from 3 mice	Slice	W=0.7428 df=10 p=0.0029 W=0.9661 df=16 p=0.7113	F=4.349 df1=1 df2=24 p=0.0313	Two-tailed unpaired t-test	t=3.617 df=24	p=0.0014	**	Figure S16Q	Fluorescence intensity in S1 between sham and stimulation	control n=6 slices from 3 mice PTSD n=9 slices from 3 mice	Slice	W=0.956 df=6 p=0.7881 W=0.958 df=9 p=0.7777	F=6.911 df1=1 df2=13 p=0.0477	Two-tailed unpaired t-test	t=2.366 df=13	p=0.0342	*	Figure S16Q	Fluorescence intensity in M2 between sham and stimulation	control n=10 slices from 3 mice PTSD n=9 slices from 3 mice	Slice	W=0.9519 df=10 p=0.6915 W=0.8985 df=9 p=0.2435	F=1.73 df1=1 df2=17 p=0.4309	Two-tailed unpaired t-test	t=0.8623 df=17	p=0.4005	ns	Figure S16Q	Fluorescence intensity in ACC between sham and stimulation	control n=8 slices from 3 mice PTSD n=10 slices from 3 mice	Slice	W=0.938 df=8 p=0.5918 W=0.9138 df=10 p=0.3084	F=6.35 df1=1 df2=16 p=0.0235	Two-tailed unpaired t-test	t=0.7565 df=16	p=0.4603	ns	Figure S17H	Spine head max diameter	control n=10 slices in 3 mice PTSD n=11 slices in 3 mice	Slice	W=0.9405 df=10 p=0.5584 W=0.9308 df=11 p=0.4187	F=3.552 df1=1 df2=19 p=0.0698	Two-tailed unpaired t-test	t=0.4347 df=19	p=0.6687	ns	Figure S17I	Spine head volume	control n=10 slices in 3 mice PTSD n=11 slices in 3 mice	Slice	W=0.9514 df=8 p=0.6850 W=0.8972 df=7 p=0.1709	F=4.42 df1=1 df2=19 p=0.0354	Two-tailed unpaired t-test	t=2.752 df=19	p=0.0127	*	Figure S17J	Spine volume
Figure S6G	percentage of mCherry+ Arc+ in mCherry+ cells in PoM	PTSD mCherry n=5	mouse	W=0.8805 df=5 p=0.8708	F=1.731 df1=1 df2=8 p=0.6082	Two-tailed unpaired t-test	t=2.702 df=8	p=0.0270	*																																																																																																																																																																																																		
		PTSD hM4D(Gi) n=5		W=0.3117 df=5 p=0.2697						Figure S8F	density of TRN GFP+ Arc+ along the bregma	control n=8 PTSD n=8	mouse	Not all subgroups meets p > 0.05	Not all subgroups meets p > 0.05	Friedman's M test	χ <sup>2</sup> =74.387 df=1	p < 0.0001	****	Figure S8G	Percentage of GFP+ Arc+ in GFP+ cells in TRN	control n=8 PTSD n=8	mouse	W=0.9618 df=8 p=0.8268 W=0.9493 df=8 p=0.7040	F=7.689 df1=1 df2=14 p=0.0153	Two-tailed unpaired t-test	t=5.207 df=14	p=0.0001	***	Figure S9F	density of ZI GFP+ Arc+ along the bregma	control n=8 PTSD n=7	mouse	W=0.9722 df=8 p=0.9145 W=0.9512 df=7 p=0.7402	F=1.364 df1=1 df2=13 p=0.7213	Two-tailed unpaired t-test	t=0.6605 df=13	p=0.5205	ns	Figure S9G	Percentage of GFP+ Arc+ in GFP+ cells in ZI	control n=8 PTSD n=7	mouse	W=0.7143 df=8 p=0.0033 W=0.8056 df=7 p=0.0465	F=1.033 df1=1 df2=13 p=0.9850	Mann-Whitney U test	U=21	p=0.4634	ns	Figure S10C	Max amplitude of oIPSC in PoM neurons received inputs from TRN	control n=7 PTSD n=8	mouse	W=0.9455 df=7 p=0.6887 W=0.6383 df=8 p=0.0004	F=1.908 df1=1 df2=13 p=0.4176	Two-tailed unpaired t-test	t=2.933 df=13	p=0.0116	*	Figure S10F	Max amplitude of oIPSC in PoM neurons received inputs from ZI	control n=7 PTSD n=8	mouse	W=0.9493 df=7 p=0.7236 W=0.9277 df=8 p=0.4954	F=1.417 df1=1 df2=13 p=0.6546	Two-tailed unpaired t-test	t=2.938 df=13	p=0.7735	ns	Figure S12G	Density of mCherry+ Arc+ cells in TRN	control n=6 PTSD n=6	mouse	W=0.9546 df=6 p=0.7776 W=0.7919 df=6 p=0.0497	F=5.376 df1=1 df2=10 p=0.0886	Mann-Whitney U test	U=0	p=0.0022	**	Figure S12H	Percentage of mCherry+ cells in TRN	control n=6 PTSD n=6	mouse	W=0.9561 df=6 p=0.7894 W=0.982 df=6 p=0.9611	F=3.619 df1=1 df2=10 p=0.1844	Two-tailed unpaired t-test	t=1.376 df=10	p=1.1990	ns	Figure S12I	Percentage of mCherry+ Arc+ in mCherry+ cells in TRN	control n=6 PTSD n=6	mouse	W=0.9409 df=6 p=0.6661 W=0.9721 df=6 p=0.9062	F=2.625 df1=1 df2=10 p=0.3130	Two-tailed unpaired t-test	t=4.513 df=10	p=0.0011	**	Figure S15C	sIPSC frequency between baseline and light on	control n=5 PTSD n=5	neuron	W=0.9108 df=5 p=0.4727 W=0.8102 df=5 p=0.0978	F=1.86 df1=1 df2=18 p=0.5625	Two-tailed unpaired t-test	t=1.261 df=8	p=0.0229	*	Figure S15C	sIPSC frequency between light on and light off	control n=5 PTSD n=5	neuron	W=0.8102 df=5 p=0.0978 W=0.7183 df=5 p=0.0147	F=6.145 df1=1 df2=18 p=0.1066	Two-tailed unpaired t-test	t=2.831 df=8	p=0.0079	**	Figure S16Q	Fluorescence intensity in FrA between sham and stimulation	control n=6 slices from 3 mice PTSD n=14 slices from 3 mice	Slice	W=0.9098 df=6 p=0.4349 W=0.9395 df=14 p=0.4119	F=3.373 df1=1 df2=18 p=0.1874	Two-tailed unpaired t-test	t=4.713 df=18	p=0.0002	***	Figure S16Q	Fluorescence intensity in PrI between sham and stimulation	control n=10 slices from 3 mice PTSD n=16 slices from 3 mice	Slice	W=0.7428 df=10 p=0.0029 W=0.9661 df=16 p=0.7113	F=4.349 df1=1 df2=24 p=0.0313	Two-tailed unpaired t-test	t=3.617 df=24	p=0.0014	**	Figure S16Q	Fluorescence intensity in S1 between sham and stimulation	control n=6 slices from 3 mice PTSD n=9 slices from 3 mice	Slice	W=0.956 df=6 p=0.7881 W=0.958 df=9 p=0.7777	F=6.911 df1=1 df2=13 p=0.0477	Two-tailed unpaired t-test	t=2.366 df=13	p=0.0342	*	Figure S16Q	Fluorescence intensity in M2 between sham and stimulation	control n=10 slices from 3 mice PTSD n=9 slices from 3 mice	Slice	W=0.9519 df=10 p=0.6915 W=0.8985 df=9 p=0.2435	F=1.73 df1=1 df2=17 p=0.4309	Two-tailed unpaired t-test	t=0.8623 df=17	p=0.4005	ns	Figure S16Q	Fluorescence intensity in ACC between sham and stimulation	control n=8 slices from 3 mice PTSD n=10 slices from 3 mice	Slice	W=0.938 df=8 p=0.5918 W=0.9138 df=10 p=0.3084	F=6.35 df1=1 df2=16 p=0.0235	Two-tailed unpaired t-test	t=0.7565 df=16	p=0.4603	ns	Figure S17H	Spine head max diameter	control n=10 slices in 3 mice PTSD n=11 slices in 3 mice	Slice	W=0.9405 df=10 p=0.5584 W=0.9308 df=11 p=0.4187	F=3.552 df1=1 df2=19 p=0.0698	Two-tailed unpaired t-test	t=0.4347 df=19	p=0.6687	ns	Figure S17I	Spine head volume	control n=10 slices in 3 mice PTSD n=11 slices in 3 mice	Slice	W=0.9514 df=8 p=0.6850 W=0.8972 df=7 p=0.1709	F=4.42 df1=1 df2=19 p=0.0354	Two-tailed unpaired t-test	t=2.752 df=19	p=0.0127	*	Figure S17J	Spine volume	control n=10 slices in 3 mice PTSD n=11 slices in 3 mice	Slice	W=0.9546 df=10 p=0.7227 W=0.9524 df=11 p=0.6748	F=1.399 df1=1 df2=19 p=0.6248	Two-tailed unpaired t-test	t=2.458 df=19	p=0.0237	*				
Figure S8F	density of TRN GFP+ Arc+ along the bregma	control n=8 PTSD n=8	mouse	Not all subgroups meets p > 0.05	Not all subgroups meets p > 0.05	Friedman's M test	χ <sup>2</sup> =74.387 df=1	p < 0.0001	****																																																																																																																																																																																																		
Figure S8G	Percentage of GFP+ Arc+ in GFP+ cells in TRN	control n=8 PTSD n=8	mouse	W=0.9618 df=8 p=0.8268 W=0.9493 df=8 p=0.7040	F=7.689 df1=1 df2=14 p=0.0153	Two-tailed unpaired t-test	t=5.207 df=14	p=0.0001	***																																																																																																																																																																																																		
Figure S9F	density of ZI GFP+ Arc+ along the bregma	control n=8 PTSD n=7	mouse	W=0.9722 df=8 p=0.9145 W=0.9512 df=7 p=0.7402	F=1.364 df1=1 df2=13 p=0.7213	Two-tailed unpaired t-test	t=0.6605 df=13	p=0.5205	ns																																																																																																																																																																																																		
Figure S9G	Percentage of GFP+ Arc+ in GFP+ cells in ZI	control n=8 PTSD n=7	mouse	W=0.7143 df=8 p=0.0033 W=0.8056 df=7 p=0.0465	F=1.033 df1=1 df2=13 p=0.9850	Mann-Whitney U test	U=21	p=0.4634	ns																																																																																																																																																																																																		
Figure S10C	Max amplitude of oIPSC in PoM neurons received inputs from TRN	control n=7 PTSD n=8	mouse	W=0.9455 df=7 p=0.6887 W=0.6383 df=8 p=0.0004	F=1.908 df1=1 df2=13 p=0.4176	Two-tailed unpaired t-test	t=2.933 df=13	p=0.0116	*																																																																																																																																																																																																		
Figure S10F	Max amplitude of oIPSC in PoM neurons received inputs from ZI	control n=7 PTSD n=8	mouse	W=0.9493 df=7 p=0.7236 W=0.9277 df=8 p=0.4954	F=1.417 df1=1 df2=13 p=0.6546	Two-tailed unpaired t-test	t=2.938 df=13	p=0.7735	ns																																																																																																																																																																																																		
Figure S12G	Density of mCherry+ Arc+ cells in TRN	control n=6 PTSD n=6	mouse	W=0.9546 df=6 p=0.7776 W=0.7919 df=6 p=0.0497	F=5.376 df1=1 df2=10 p=0.0886	Mann-Whitney U test	U=0	p=0.0022	**																																																																																																																																																																																																		
Figure S12H	Percentage of mCherry+ cells in TRN	control n=6 PTSD n=6	mouse	W=0.9561 df=6 p=0.7894 W=0.982 df=6 p=0.9611	F=3.619 df1=1 df2=10 p=0.1844	Two-tailed unpaired t-test	t=1.376 df=10	p=1.1990	ns																																																																																																																																																																																																		
Figure S12I	Percentage of mCherry+ Arc+ in mCherry+ cells in TRN	control n=6 PTSD n=6	mouse	W=0.9409 df=6 p=0.6661 W=0.9721 df=6 p=0.9062	F=2.625 df1=1 df2=10 p=0.3130	Two-tailed unpaired t-test	t=4.513 df=10	p=0.0011	**																																																																																																																																																																																																		
Figure S15C	sIPSC frequency between baseline and light on	control n=5 PTSD n=5	neuron	W=0.9108 df=5 p=0.4727 W=0.8102 df=5 p=0.0978	F=1.86 df1=1 df2=18 p=0.5625	Two-tailed unpaired t-test	t=1.261 df=8	p=0.0229	*																																																																																																																																																																																																		
Figure S15C	sIPSC frequency between light on and light off	control n=5 PTSD n=5	neuron	W=0.8102 df=5 p=0.0978 W=0.7183 df=5 p=0.0147	F=6.145 df1=1 df2=18 p=0.1066	Two-tailed unpaired t-test	t=2.831 df=8	p=0.0079	**																																																																																																																																																																																																		
Figure S16Q	Fluorescence intensity in FrA between sham and stimulation	control n=6 slices from 3 mice PTSD n=14 slices from 3 mice	Slice	W=0.9098 df=6 p=0.4349 W=0.9395 df=14 p=0.4119	F=3.373 df1=1 df2=18 p=0.1874	Two-tailed unpaired t-test	t=4.713 df=18	p=0.0002	***																																																																																																																																																																																																		
Figure S16Q	Fluorescence intensity in PrI between sham and stimulation	control n=10 slices from 3 mice PTSD n=16 slices from 3 mice	Slice	W=0.7428 df=10 p=0.0029 W=0.9661 df=16 p=0.7113	F=4.349 df1=1 df2=24 p=0.0313	Two-tailed unpaired t-test	t=3.617 df=24	p=0.0014	**																																																																																																																																																																																																		
Figure S16Q	Fluorescence intensity in S1 between sham and stimulation	control n=6 slices from 3 mice PTSD n=9 slices from 3 mice	Slice	W=0.956 df=6 p=0.7881 W=0.958 df=9 p=0.7777	F=6.911 df1=1 df2=13 p=0.0477	Two-tailed unpaired t-test	t=2.366 df=13	p=0.0342	*																																																																																																																																																																																																		
Figure S16Q	Fluorescence intensity in M2 between sham and stimulation	control n=10 slices from 3 mice PTSD n=9 slices from 3 mice	Slice	W=0.9519 df=10 p=0.6915 W=0.8985 df=9 p=0.2435	F=1.73 df1=1 df2=17 p=0.4309	Two-tailed unpaired t-test	t=0.8623 df=17	p=0.4005	ns																																																																																																																																																																																																		
Figure S16Q	Fluorescence intensity in ACC between sham and stimulation	control n=8 slices from 3 mice PTSD n=10 slices from 3 mice	Slice	W=0.938 df=8 p=0.5918 W=0.9138 df=10 p=0.3084	F=6.35 df1=1 df2=16 p=0.0235	Two-tailed unpaired t-test	t=0.7565 df=16	p=0.4603	ns																																																																																																																																																																																																		
Figure S17H	Spine head max diameter	control n=10 slices in 3 mice PTSD n=11 slices in 3 mice	Slice	W=0.9405 df=10 p=0.5584 W=0.9308 df=11 p=0.4187	F=3.552 df1=1 df2=19 p=0.0698	Two-tailed unpaired t-test	t=0.4347 df=19	p=0.6687	ns																																																																																																																																																																																																		
Figure S17I	Spine head volume	control n=10 slices in 3 mice PTSD n=11 slices in 3 mice	Slice	W=0.9514 df=8 p=0.6850 W=0.8972 df=7 p=0.1709	F=4.42 df1=1 df2=19 p=0.0354	Two-tailed unpaired t-test	t=2.752 df=19	p=0.0127	*																																																																																																																																																																																																		
Figure S17J	Spine volume	control n=10 slices in 3 mice PTSD n=11 slices in 3 mice	Slice	W=0.9546 df=10 p=0.7227 W=0.9524 df=11 p=0.6748	F=1.399 df1=1 df2=19 p=0.6248	Two-tailed unpaired t-test	t=2.458 df=19	p=0.0237	*																																																																																																																																																																																																		

**Table S2. Quantification data for PoM neurons manipulated in the experiments.**

Quantification data for PoM neurons manipulated in the experiments							
Figure	PoM	cell number		Figure	TNR	cell number	
		left	right			left	right
Figure 3	Control-eGFP-1	441	368	Figure 5	PTSD-eYFP-1	203	429
Figure 3	Control-eGFP-2	182	202	Figure 5	PTSD-eYFP-2	446	498
Figure 3	Control-eGFP-3	225	314	Figure 5	PTSD-eYFP-3	317	255
Figure 3	Control-eGFP-4	227	365	Figure 5	PTSD-eYFP-4	331	248
Figure 3	Control-eGFP-5	586	396	Figure 5	PTSD-eYFP-5	305	297
Figure 3	Control-eGFP-6	332	285	Figure 5	PTSD-eYFP-6	331	305
Figure 3	Control-eGFP-7	265	224	Figure 5	control-eYFP-1	321	288
Figure 3	Control-eGFP-8	283	302	Figure 5	control-eYFP-2	445	467
Figure 3	Control-eGFP-9	266	312	Figure 5	control-eYFP-3	399	428
Figure 3	Control-eGFP-10	374	402	Figure 5	control-eYFP-4	433	418
Figure 3	PTSD-eGFP-1	227	334	Figure 5	control-eYFP-5	447	469
Figure 3	PTSD-eGFP-2	265	343	Figure 5	control-eYFP-6	454	437
Figure 3	PTSD-eGFP-3	373	196	Figure 5	PTSD-SSFO-1	441	396
Figure 3	PTSD-eGFP-4	165	294	Figure 5	PTSD-SSFO-2	296	365
Figure 3	PTSD-eGFP-5	199	261	Figure 5	PTSD-SSFO-3	331	453
Figure 3	PTSD-eGFP-6	234	479	Figure 5	PTSD-SSFO-4	361	378
Figure 3	PTSD-eGFP-7	336	452	Figure 5	PTSD-SSFO-5	412	388
Figure 3	PTSD-Kir2.1-1	415	182	Figure 5	PTSD-SSFO-6	318	386
Figure 3	PTSD-Kir2.1-2	383	302	Figure 5	PTSD-SSFO-7	279	345
Figure 3	PTSD-Kir2.1-3	241	293	Figure 5	PTSD-SSFO-8	336	367
Figure 3	PTSD-Kir2.1-4	119	434	Figure 5	Control-mCherry-1	105	97
Figure 3	PTSD-Kir2.1-5	285	221	Figure 5	Control-mCherry-2	106	174
Figure 3	PTSD-Kir2.1-6	334	286	Figure 5	Control-mCherry-3	231	174
Figure 3	PTSD-Kir2.1-7	281	314	Figure 5	Control-mCherry-4	94	186
Figure 3	control-mcherry-1	196	241	Figure 5	Control-mCherry-5	261	177
Figure 3	control-mcherry-2	264	331	Figure 5	Control-mCherry-6	195	167
Figure 3	control-mcherry-3	222	219	Figure 5	PTSD-mCherry-1	225	196
Figure 3	control-mcherry-4	316	273	Figure 5	PTSD-mCherry-2	165	118
Figure 3	control-mcherry-5	326	296	Figure 5	PTSD-mCherry-3	233	199
Figure 3	control-mcherry-6	221	162	Figure 5	PTSD-mCherry-4	105	67
Figure 3	control-mcherry-7	194	225	Figure 5	PTSD-mCherry-5	105	173
Figure 3	control-mcherry-8	203	313	Figure 5	PTSD-mCherry-6	157	106
Figure 3	control-mcherry-9	334	296	Figure 5	PTSD-mCherry-7	178	164
Figure 3	control-mcherry-10	345	217	Figure 5	PTSD-hM3D(Gq)-1	142	113
Figure 3	PTSD-mcherry-1	297	332	Figure 5	PTSD-hM3D(Gq)-2	133	175
Figure 3	PTSD-mcherry-2	278	321	Figure 5	PTSD-hM3D(Gq)-3	152	151
Figure 3	PTSD-mcherry-3	257	196	Figure 5	PTSD-hM3D(Gq)-4	72	122
Figure 3	PTSD-mcherry-4	264	354	Figure 5	PTSD-hM3D(Gq)-5	84	106
Figure 3	PTSD-mcherry-5	355	401	Figure 5	PTSD-hM3D(Gq)-6	138	170
Figure 3	PTSD-mcherry-6	223	312	Figure 5	PTSD-hM3D(Gq)-7	182	141
Figure 3	PTSD-hM4D(Gi)-1	284	176	Figure 5	PTSD-hM3D(Gq)-8	102	144
Figure 3	PTSD-hM4D(Gi)-2	265	239				
Figure 3	PTSD-hM4D(Gi)-3	284	327				
Figure 3	PTSD-hM4D(Gi)-4	266	317				
Figure 3	PTSD-hM4D(Gi)-5	321	331				
Figure 3	PTSD-hM4D(Gi)-6	336	402				
Figure 5	Control-mcherry-1	121	143				
Figure 5	Control-mcherry-2	197	265				
Figure 5	Control-mcherry-3	135	176				
Figure 5	Control-mcherry-4	244	231				
Figure 5	Control-mcherry-5	263	287				
Figure 5	Control-mcherry-6	196	346				
Figure 5	PTSD-mcherry-1	221	169				
Figure 5	PTSD-mcherry-2	278	265				
Figure 5	PTSD-mcherry-3	179	264				
Figure 5	PTSD-mcherry-4	297	104				
Figure 5	PTSD-mcherry-5	268	298				
Figure 5	PTSD-mcherry-6	243	268				
Figure 5	PTSD-mcherry-7	261	213				
Figure 5	PTSD-hM3D(Gq)-1	417	396				
Figure 5	PTSD-hM3D(Gq)-2	214	275				
Figure 5	PTSD-hM3D(Gq)-3	146	385				
Figure 5	PTSD-hM3D(Gq)-4	253	283				
Figure 5	PTSD-hM3D(Gq)-5	292	143				
Figure 5	PTSD-hM3D(Gq)-6	256	281				
Figure 5	PTSD-hM3D(Gq)-7	182	201				
Figure 5	PTSD-hM3D(Gq)-8	283	266				

Quantification data for PoM neurons manipulated in the experiments							
Figure	PoM	cell number		Figure	TNR	cell number	
		left	right			left	right
Figure 7	Control-eYFP-1	176	368				
Figure 7	Control-eYFP-2	227	231				
Figure 7	Control-eYFP-3	406	277				
Figure 7	Control-eYFP-4	365	377				
Figure 7	Control-eYFP-5	173	113				
Figure 7	Control-eYFP-6	268	289				
Figure 7	Control-eYFP-7	223	331				
Figure 7	Control-eYFP-8	278	321				
Figure 7	PTSD-eYFP-1	365	272				
Figure 7	PTSD-eYFP-2	297	286				
Figure 7	PTSD-eYFP-3	665	387				
Figure 7	PTSD-eYFP-4	423	344				
Figure 7	PTSD-eYFP-5	512	466				
Figure 7	PTSD-eYFP-6	279	211				
Figure 7	PTSD-eYFP-7	334	297				
Figure 7	PTSD-eYFP-8	361	301				
Figure 7	PTSD-eYFP-9	336	299				
Figure 7	PTSD-eNpHR-1	273	305				
Figure 7	PTSD-eNpHR-2	261	173				
Figure 7	PTSD-eNpHR-3	449	483				
Figure 7	PTSD-eNpHR-4	301	421				
Figure 7	PTSD-eNpHR-5	291	341				
Figure 7	PTSD-eNpHR-6	406	378				
Figure 7	PTSD-eNpHR-7	278	336				
Figure 7	PTSD-eNpHR-8	325	296				

**Table S3. Antibodies list.**

<b>Antibodies</b>		
<b>REAGENT or RESOURCE</b>	<b>SOURCE</b>	<b>IDENTIFIER</b>
Mouse anti-MAP2	Sigma-Aldrich	Cat#M3696; RRID: AB_1840999
Rabbit anti-Arc	Synaptic Systems	Cat#156 003; RRID: AB_887694
Rabbit anti-NeuN	Sigma-Aldrich	Cat#ABN78; RRID:AB_10807945
Goat anti-rabbit 488	Invitrogen	Cat# ab150077; RRID:AB_2630356
Goat anti-mouse 594	Invitrogen	Cat#R37121; RRID: AB_2556549
Goat anti-rabbit 647	Jackson	Cat# ab150083; RRID: AB_2714032

**Supplementary Movie S1.** Representative video showing the defensive behaviors of control and PTSD mice provoked by a whisker stimulation.