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Supplementary Materials for

Reversal of hyperactive higher-order thalamus attenuates defensiveness in a mouse model of PTSD

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

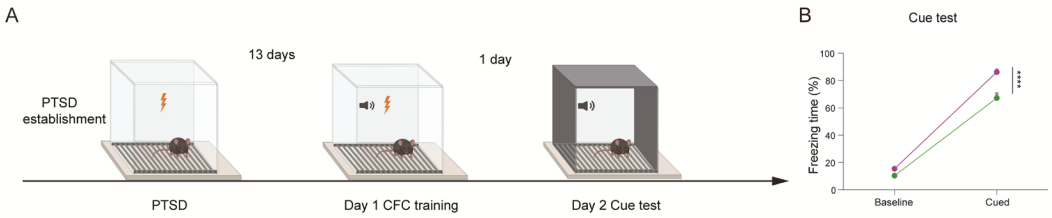


Fig. S1. Excessive defensive behavior in PTSD mice caused by auditory stimulation. (**A**) Diagram of Day 2 cue test. (**B**) Elevated freezing time on Day 2 cue test in PTSD mice (n = 10 mice for each group). ****$P < 0.0001$, by Two-way ANOVA. Data are presented as the mean ± S.E.M. See Table S1 for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

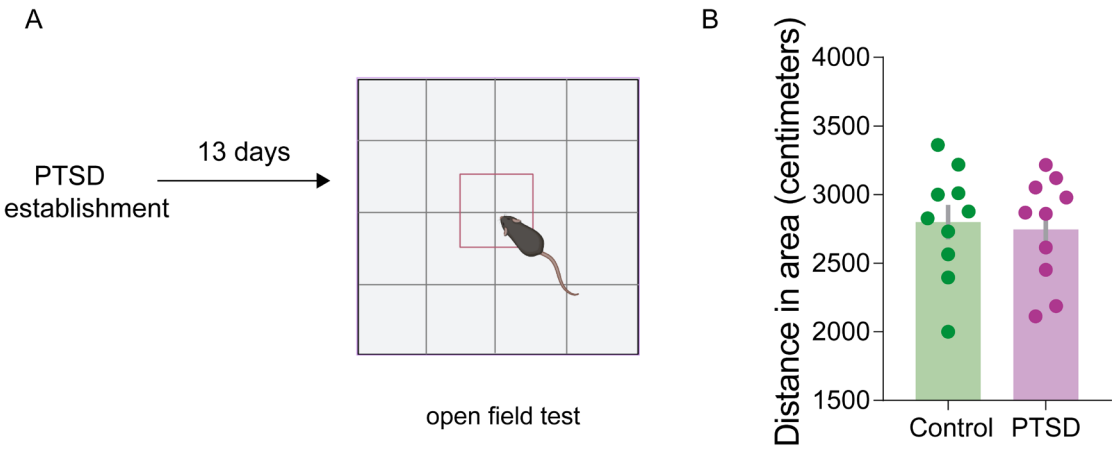


Fig. S2. PTSD stress did not influence the locomotor activity. (**A**) Diagram of the open field test 13 days after the PTSD establishment. (**B**) Histogram of the distance in the open field between control and PTSD mice (n = 10 mice for each group). By Two-tailed unpaired t-test. Data are presented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

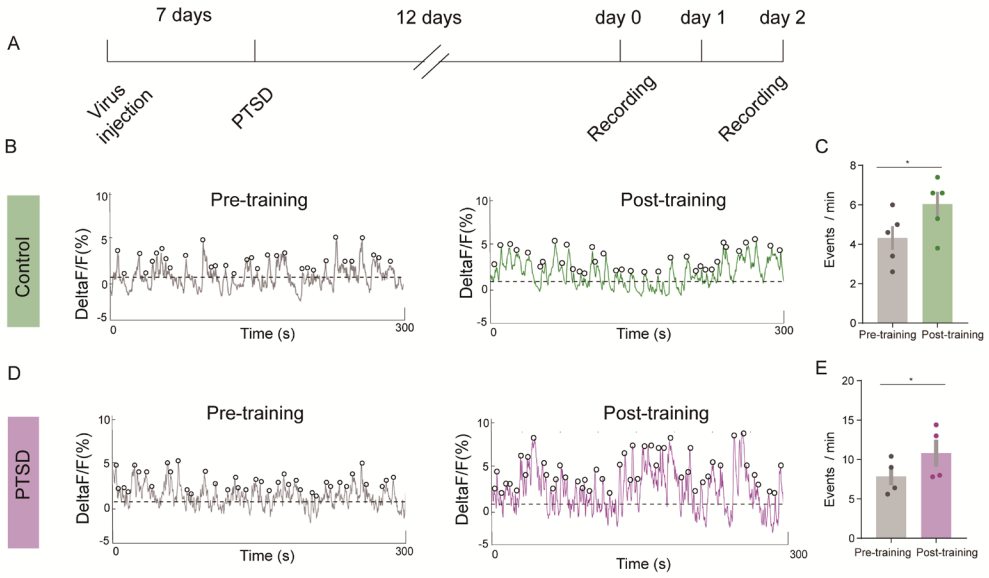


Fig. S3. Increased neuronal activity of PoM following CFC training. (**A**) Schematic of fiber photometry. (**B**) Schematic and representative traces of tests of fiber photometry in PoM of control mice were subjected to environmental stimulation on the day before training and 2nd day after training. (**C**) Frequency of spontaneous calcium events in PoM of control mice on the day before and 2nd 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 2nd day after training. (**E**) Frequency of spontaneous calcium events in PoM of PTSD mice on the day before training and 2nd 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.

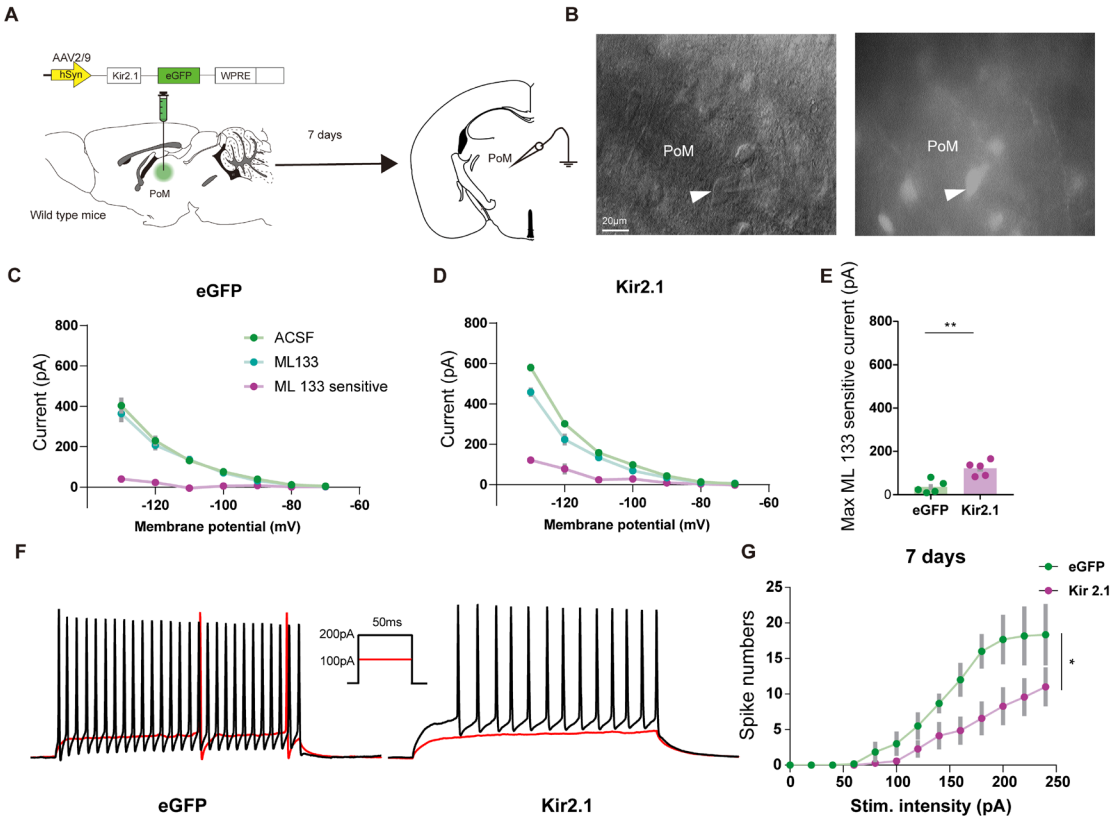


Fig. S4. Kir2.1 overexpression reduced the excitability of PoM neurons. (**A**) Schematic of virus injection and patch clamp recordings. (**B**) Representative images of patch clamp recordings of PoM neurons with Kir2.1 overexpression. Scale bar: 20μm. Left: Patch clamp recording of the PoM neuron in 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 ± S.E.M. See Table S1 for detailed statistical information.

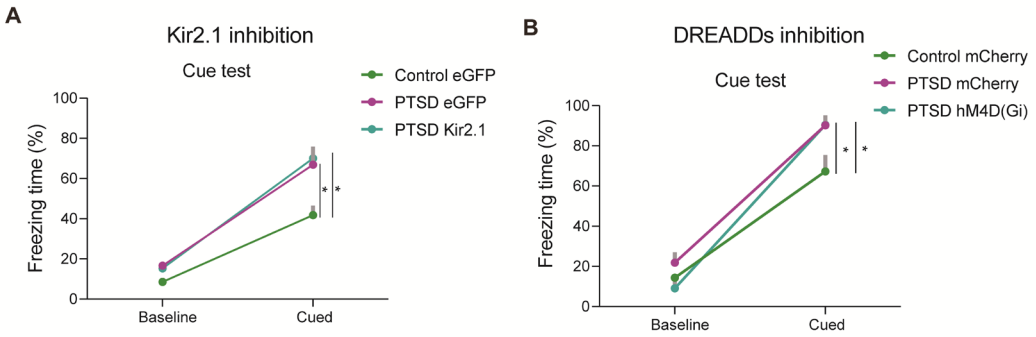


Fig. S5. Failure of PoM inhibition to correct auditory-related defensive response in PTSD mice. (**A**) Freezing time in day 2 cue test between eGFP-expressing control mice, eGFP-expressing PTSD mice and Kir2.1-expressing PTSD mice (n = 7 mice for control eGFP group, n = 6 mice for PTSD eGFP group and 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 ± S.E.M. See Table S1 for detailed statistical information.

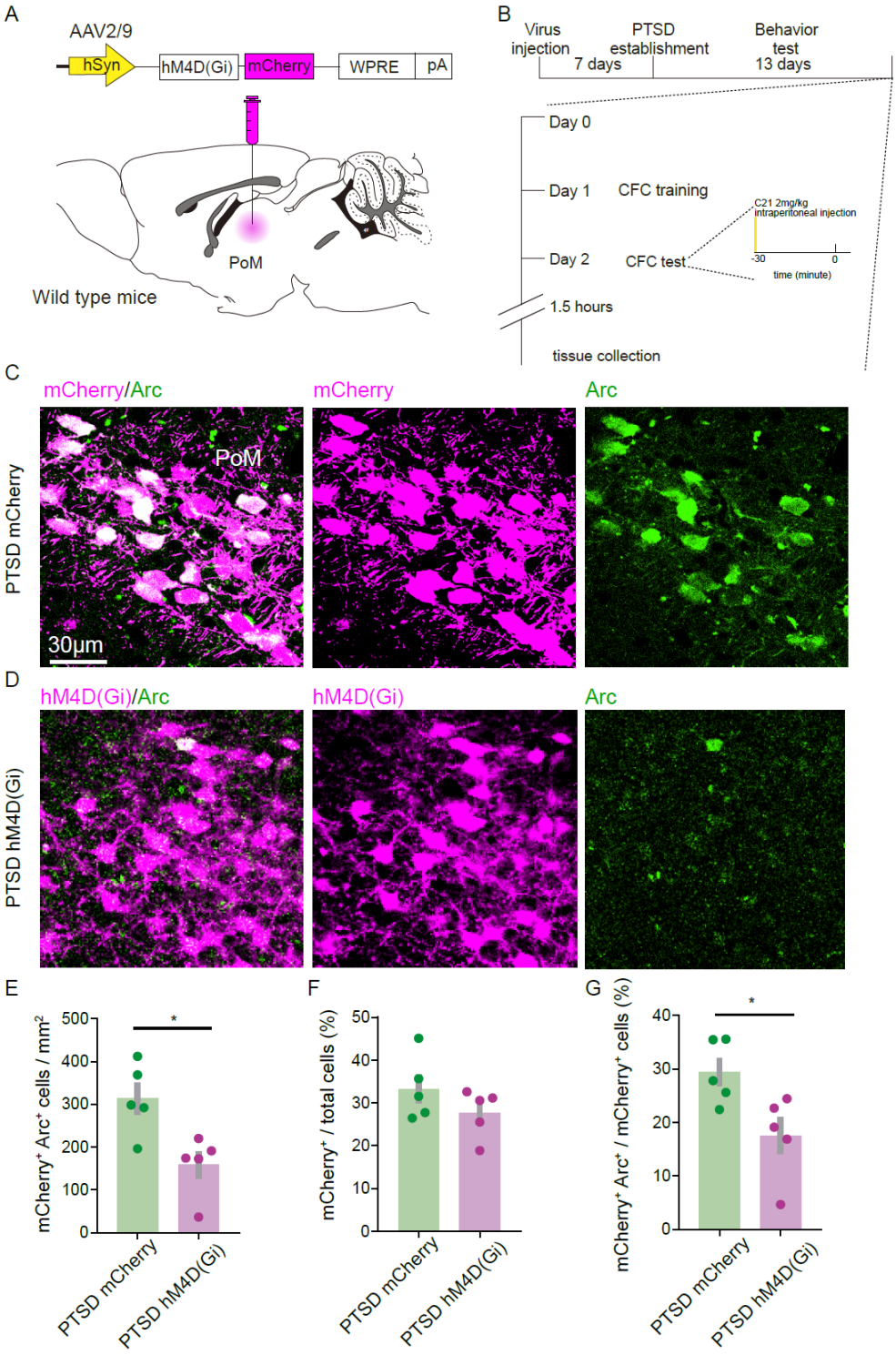


Fig. S6. hM4D(Gi) suppressed Arc expression in PoM neurons in PTSD mice. (A) Schematic of the viral strategy to chemo-genetically inhibit PoM neurons. (**B**) Schematic of behavioral tests and immunohistochemical fluorescence staining. (**C**) Representative images showing mCherry expression and Arc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

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

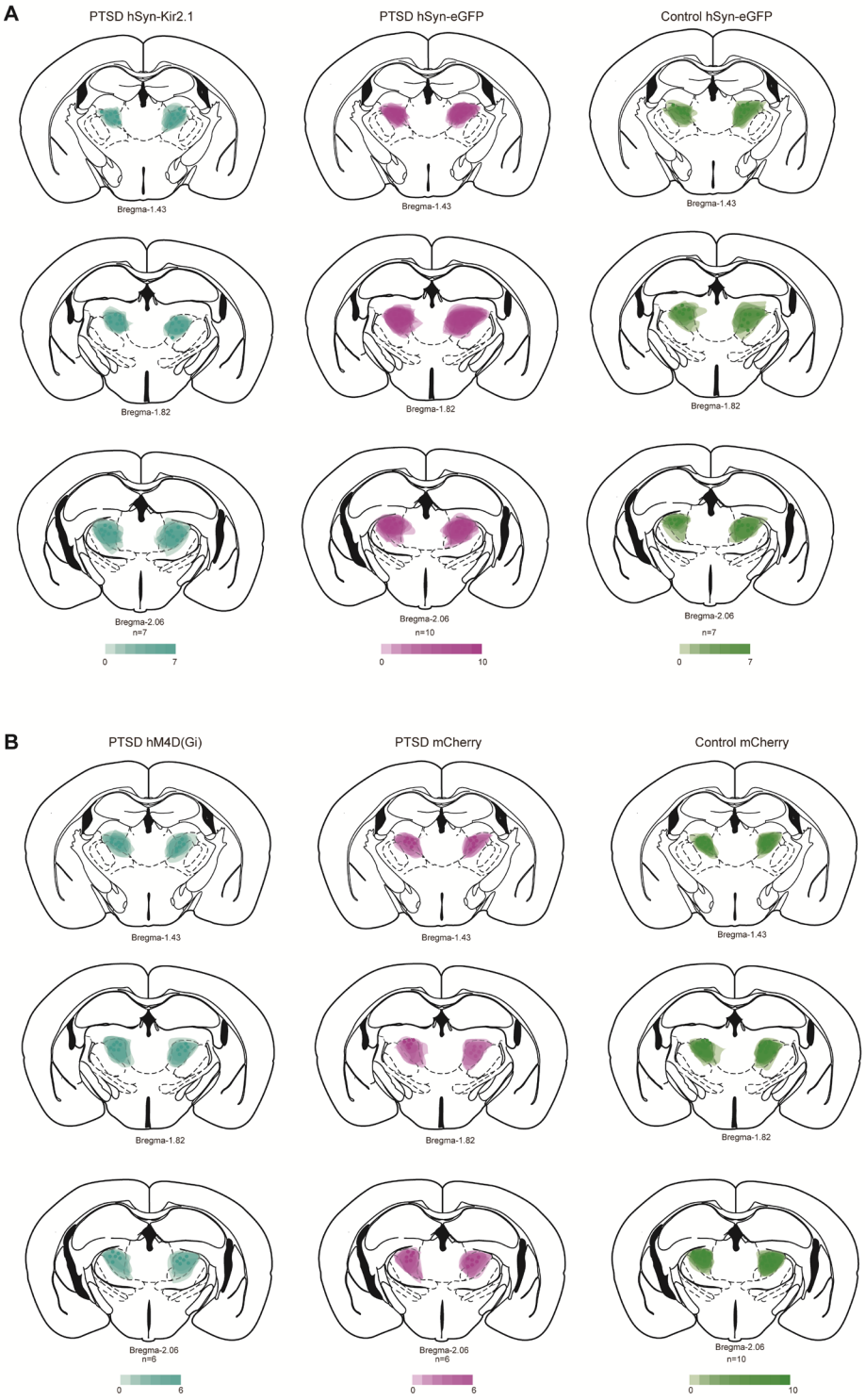


Fig. S7. Verification of the region showing virus infection for PoM inhibition. (**A**) Schematic showing areas expressing Kir2.1 or eGFP in PoM. (**B**) Schematic showing areas expressing hM4D(Gi) or mCherry in PoM.

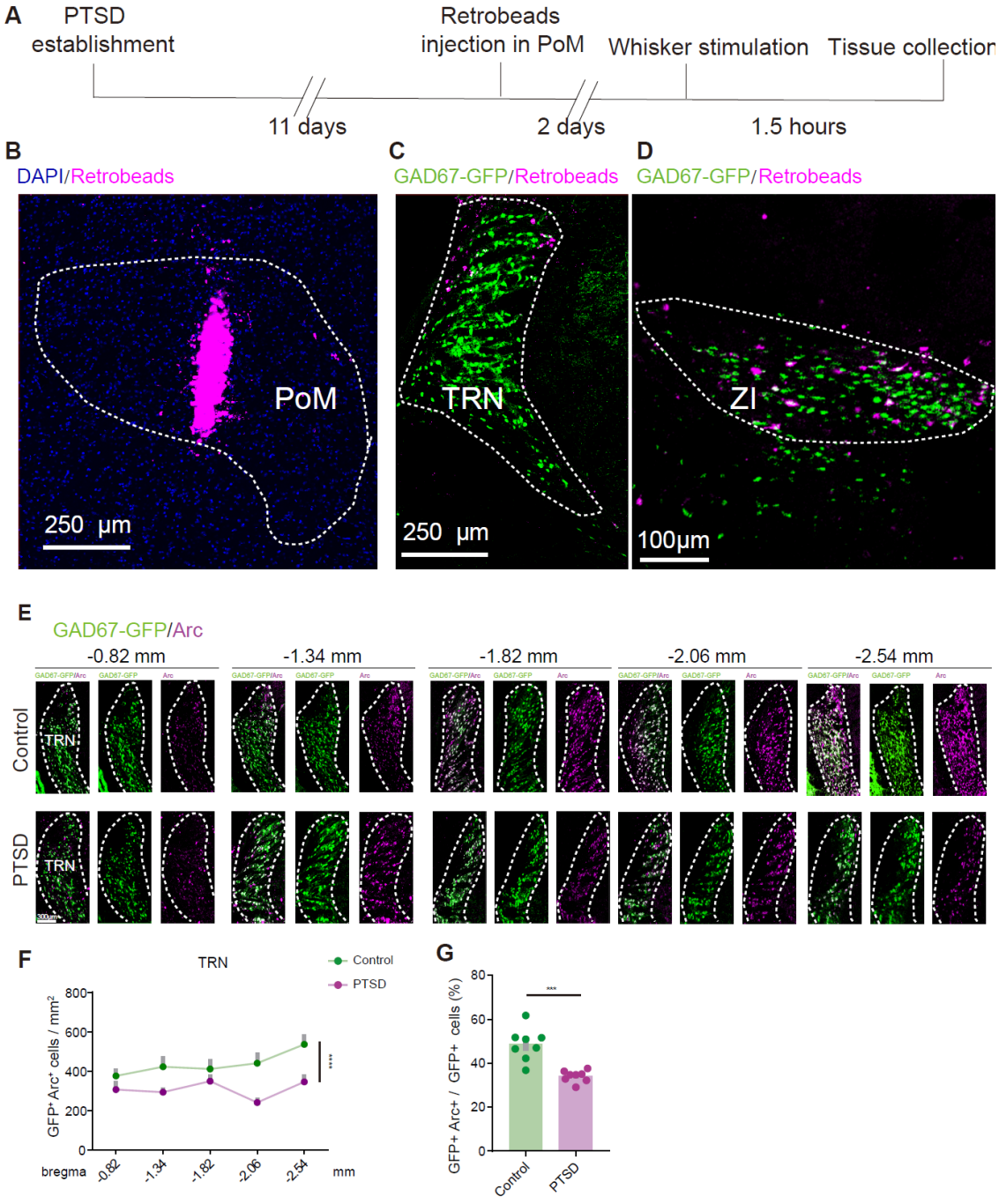


Fig. S8. Reduced Arc expression in TRN in PTSD mice. (**A**) Schematic for retrobeads injection in PoM and tissue collection strategy. (**B**) Representative images of injection sites in PoM. Scale bar: 250μm. (**C**) Representative images of GAD67-GFP and retrobeads in TRN. Scale bar: 250μm. (**D**) Representative images of GAD67-GFP and retrobeads in ZI. Scale bar:100μm. (**E**) Representative images of GAD67 and Arc in TRN along the anterior (A) to posterior (P) axis between control and PTSD mice. Scale bar: 300μm. (**F**) The number of GFP^+ Arc^+ 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^+ Arc^+ cells in GFP^+ 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 ± S.E.M. See Table S1 for detailed statistical information.

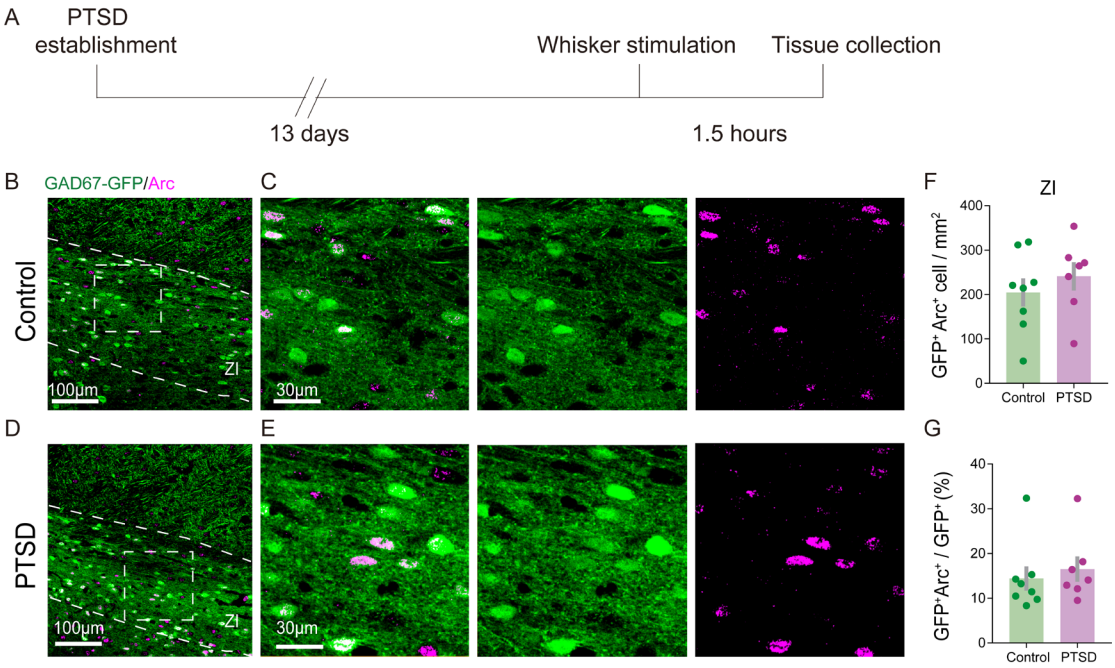


Fig. S9. Intact Arc expression in ZI in PTSD mice. (**A**) Schematic for tissue collection. (**B-C**) Representative images of GAD67 and Arc in ZI in control mice. Scale bar: 100 μm and 30 μm. (**D-E**) Representative images of GAD67 and Arc in ZI in PTSD mice. Scale bar: 100 μm and 30 μm. (**F**) The number of GFP^+ Arc^+ 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^+ Arc^+ cells in GFP^+ 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 ± S.E.M. See Table S1 for detailed statistical information.

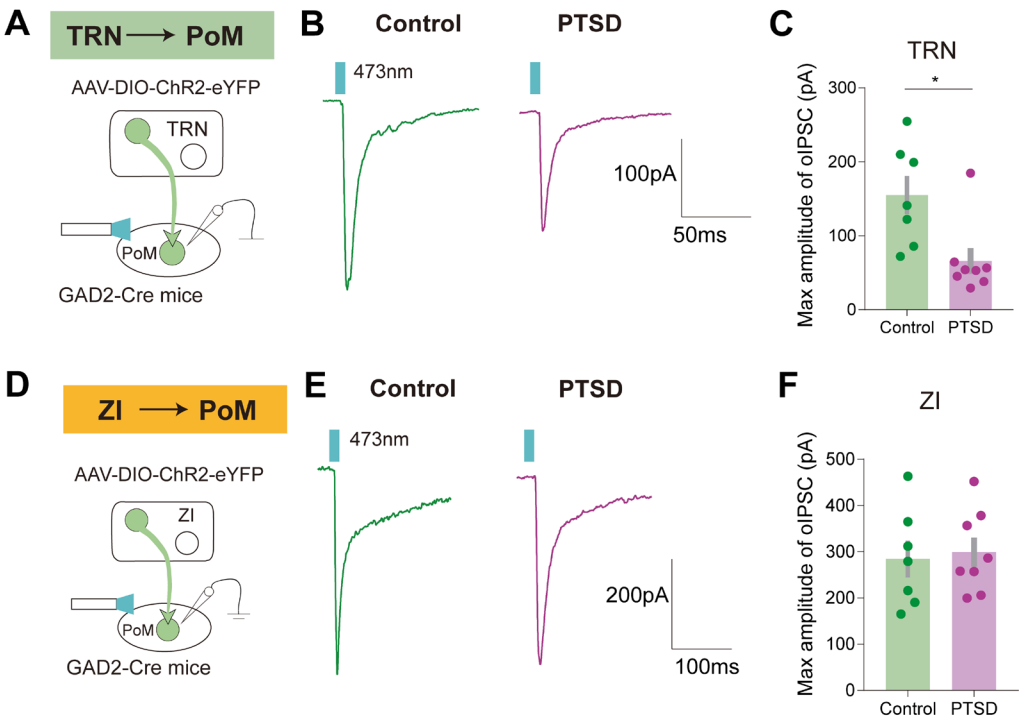
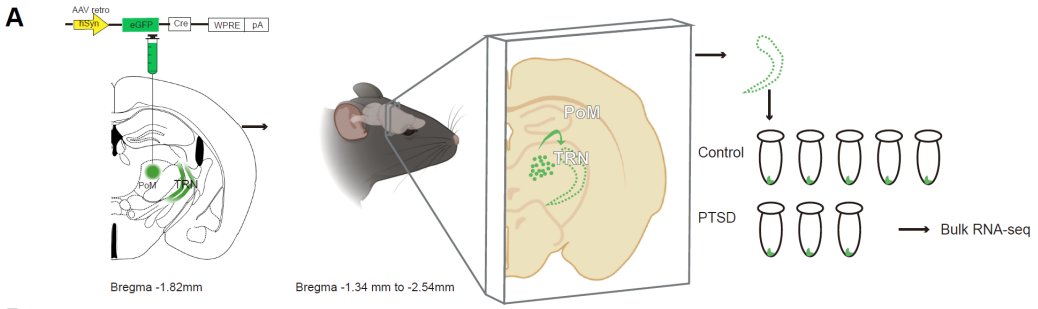


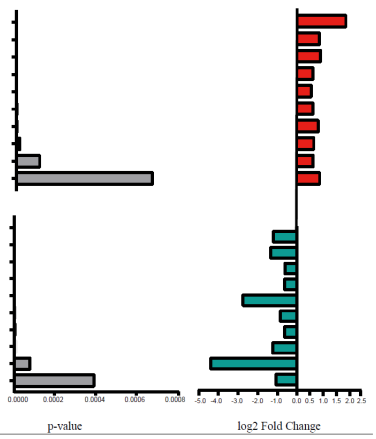
Fig. S10. Impaired TRN inhibitory inputs to PoM in PTSD mice. (**A**) Schematic of the viral strategy and slice recordings in PoM evoked by photoactivation of TRN terminals. (**B**) Representative oIPSC evoked by 473nm light stimulation in PoM neurons received inputs from TRN in control and PTSD mice. (**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 ± S.E.M. See Table S1 for detailed statistical information.



B

Differently expressed genes in PTSD mice

Gene	p-value
Top upregulated genes	
Slc19a3	∝0
Clic5	∝0
Adgrl4	2.21E-12
Abcc9	1.56E-09
Notch4	3.55E-07
Spry4	1.33E-06
Tgm2	1.96E-06
Rhobtb1	1.62E-05
Esam	1.14E-04
Grm2	6.70E-04
Top downregulated genes	
Rasl11b	1.25E-14
Dbp	3.23E-12
Mog	2.57E-08
Shroom3	5.17E-08
Satb2	6.45E-07
Slc45a3	1.30E-06
Lgi4	2.17E-06
Cdh3	4.33E-06
9130024F11Rik	7.61E-05
Vxn	3.85 E-04



C

Enriched gene sets in PTSD mice

Gene set	FDR
Calcium signaling pathway	0.001
cGMP-PKG signaling pathway	0.006
Focal adhesion	0.006
VEGF signaling pathway	0.099
PI3K-Akt signaling pathway	0.022
HIF-1 signaling pathway	0.025
Platelet activation	0.025
Apelin signaling pathway	0.027
Fluid shear stress and atherosclerosis	0.027
Oxytocin signaling pathway	0.027
Rap1 signaling pathway	0.047
Ras signaling pathway	0.047
Arginine biosynthesis	0.047
Phototransduction	0.059
MAPK signaling pathway	0.062
Arginine and proline metabolism	0.102

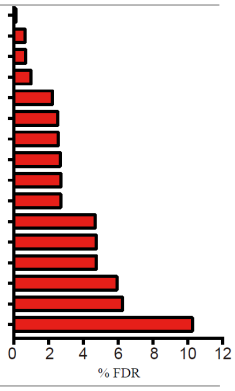


Fig. S11. Dysregulated genes and pathways of somatosensory TRN in PTSD mice. (**A**) Schematic of the viral strategy and tissue collection for bulk RNA seq from 6 control mice and 3 PTSD mice. (**B**) Table of the top ten upregulated (top) and downregulated (bottom) differentially expressed genes. Differential 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%.

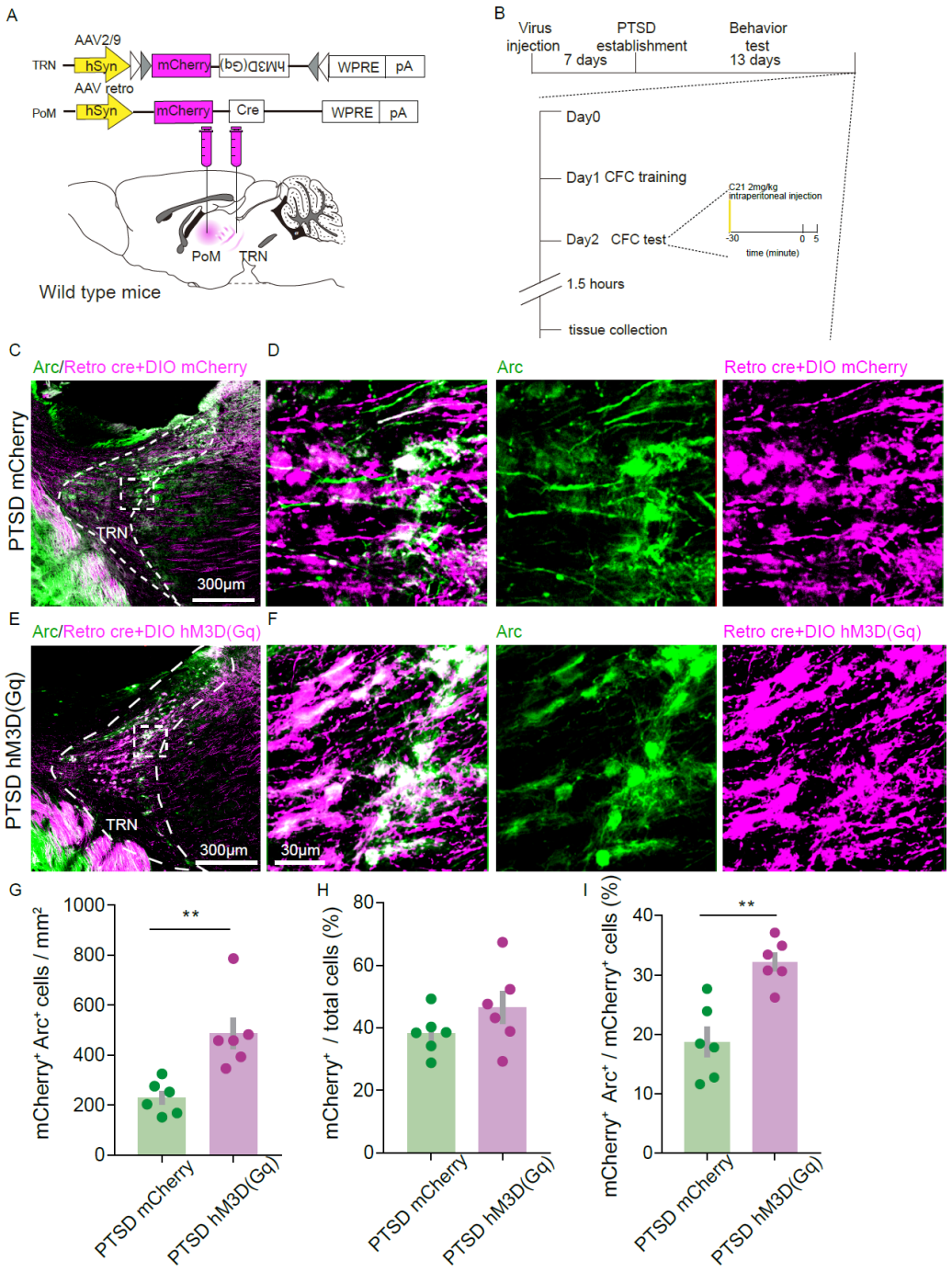


Fig. S12. hM3D(Gq) increased Arc expression in TRN neurons in PTSD mice. (**A**) Schematic of the viral strategy to chemo-genetically activate TRN neurons projecting to PoM. (**B**) Schematic of behavioral tests and immunohistochemical fluorescence staining. (**C-D**) Representative images showing mCherry 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 $mCherry^+$ Arc^+ cells (n = 6 mice for each group). (**H**) Percentage of $mCherry^+$ cells in TRN cells (n = 6 mice for each group). (**I**)

Percentage of mCherry^{+} Arc^{+} cells in mCherry^{+} 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.

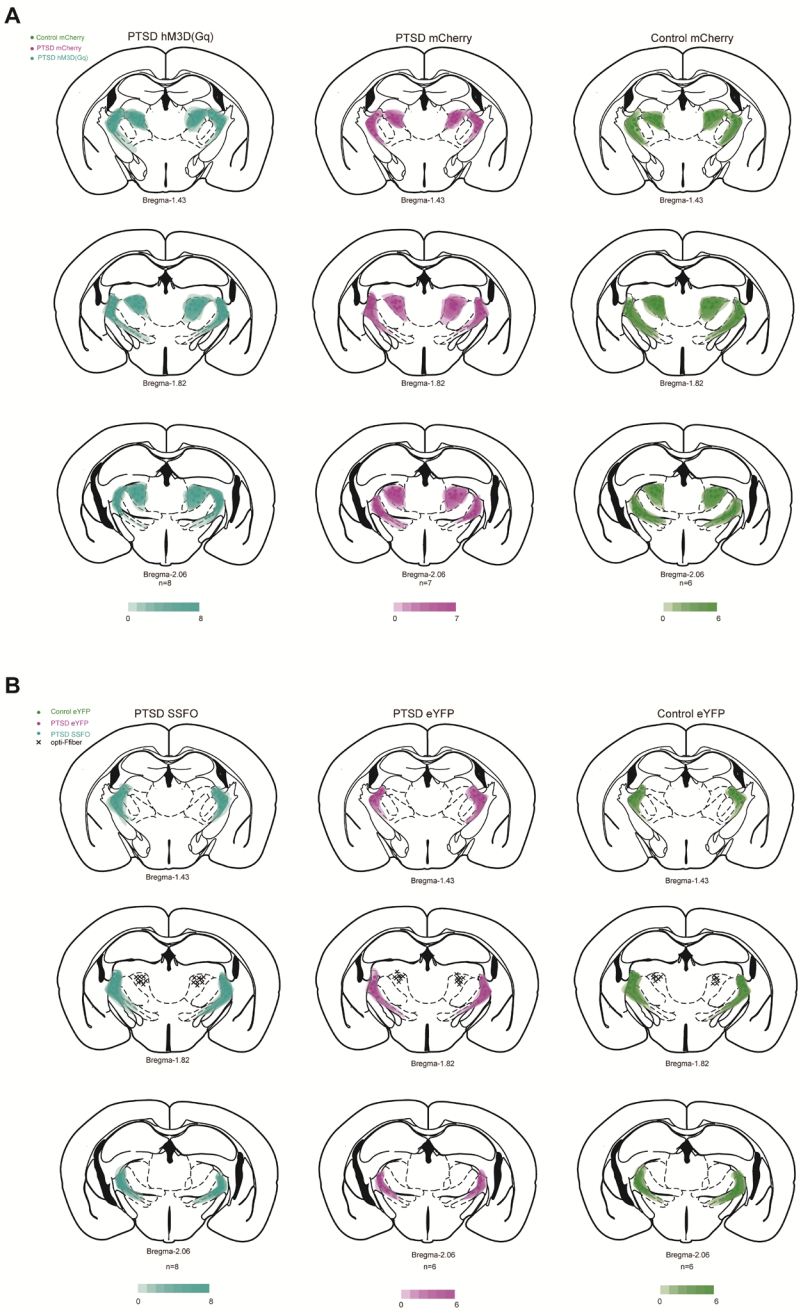


Fig. S13. Verification of the region showing virus infection for optogenetics and DREADDs activation. (**A**) Schematic showing areas expressing hM3D(Gq) or mCherry in TRN or PoM. (**B**) Schematic showing areas expressing SSFO or eYFP in TRN.

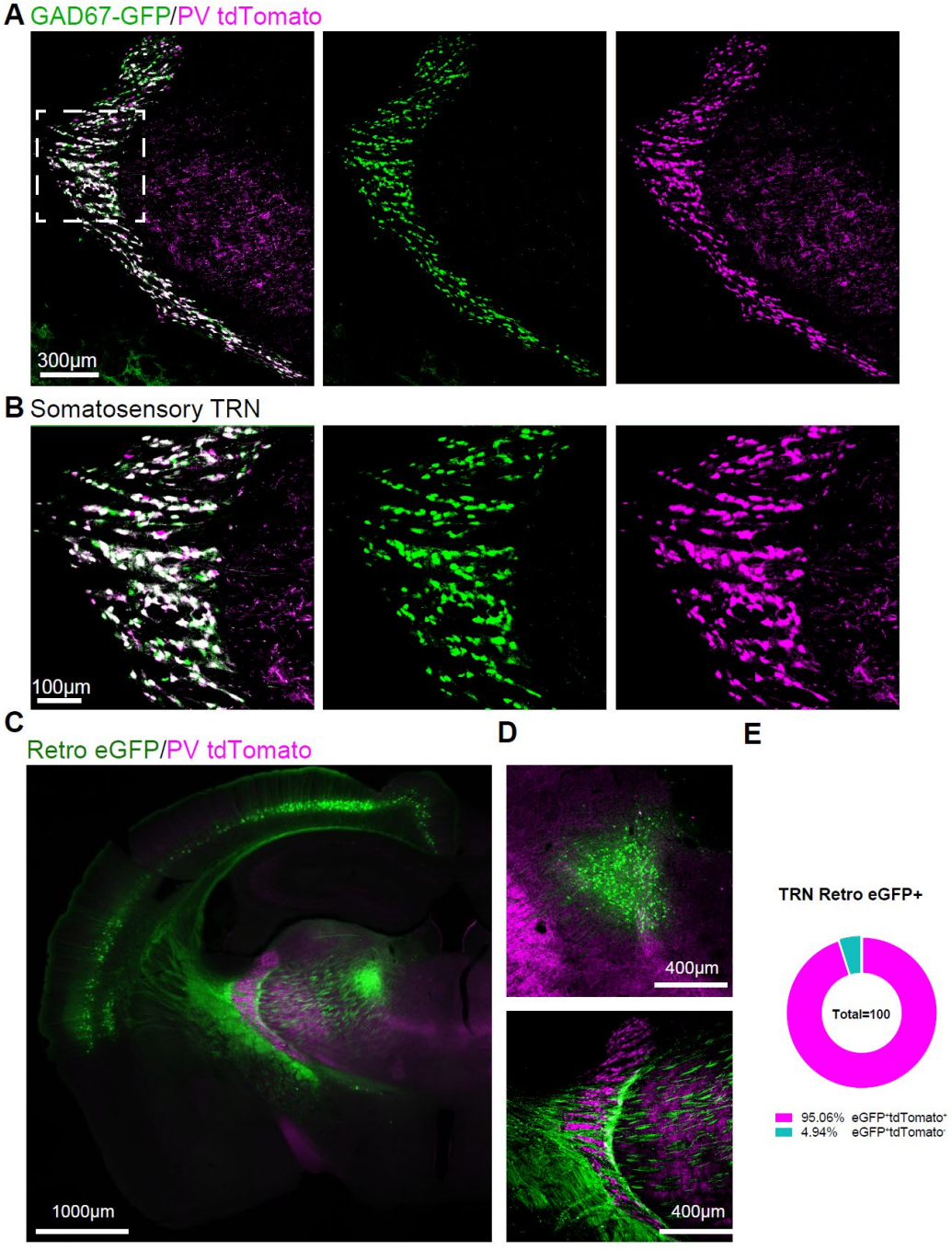


Fig. S14. Verification of PV expression in TRN and PoM projecting cells. (A) Representative images of TRN neurons in GAD67-GFP$^+$ and PV tdTomato$^+$ mice. Scale bar: 300 μm. **(B)** Representative images of neurons in somatosensory TRN in GAD67-GFP$^+$ and PV tdTomato$^+$ mice. Scale bar: 100 μm. **(C)** A low-magnification image showing eGFP expression in PV tdTomato$^+$ 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$^+$ tdTomato$^+$ cells in eGFP$^+$ cells in TRN.

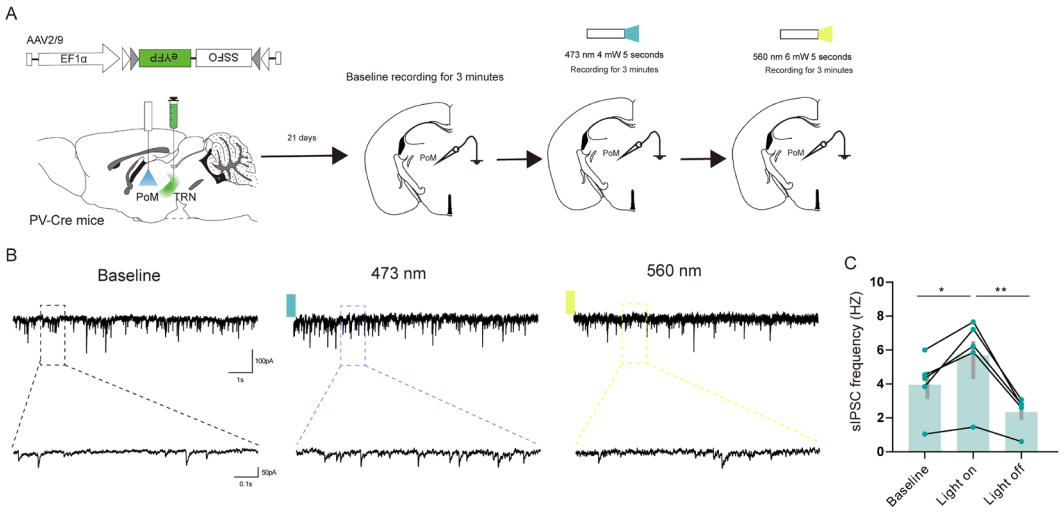


Fig. S15. SSFO enhanced the inhibitory input to PoM. (A) Schematic of the viral strategy and slice recordings. **(B)** Representative sIPSCs of PoM neurons received illuminating of 473nm and 560nm light. Left: Baseline of sIPSCs. Middle: sIPSCs with 473nm light stimulation. Right: sIPSCs with 560nm light stimulation. **(C)** Summary of the frequency of sISPCs (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 ± S.E.M. See Table S1 for detailed statistical information.

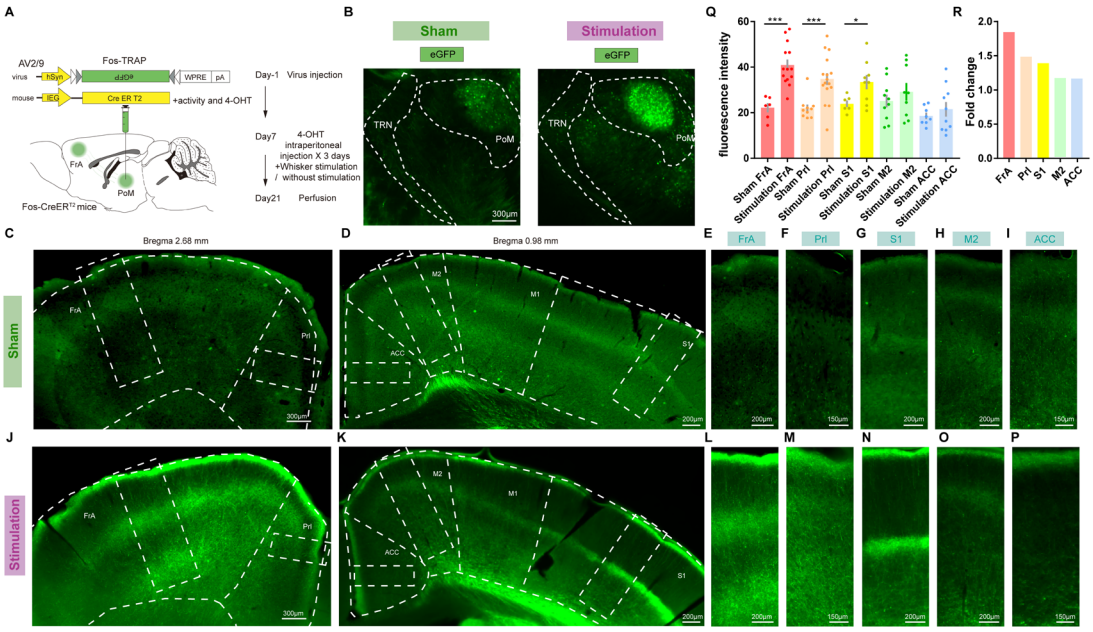


Fig. S16. TRAPing axons of PoM cells responding to tactile threats in the cortex. (**A**) Schematic of Fos-TRAP strategy for anterograde tracing of the downstream regions of PoM. (**B**) Representative images showing eGFP expression in PoM under low magnification in a mouse with sham stimulation and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

in a mouse with whisker stimulation. Scale bar: 200μm. (**O**) Representative images of M2 under high magnification in a mouse with whisker stimulation. Scale bar: 200μm. (**P**) Representative images of ACC under high magnification in a mouse with whisker stimulation. Scale bar: 150μm. (**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 ± S.E.M. See Table S1 for detailed statistical information.

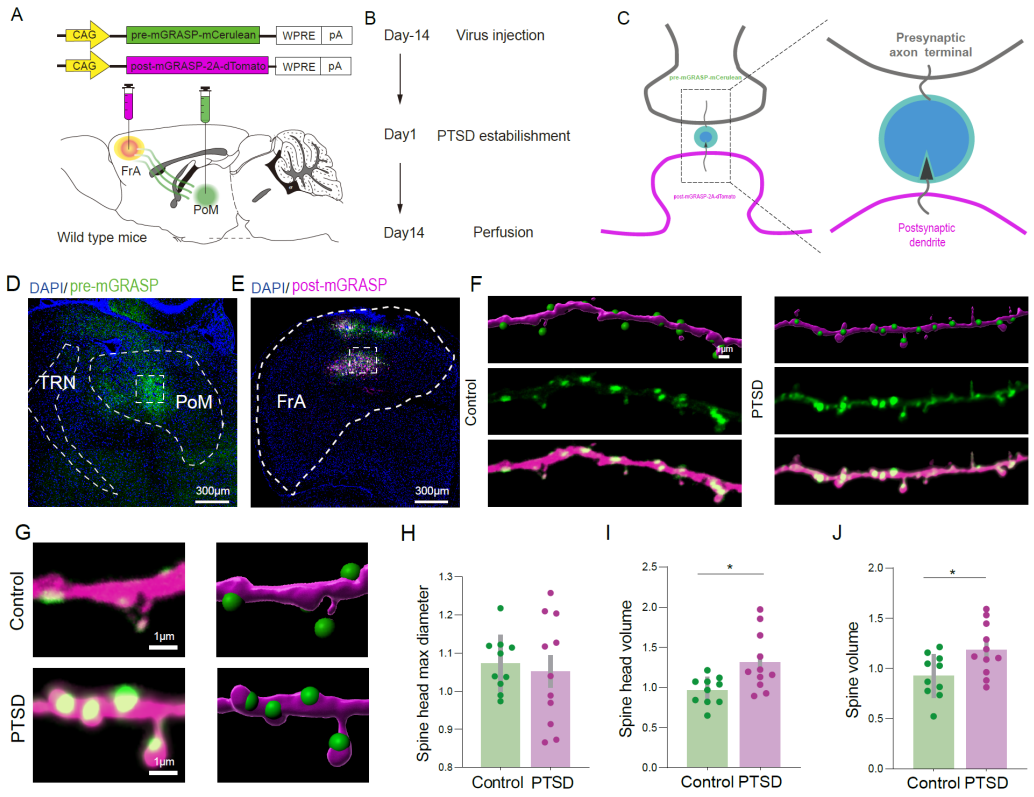


Fig. S17. Altered spine morphology in the PoM-FrA pathway in PTSD mice. (**A**) Schematic of viral strategy to selectively label synapses in FrA originating from PoM. (**B**) Schematic of sample preparation. (**C**) Schematic of dual-enhanced green fluorescent protein reconstitution across synaptic partners (dual-eGRASP). (**D**) Representative images showing pre-mGRASP expression in PoM. Scale bar: 300μm. (**E**) Representative images showing post-mGRASP expression in FrA. Scale bar: 300μm. (**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 ± S.E.M. See Table S1 for detailed statistical information.

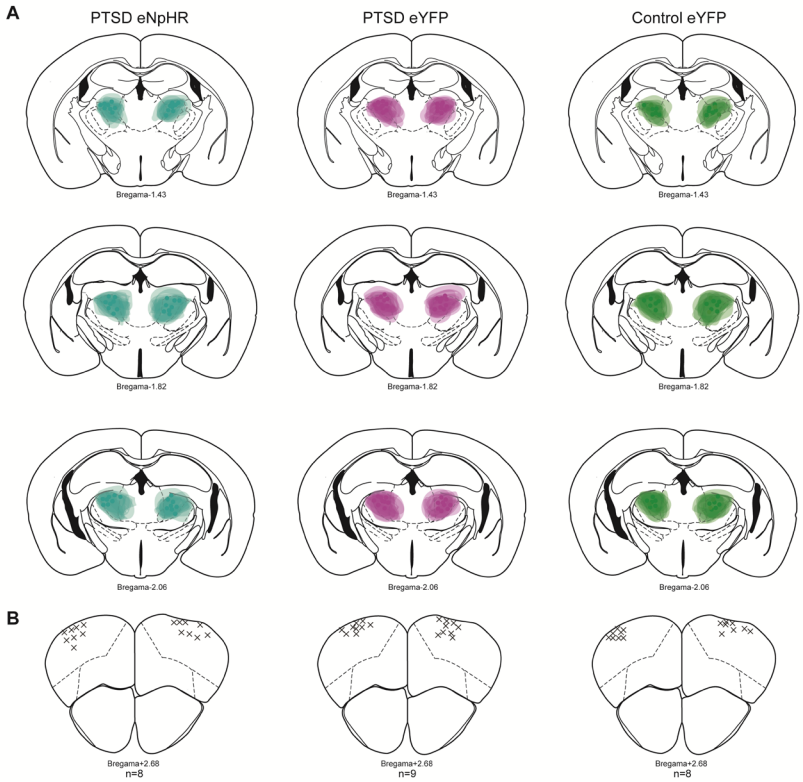


Fig. S18. Verification of the region showing virus infection and fiber placements for optogenetic inhibition. (**A**) Schematic showing areas expressing eNpHR in PoM. (**B**) Schematic showing optical fiber site in FrA

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	mouse	W=0.8957 df=10 p=0.1963	F=11.33 df1=1 df2=17 p=0.0014	Two-tailed unpaired t-test	t=3.122 df=17	p=0.0062	**
		PTSD n=9		W=0.8664 df=9 p=0.1123					
Figure 2G	VPM Arc	control n=10	mouse	W=0.9648 df=10 p=0.8389	F=1.976 df1=1 df2=17 p=0.3303	Two-tailed unpaired t-test	t=0.469 df=17	p=0.6450	ns
		PTSD n=9		W=0.958 df=9 p=0.7767					
Figure 2J	Area under cure in fiber recording	control n=7	mouse	W=0.9625 df=7 p=0.1594	F=1.849 df1=1 df2=10 p=0.3303	Two-tailed unpaired t-test	t=2.538 df=10	p=0.0294	*
		PTSD n=5		W=0.8784 df=5 p=0.3022					
Figure 2N	Event frequency in the Day 2 CFC test in fiber recording	control n=5	mouse	W=0.8853 df=5 p=0.3342	F=6.116 df1=1 df2=7 p=0.1127	Two-tailed unpaired t-test	t=2.89 df=7	p=0.0233	*
		PTSD n=4		W=0.8317 df=4 p=0.1724					
Figure 2P	Event frequency in the Day 7 CFC test in fiber recording	control n=5	mouse	W=0.9385 df=5 p=0.6557	F=41.39 df1=1 df2=7 p=0.0036	Two-tailed unpaired t-test	t=2.629 df=7	p=0.0339	*
		PTSD n=4		W=0.8844 df=4 p=0.3577					
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	$p_{\text{Control eGFP vs. PTSD eGFP}}$<0.0001	****
		PTSD eGFP n=7		W=0.9671 df=7 p=0.8766				$p_{\text{Control eGFP vs. PTSD Kir2.1}}$=0.4826	
		PTSD Kir2.1 n=7		W=0.9654 df=7 p=0.8632				$p_{\text{PTSD eGFP vs. PTSD Kir2.1}}$<0.0001	
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	$p_{\text{Control eGFP vs. PTSD eGFP}}$=0.0006	***
		PTSD eGFP n=7		W=0.941 df=7 p=0.6475				$p_{\text{Control eGFP vs. PTSD Kir2.1}}$=0.4084	
		PTSD Kir2.1 n=7		W=0.9468 df=7 p=0.7008				$p_{\text{PTSD eGFP vs. PTSD Kir2.1}}$<0.0227	
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	$p_{\text{Control eGFP vs. PTSD eGFP}}$=0.0165	*
		PTSD eGFP n=7		W=0.971 df=7 p=0.9056				$p_{\text{Control eGFP vs. PTSD Kir2.1}}$=0.9749	
		PTSD Kir2.1 n=7		W=0.9613 df=7 p=0.8297				$p_{\text{PTSD eGFP vs. PTSD Kir2.1}}$=0.0421	
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	$p_{\text{Control mCherry vs. PTSD mCherry}}$=0.0016	***
		PTSD mCherry n=6		W=0.9076 df=6 p=0.4207				$p_{\text{Control mcheery vs. PTSD hM4D (Gi)}}$=0.8439	
		PTSD hM4D (Gi) n=6		W=0.9713 df=6 p=0.9009				$p_{\text{PTSD mcheery vs. PTSD hM4D (Gi)}}$=0.0014	
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	$p_{\text{Control mCherry vs. PTSD mCherry}}$=0.0002	***
		PTSD mCherry n=6		W=0.9063 df=6 p=0.4128				$p_{\text{Control mcheery vs. PTSD hM4D (Gi)}}$=0.3481	
		PTSD hM4D (Gi) n=6		W=0.9776 df=6 p=0.9390				$p_{\text{PTSD mcheery vs. PTSD hM4D (Gi)}}$=0.0117	
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	$p_{\text{Control mCherry vs. PTSD mCherry}}$=0.0014	***
		PTSD mCherry n=6		W=0.9172 df=6 p=0.4854				$p_{\text{Control mcheery vs. PTSD hM4D (Gi)}}$=0.7170	
		PTSD hM4D (Gi) n=6		W=0.9225 df=6 p=0.5232				$p_{\text{PTSD mcheery vs. PTSD hM4D (Gi)}}$=0.0008	
Figure 4E	Density of TRN GFP+ Arc+	control n=12	mouse	W=0.9548 df=12 p=0.7077	F=8.621 df1=1 df2=22 p=0.0012	Two-tailed unpaired t-test	t=5.271 df=22	p<0.0001	****
		PTSD n=12		W=0.9344 df=12 p=0.4292					
Figure 4F	Percentaege of GFP+ Arc+ / GFP+ cells in TRN	control n=12	mouse	W=0.9637 df=12 p=0.8354	F=8.134 df1=1 df2=22 p=0.0016	Two-tailed unpaired t-test	t=5.258 df=22	p<0.0001	****
		PTSD n=12		W=0.9531 df=12 p=0.6824					
Figure 4I	TRN-PoM PPR	50ms control n=7	neuron	W=0.908 df=7 p=0.3825	F=8.127 df1=1 df2=13 p=0.0208	Mann-Whitney U test	U=6	p=0.0093	**
		50ms PTSD n=8		W=0.7552 df=8 p=0.0093					
		100ms control n=7	neuron	W=0.9489 df=7 p=0.7195	F=15 df1=1 df2=13 p=0.0040	Two-tailed unpaired t-test	t=3.21 df=13	p=0.0068	**
		100ms PTSD n=8		W=0.8858 df=8 p=0.2138					
		200ms control n=7	neuron	W=0.9087 df=7 p=0.3868	F=4.801 df1=1 df2=13 p=0.0744	Two-tailed unpaired t-test	t=5.293 df=13	p=0.0001	***
		200ms PTSD n=8		W=0.8987 df=8 p=0.2810					
		control n=7	neuron	Not all subgroups meets p> 0.05	Not all subgroups meets p>0.05	Friedman's M test	X^2=21.000 df=1	p < 0.0001	****
		PTSD n=8							

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	Two-tailed unpaired t-test	t=0.7605 df=13	p=0.4606	ns
		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	Two-tailed unpaired t-test	t=1.236 df=13	p=0.4606	ns
		200ms PTSD n=7		W=0.8403 df=7 p=0.1000					
		control n=8	neuron	Not all subgroups meets p> 0.05	Not all subgroups meets p>0.05	Friedman's M test	X^2=2.333 df=1	p =0.2385	ns
		PTSD n=7							
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	$p_{\text{Control mCherry vs. PTSD mCherry}}$<0.0001	****
		PTSD mCherry n=7		W=0.9666 df=7 p=0.8733				$p_{\text{Control mCherry vs. PTSD hM3D (Gq)}}$=0.9242	
		PTSD hM3D (Gq) n=8		W=0.8352 df=8 p=0.0672				$p_{\text{PTSD mCherry vs. PTSD hM3D (Gq)}}$<0.0001	
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	$p_{\text{Control mCherry vs. PTSD mCherry}}$<0.0001	****
		PTSD mCherry n=7		W=0.8351 df=7 p=0.0895				$p_{\text{Control mCherry vs. PTSD hM3D (Gq)}}$=0.1324	
		PTSD hM3D (Gq) n=8		W=0.9689 df=8 p=0.8894				$p_{\text{PTSD mCherry vs. PTSD hM3D (Gq)}}$<0.0001	
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	$p_{\text{Control mCherry vs. PTSD mCherry}}$=0.0022	****
		PTSD mCherry n=7		W=0.8048 df=7 p=0.0457				$p_{\text{Control mCherry vs. PTSD hM3D (Gq)}}$>0.9999	
		PTSD hM3D (Gq) n=8		W=0.8818 df=8 p=0.1959				$p_{\text{PTSD mCherry vs. PTSD hM3D (Gq)}}$=0.0082	
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	$p_{\text{Control eYFP vs. PTSD eYFP}}$=0.0123	***
		PTSD eYFP n=6		W=0.7709 df=6 p=0.0317				$p_{\text{Control eYFP vs. PTSD SSFO}}$>0.9999	
		PTSD SSFO n=8		W=0.935 df=8 p=0.5630				$p_{\text{PTSD eYFP vs. PTSD SSFO}}$=0.0054	
Figure 5K	Freezing time (%) in the Day 2 CFC test PTSD SSFO	off n=8	mouse	W=0.7526 df=8 p=0.0087	F=2.987 df1=1 df2=14 p=0.1721	Wilcoxon signed-rank test	rs=0.6667 p=0.0415	p=0.0078	**
		on n=8		W=0.7872 df=8 p=0.0208					
Figure 5K	Freezing time (%) in the Day 2 CFC test Control eYFP	off n=6	mouse	W=0.9564 df=6 p=0.7913	F=9.1114 df1=1 df2=10 p=0.0299	Wilcoxon signed-rank test	rs=0.2571 p=0.3292	p=0.4375	ns
		on n=6		W=0.7895 df=6 p=0.0472					
Figure 5K	Freezing time (%) in the Day 2 CFC test PTSD eYFP	off n=6	mouse	W=0.8184 df=6 p=0.70854	F=1.08 df1=1 df2=10 p=0.9351	Two-tailed paired t-test	t=0.02308 df=10	p=0.9825	ns
		on n=6		W=0.9537 df=6 p=0.7704					
Figure 5L	Freezing time (%) in the Day 7 CFC test Control eYFP	off n=6	mouse	W=0.7557 df=6 p=0.0226	F=1.657 df1=1 df2=10 p=0.5931	Wilcoxon signed-rank test	rs=0.6 p=0.1208	p=0.0625	ns
		on n=6		W=0.7896 df=6 p=0.0472					
Figure 5L	Freezing time (%) in the Day 7 CFC test PTSD SSFO	off n=8	mouse	W=0.9134 df=8 p=0.3786	F=3.225 df1=1 df2=14 p=0.1452	Two-tailed paired t-test	t=2.738 df=14	p=0.0160	*
		on n=8		W=0.8959 df=8 p=0.2651					
Figure 5L	Freezing time (%) in the Day 7 CFC test PTSD eYFP	off n=6	mouse	W=0.6638 df=6 p=0.0025	F=1.03 df1=1 df2=10 p=0.9753	Wilcoxon signed-rank test	rs=0.7143 p=0.0681	p=0.2188	ns
		on n=6		W=0.908 df=6 p=0.4233					
Figure 6Q	CNQX in FrA	baseline n=5 neurons from 3 mice	neuron	W=0.8666 df=6 p=0.2529	F=859.2 df1=1 df2=8 p<0.0001	Two-tailed paired t-test	t=5.607 df=4	p=0.0050	**
		CNQX n=5 neurons from 3 mice		W=0.8601 df=6 p=0.2286					
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	$p_{\text{Control eYFP vs. PTSD eYFP}}$<0.0001	****
		PTSD eNPHR n=9		W=0.9258 df=9 p=0.4423				$p_{\text{Control eYFP vs. PTSD eNPHR}}$=0.7749	
		PTSD eYFP n=8		W=0.8774 df=8 p=0.1780				$p_{\text{PTSD eYFP vs. PTSD eNPHR}}$<0.0001	
Figure 7E	Freezing time (%) in the Day 2 CFC test control eYFP	off n=8	mouse	W=0.8476 df=8 p=0.0901	F=8.437 df1=1 df2=14 p=0.0116	Wilcoxon signed-rank test	rs=0.4524 p=0.1337	p=0.1094	ns
		on n=8		W=0.734 df=8 p=0.0054					
Figure 7E	Freezing time (%) in the Day 2 CFC test PTSD eYFP	off n=9	mouse	W=0.9538 df=9 p=0.7316	F=1.621 df1=1 df2=16 p=0.0116	Two-tailed paired t-test	t=1.441 df=8	p=0.1876	ns
		on n=9		W=0.9357 df=9 p=0.5375					
Figure 7E	Freezing time (%) in the Day 2 CFC test PTSD eNPHR	off n=8	mouse	W=0.9525 df=8 p=0.7364	F=3.164 df1=1 df2=14 p=0.1628	Wilcoxon signed-rank test	rs=0.04762 p=0.4674	p=0.0156	*
		on n=8		W=0.7295 df=8 p=0.1148					

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	neuron			Two-way ANOVA		p=0.0462	*
		PTSD n=7							
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	$p_{\text{Control eGFP vs. PTSD eGFP}}$=0.0090	**
		PTSD eGFP n=6		W=0.9491 df=6 p=0.7329				$p_{\text{Control eGFP vs. PTSD Kir2.1}}$=0.0228	
		PTSD Kir2.1 n=7		W=0.9376 df=7 p=0.6176				$p_{\text{PTSD eGFP vs. PTSD Kir2.1}}$=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	$p_{\text{Control eGFP vs. PTSD eGFP}}$=0.0324	**
		PTSD eGFP n=6		W=0.8482 df=6 p=0.1522				$p_{\text{Control eGFP vs. PTSD Kir2.1}}$=0.0119	
		PTSD Kir2.1 n=7		W=0.9079 df=7 p=0.3813				$p_{\text{PTSD eGFP vs. PTSD Kir2.1}}$=0.9320	
Figure S5A	Freezing time (%) in Cue test in Kir	Control eGFP n=7	mouse			Two-way ANOVA		$p_{\text{Control eGFP vs. PTSD eGFP}}$=0.0324	**
		PTSD eGFP n=6						$p_{\text{Control eGFP vs. PTSD Kir2.1}}$=0.0119	
		PTSD Kir2.1 n=7						$p_{\text{PTSD eGFP vs. PTSD Kir2.1}}$=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	$p_{\text{Control eGFP vs. PTSD mCherry}}$=0.2520	ns
		PTSD mCherry n=5		W=0.8825 df=5 p=0.3206				$p_{\text{Control eGFP vs. PTSD hM4D(Gi)}}$=0.5039	
		PTSD hM4D(Gi) n=5		W=0.8959 df=5 p=0.3876				$p_{\text{PTSD eGFP vs. PTSDhM4D(Gi)}}$=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	$p_{\text{Control eGFP vs. PTSD mCherry}}$=0.5221	ns
		PTSD mCherry n=5		W=0.8797 df=5 p=0.3079				$p_{\text{Control eGFP vs. PTSD hM4D(Gi)}}$=0.1848	
		PTSD hM4D(Gi) n=5		W=0.7577 df=5 p=0.0350				$p_{\text{PTSD eGFP vs. PTSDhM4D(Gi)}}$>0.9999	
Figure S5B	Freezing time (%) in Cue test in Gi	Control mCherry n=9	mouse			Two-way ANOVA		$p_{\text{Control eGFP vs. PTSD mCherry}}$=0.0189	*
		PTSD mCherry n=5						$p_{\text{Control eGFP vs. PTSD hM4D(Gi)}}$=0.0175	
		PTSD hM4D(Gi) n=5						$p_{\text{PTSD eGFP vs. PTSDhM4D(Gi)}}$=0.9996	
Figure S6E	Density of mCherry+ Arc+ cells in PoM	PTSD mCherry n=5	mouse	W=0.9646 df=5 p=0.8397	F=1.348 df1=1 df2=8 p=0.7793	Two-tailed unpaired t-test	t=3.182 df=8	p=0.0130	*
		PTSD hM4D(Gi) n=5		W=0.7932 df=5 p=0.0712					

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	mouse	Not all subgroups meets p> 0.05	Not all subgroups meets p>0.05	Friedman's M test	X^2=74.387 df=1	p < 0.0001	****
		PTSD n=8							
Figure S8G	Percentage of GFP^+Arc^+in GFP^+ cells in TRN	control n=8	mouse	W=0.9618 df=8 p=0.8268	F=7.689 df1=1 df2=14 p=0.0153	Two-tailed unpaired t-test	t=5.207 df=14	p=0.0001	***
		PTSD n=8		W=0.9493 df=8 p=0.7040					
Figure S9F	density of ZI GFP^+ Arc^+ along the bregma	control n=8	mouse	W=0.9722 df=8 p=0.9145	F=1.364 df1=1 df2=13 p=0.7213	Two-tailed unpaired t-test	t=0.6605 df=13	p=0.5205	ns
		PTSD n=7		W=0.9512 df=7 p=0.7402					
Figure S9G	Percentage of GFP^+Arc^+in GFP^+ cells in ZI	control n=8	mouse	W=0.7143 df=8 p=0.0033	F=1.033 df1=1 df2=13 p=0.9850	Mann-Whitney U test	U=21	p=0.4634	ns
		PTSD n=7		W=0.8056 df=7 p=0.0465					
Figure S10C	Max amplitude of oIPSC in PoM neurons received inputs from TRN	control n=7	mouse	W=0.9455 df=7 p=0.6887	F=1.908 df1=1 df2=13 p=0.4176	Two-tailed unpaired t-test	t=2.933 df=13	p=0.0116	*
		PTSD n=8		W=0.6383 df=8 p=0.0004					
Figure S10F	Max amplitude of oIPSC in PoM neurons received inputs from ZI	control n=7	mouse	W=0.9493 df=7 p=0.7236	F=1.417 df1=1 df2=13 p=0.6546	Two-tailed unpaired t-test	t=2.938 df=13	p=0.7735	ns
		PTSD n=8		W=0.9277 df=8 p=0.4954					
Figure S12G	Density of mCherry+ Arc+ cells in TRN	control n=6	mouse	W=0.9546 df=6 p=0.7776	F=5.376 df1=1 df2=10 p=0.0886	Mann-Whitney U test	U=0	p=0.0022	**
		PTSD n=6		W=0.7919 df=6 p=0.0497					
Figure S12H	Percentage of mCherry+ cells in TRN	control n=6	mouse	W=0.9561 df=6 p=0.7894	F=3.619 df1=1 df2=10 p=0.1844	Two-tailed unpaired t-test	t=1.376 df=10	p=0.1990	ns
		PTSD n=6		W=0.982 df=6 p=0.9611					
Figure S12I	Percentage of mCherry+ Arc+ in mCherry+ cells in TRN	control n=6	mouse	W=0.9409 df=6 p=0.6661	F=2.625 df1=1 df2=10 p=0.3130	Two-tailed unpaired t-test	t=4.513 df=10	p=0.0011	**
		PTSD n=6		W=0.9721 df=6 p=0.9062					
Figure S15C	sIPSC frequceny between baseline and light on	control n=5	neuron	W=0.9108 df=5 p=0.4727	F=1.86 df1=1 df2=18 p=0.5625	Two-tailed unpaired t-test	t=1.261 df=8	p=0.0229	*
		PTSD n=5		W=0.8102 df=5 p=0.0978					
Figure S15C	sIPSC frequceny between light on and light off	control n=5	neuron	W=0.8102 df=5 p=0.0978	F=6.145 df1=1 df2=18 p=0.1066	Two-tailed unpaired t-test	t=2.831 df=8	p=0.0079	**
		PTSD n=5		W=0.7183 df=5 p=0.0147					
Figure S16Q	Fluorescence intensity in FrA between sham and stimulation	control n=6 slices from 3 mice	Slice	W=0.9098 df=6 p=0.4349	F=3.373 df1=1 df2=18 p=0.1874	Two-tailed unpaired t-test	t=4.713 df=18	p=0.0002	***
		PTSD n=14 slices from 3 mice		W=0.9395 df=14 p=0.4119					
Figure S16Q	Fluorescence intensity in Prl between sham and stimulation	control n=10 slices from 3 mice	Slice	W=0.7428 df=10 p=0.0029	F=4.349 df1=1 df2=24 p=0.0313	Two-tailed unpaired t-test	t=3.617 df=24	p=0.0014	**
		PTSD n=16 slices from 3 mice		W=0.9661 df=16 p=0.7113					
Figure S16Q	Fluorescence intensity in S1 between sham and stimulation	control n=6 slices from 3 mice	Slice	W=0.956 df=6 p=0.7881	F=6.911 df1=1 df2=13 p=0.0477	Two-tailed unpaired t-test	t=2.366 df=13	p=0.0342	*
		PTSD n=9 slices from 3 mice		W=0.958 df=9 p=0.7777					
Figure S16Q	Fluorescence intensity in M2 between sham and stimulation	control n=10 slices from 3 mice	Slice	W=0.9519 df=10 p=0.6915	F=1.73 df1=1 df2=17 p=0.4309	Two-tailed unpaired t-test	t=0.8623 df=17	p=0.4005	ns
		PTSD n=9 slices from 3 mice		W=0.8985 df=9 p=0.2435					
Figure S16Q	Fluorescence intensity in ACC between sham and stimulation	control n=8 slices from 3 mice	Slice	W=0.938 df=8 p=0.5918	F=6.35 df1=1 df2=16 p=0.0235	Two-tailed unpaired t-test	t=0.7565 df=16	p=0.4603	ns
		PTSD n=10 slices from 3 mice		W=0.9138 df=10 p=03084					
Figure S17H	Spine head max diameter	control n=10 slices in 3 mice	Slice	W=0.9405 df=10 p=0.5584	F=3.552 df1=1 df2=19 p=0.0698	Two-tailed unpaired t-test	t=0.4347 df=19	p=0.6687	ns
		PTSD n=11 slices in 3 mice		W=0.9308 df=11 p=0.4187					
Figure S17I	Spine head volume	control n=10 slices in 3 mice	Slice	W=0.9514 df=8 p=0.6850	F=4.42 df1=1 df2=19 p=0.0354	Two-tailed unpaired t-test	t=2.752 df=19	p=0.0127	*
		PTSD n=11 slices in 3 mice		W=0.8972 df=7 p=0.1709					
Figure S17J	Spine volume	control n=10 slices in 3 mice	Slice	W=0.9546 df=10 p=0.7227	F=1.399 df1=1 df2=19 p=0.6248	Two-tailed unpaired t-test	t=2.458 df=19	p=0.0237	*
		PTSD n=11slices in 3 mice		W=0.9524 df=11 p=0.6748					

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.

Antibodies		
REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
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.