

Prefrontal engrams of long-term fear memory perpetuate pain perception

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Supplementary table 1: Detailed information on statistical parameters for all comparisons in main figures and Extended Data figures.

Figure	Group name	F and P values	Stats test
Fig. 1b	Common cells	F (3, 15) = 28.32, P < 0.0001 Foot Shock (stimulus 1) followed by Foot Shock (stimulus 2) vs Capsaicin (stimulus 1) followed by Fear Recall (stimulus 2) P < 0.0001 Foot Shock (stimulus 1) followed by Foot Shock (stimulus 2) vs Fear recall (stimulus 1) followed by Capsaicin (stimulus 2) P < 0.0001 Fear Recall (stimulus 1) followed by Fear Recall (stimulus 2) vs Capsaicin (stimulus 1) followed by Fear Recall (stimulus 2) P = 0.0004 Fear Recall (stimulus 1) followed by Fear Recall (stimulus 2) vs Fear recall (stimulus 1) followed by Capsaicin (stimulus 2) P = 0.0404	One-way ANOVA with Tukey's multiple comparisons
Fig. 1c	Proportion of common glutamatergic cells	F (3, 6) = 17.09, P = 0.0024 SATB2 p=0.0034 LHX2 p=0.9636 Ctip2 p=0.0458 TLE4 p=0.9087	Two-way ANOVA with Šidák correction for multiple comparisons
	Proportion of common gabaergic cells	F (2, 4) = 11.10, P = 0.0233 SOM p=0.0263 PV p=0.8112 VIP p=0.0224	
Fig. 1e	Activated neurons	P = 0.0270	Unpaired t-test, two-tailed
	Fear recall	P = 0.0045	Paired t-test, two-tailed
	Tonic pain	P = 0.0002	Paired t-test
Fig. 1f	Activated neurons	P < 0.0001	Unpaired t-test
	Fear recall	P = 0.0069	Paired t-test
	Tonic pain	P = 0.0019	
Fig. 2b	Proportion of all units with behavior locked firing increase	Fear/Pain Modality: F(1, 6)=5.799, P=0.0527 Incr-/Decreased Activity: F(2, 12)=33.50, P<0.0001 Interaction Factor: F(2, 12)=8.09, P =0.0060	Two-way ANOVA with Sidak's multiple comparisons test
Fig. 2c	Proportion of putative principal neurons with behavior locked firing increase	Fear/Pain Modality: F(1, 6)=6.531, P=0.0432 Incr-/Decreased Activity: F(2, 12)=28.09, P<0.0001 Interaction Factor: F(2, 12)= 7.00, P=0.0097	

	Proportion of putative interneurons with behavior locked firing increase	Fear/Pain Modality: $F(1, 5) = 1.305$, $P = 0.305$ Incr-/Decreased Activity: $F(2, 10) = 0.1023$, $P = 0.904$ Interaction Factor: $F(2, 10) = 0.7078$, $P = 0.516$	
Fig. 2d	Proportion of units with behavior locked firing increase	$P = 0.6778$	Unpaired t-test
Fig. 3a	Silencing of random prefrontal neurons: Fear recall	$P = 0.077$	Paired t-test, two-tailed
	Silencing of random prefrontal neurons: Tonic pain	$P = 0.839$	
	Activation of random prefrontal neurons: Fear recall	$P = 0.2591$	
	Activation of random prefrontal neurons: Tonic pain	$P = 0.3792$	
Fig. 3b	White noise Fear recall engram	$F(2, 27) = 18.00$, $P < 0.0001$ 240 s: Laser off vs Baseline $P = 0.2357$ 240 s: Laser on vs Baseline $P = 0.1922$ 480 s: Laser off vs Baseline $P = 0.0364$ 480 s: Laser on vs Baseline $P = 0.0002$ 720 s: Laser off vs Baseline $P = 0.0004$ 720 s: Laser on vs Baseline $P < 0.0001$ 960 s: Laser off vs Baseline $P = 0.004$ 960 s: Laser on vs Baseline $P < 0.0001$ 1200 s: Laser off vs Baseline $P < 0.0001$ 1200 s: Laser on vs Baseline $P = 0.0001$	Two-way ANOVA with Šidák correction for multiple comparisons
	White noise Random neurons	$F(2, 30) = 15.84$, $P < 0.0001$ 240 s: Laser off vs Baseline $P = 0.0557$ 240 s: Laser on vs Baseline $P = 0.2828$ 480 s: Laser off vs Baseline $P = 0.0003$ 480 s: Laser on vs Baseline $P = 0.0009$ 720 s: Laser off vs Baseline $P = 0.0089$ 720 s: Laser on vs Baseline $P = 0.0081$ 960 s: Laser off vs Baseline $P = 0.0029$ 960 s: Laser on vs Baseline $P = 0.0010$	

		1200 s: Laser off vs Baseline $P < 0.0001$ 1200 s: Laser on vs Baseline $P = 0.0026$	
Fig. 3c	Reward learning	$F(5, 72) = 4.956$, $P = 0.0006$	One-way ANOVA
	Reward test: Number of responding trials	$P = 0.4583$	Paired t-test, two-tailed
	Reward test: % accuracy	$P = 0.204$	Wilcoxon test
Fig. 3d	Innate fear: Freezing duration	$P = 0.048$	Paired t-test, two-tailed
	Tonic pain: Duration of pain behavior	$P = 0.0197$	
Fig. 4a	Inflammatory heat hyperalgesia	$F(1, 12) = 15.74$, $P = 0.0006$ Post fear conditioning $p = 0.4283$ 2 Days post CFA $p = 0.0006$	Two-way ANOVA with Sidak's test for multiple comparisons
	Neuropathic mechanical allodynia	$F(3, 168) = 130.6$, $P < 0.0001$ 0.02g filament $p = 0.9921$ 0.07g filament $p > 0.9999$ 0.16g filament $p = 0.2019$ 0.40g filament $p = 0.0120$ 0.60g filament $p = 0.0273$ 1.00g filament $p = 0.4948$	Two-way ANOVA with Tukey's multiple comparisons
Fig. 4b	Overlap between thermal nociception and fear memory	$P < 0.0001$	Unpaired t-test, one-tailed
Fig. 4c	Overlap between tactile sensitivity and fear memory	$P = 0.0022$	
Fig. 4f	Projections	$F(148, 370) = 1.733$, $P < 0.0001$ ACC: Sham no FC vs Sham FC $p = 0.0449$ MD: Fear vs Sham FC $p = 0.039$ Fear vs SNI FC $p < 0.0001$ Sham no FC Sham FC $p = 0.0265$ Sham FC vs SNI FC $p = 0.0185$ SNI no FC vs SNI FC $p = 0.0066$ <i>For remaining p values: please see Supplementary data 1</i>	Two-way ANOVA with Tukey's test for multiple comparisons
Fig. 5b	Activated neurons	$P = 0.0006$	Unpaired t-test
	Fear recall	$P = 0.0044$	Paired t-test
	Mechanical sensitivity	$F(5, 35) = 7.245$, $P < 0.0001$ 0.02g filament $p = 0.0117$ 0.07g filament $p < 0.0001$ 0.16g filament $p < 0.0001$ 0.40g filament $p < 0.0001$ 0.60g filament $p = 0.7706$ 1.00g filament $p > 0.9999$	Two-way ANOVA with Tukey's multiple comparisons test
Fig. 5c	Activated neurons	$P < 0.0001$	Unpaired t-test
	Fear recall	$P = 0.0009$	Paired t-test
	Heat sensitivity	$P = 0.0015$	
Fig. 5d	Fear recall	$P = 0.0644$	Two-way ANOVA with Tukey's
	Mechanical sensitivity	$F(5, 25) = 0.7093$, $P = 0.6220$	

		0.02g filament p=0.6538 0.07g filament p=0.9798 0.16g filament p=0.9798 0.40g filament p=0.9798 0.60g filament p>0.9999 1.00g filament p>0.9999	multiple comparisons test
Fig. 5e	Fear recall	P = 0.9177	Paired t-test
	Heat sensitivity	P = 0.0638	
ED Fig. 1b	Prelimbic neurons tagged in homecage	P <0.0001	Unpaired t-test, two-tailed
ED Fig. 1d	Tagging of prelimbic neurons	F (3, 19) = 4.087, P = 0.0213	One-way ANOVA with Dunnet's multiple comparisons
ED Fig. 2a	Time course of Doxycycline withdrawal	F (2, 28) = 16.86, P < 0.0001 72 h: Homecage vs Capsaicin P = 0.0019	Two-way ANOVA with Sidak's test for multiple comparisons
ED Fig. 2c	Distance	P = 0.578465	Paired t-test, two-tailed
	Speed	P = 0.569085	
ED Fig. 2d	Distance	P = 0.6007	
	Speed	P = 0.2118	
ED Fig. 5a	Baseline mechanical sensitivity	F (3, 360) = 0.6322, P = 0.5947 0.02g filament: Pre conditioning uninjured paw vs pre conditioning injured paw p>0.9999 Pre conditioning uninjured paw vs post conditioning uninjured paw p>0.9999 Pre conditioning uninjured paw vs post conditioning injured paw p>0.9999 Pre conditioning injured paw vs post conditioning uninjured paw p>0.9999 Pre conditioning injured paw vs post conditioning injured paw p>0.9999 Post conditioning uninjured paw vs post conditioning injured paw p>0.9999 0.07g filament: Pre conditioning uninjured paw vs pre conditioning injured paw p=0.931 Pre conditioning uninjured paw vs post conditioning uninjured paw p>0.9999 Pre conditioning uninjured paw vs post conditioning injured paw p=0.7464 Pre conditioning injured paw vs post conditioning uninjured paw p=0.931	Two-way ANOVA with Tukey's multiple comparisons test

		Pre conditioning injured paw vs post conditioning injured paw p=0.978 Post conditioning uninjured paw vs post conditioning injured paw p=0.7464 0.16g filament: Pre conditioning uninjured paw vs pre conditioning injured paw p=0.978 Pre conditioning uninjured paw vs post conditioning uninjured paw p=0.4955 Pre conditioning uninjured paw vs post conditioning injured paw p=0.3751 Pre conditioning injured paw vs post conditioning uninjured paw p=0.7464 Pre conditioning injured paw vs post conditioning injured paw p=0.6232 Post conditioning uninjured paw vs post conditioning injured paw p=0.9971 0.4g filament: Pre conditioning uninjured paw vs pre conditioning injured paw p=0.931 Pre conditioning uninjured paw vs post conditioning uninjured paw p=0.4955 Pre conditioning uninjured paw vs post conditioning injured paw p=0.931 Pre conditioning injured paw vs post conditioning uninjured paw p=0.8524 Pre conditioning injured paw vs post conditioning injured paw p>0.9999 Post conditioning uninjured paw vs post conditioning injured paw p=0.8524 0.6g filament: Pre conditioning uninjured paw vs pre conditioning injured paw p=0.9971 Pre conditioning uninjured paw vs post conditioning uninjured paw p=0.8524 Pre conditioning uninjured paw vs post conditioning injured paw p=0.931 Pre conditioning injured paw vs post conditioning uninjured paw p=0.7464	
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		Pre conditioning injured paw vs post conditioning injured paw p=0.8524 Post conditioning uninjured paw vs post conditioning injured paw p=0.9971 1.0 g filament: Pre conditioning uninjured paw vs pre conditioning injured paw p=0.8524 Pre conditioning uninjured paw vs post conditioning uninjured paw p>0.9999 Pre conditioning uninjured paw vs post conditioning injured paw p=0.931 Pre conditioning injured paw vs post conditioning uninjured paw p=0.8524 Pre conditioning injured paw vs post conditioning injured paw p=0.9971 Post conditioning uninjured paw vs post conditioning injured paw p=0.931	
ED Fig. 5b	Neuropathic mechanical allodynia	F (1, 84) = 3.132, P = 0.0804 0.02g filament p=0.9998 0.07g filament p=0.7729 0.16g filament p=0.7729 0.40g filament p=0.7729 0.60g filament p=0.9998 1.00g filament p>0.9999	
ED Fig. 5c	Inflammatory heat hyperalgesia	F (1, 10) = 1.352, P = 0.2719 Post Fear Conditioning p=0.8760 2 Days post CFA p=0.9764	
ED Fig. 6a	Neurochemical identity of prelimbic neurons in SNI and Sham condition	F (4, 8) = 160.5, P<0.0001 SATB2 p=0.9881 Ctip2 p=0.9935 TLE4 p=0.9256 SOM p>0.9999 PV p=0.7347	
ED Fig. 6c	Projections	F (148, 370) = 1.733, P<0.0001 AIP: Sham no FC vs Sham FC p=0,004 LA: Sham no FC vs Sham FC p=0,0379 PT: Fear recall vs. SNI FC p <0,0001 Sham FC vs. SNI FC p= 0,0021 SNI no FC vs. SNI FC p <0,0001 <i>For remaining p values: please see Supplementary data 1</i>	Two-way ANOVA with Tukey's test for multiple comparisons

ED Fig. 9c	Mechanical sensitivity (6 weeks post-SNI)	$F(5, 35) = 4.391$, $P = 0.0033$ 0.02g filament $p=0.0002$ 0.07g filament $p=0.0004$ 0.16g filament $p=0.0531$ 0.40g filament $p=0.1193$ 0.60g filament $p>0.9999$ 1.00g filament $p>0.9999$	Two-way ANOVA with Tukey's multiple comparisons test
ED Fig. 9d	Heat sensitivity (2 weeks post-CFA)	$P = 0.0016$	Paired t-test