Supplementary material for:

Activation of the dorsal, but not ventral, hippocampus relieves neuropathic pain in rodents

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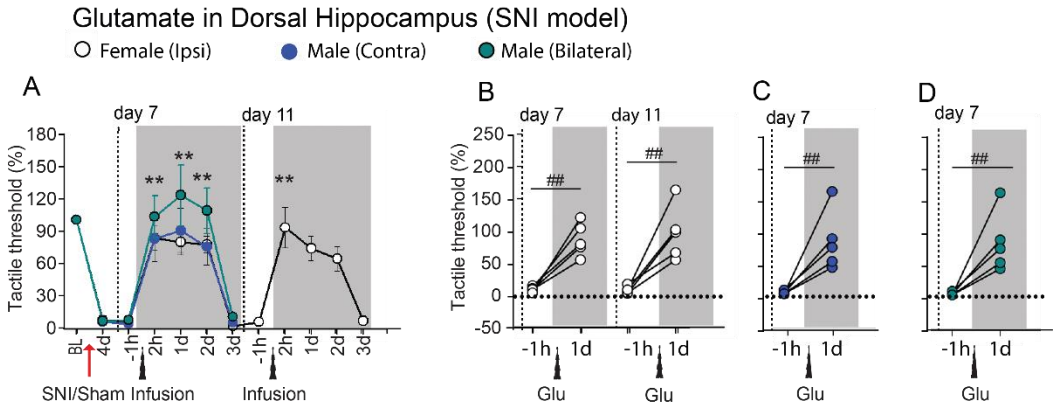
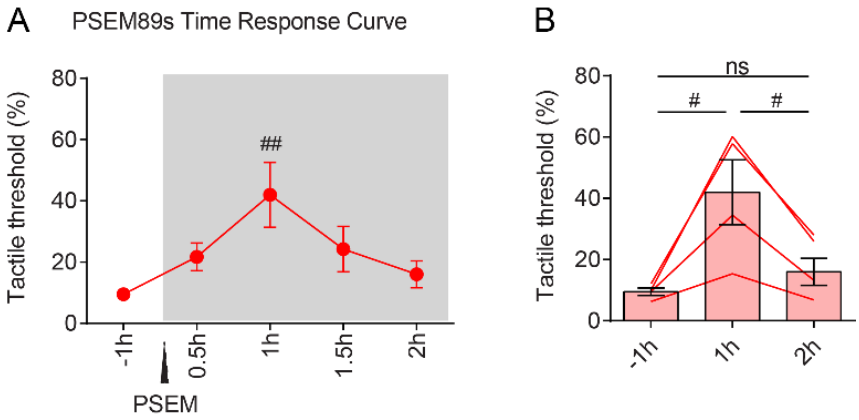
Figure S1



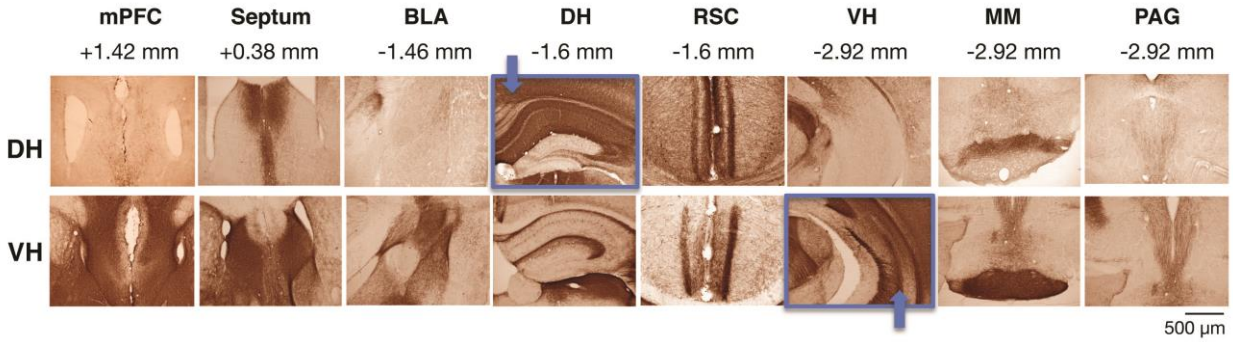
Supp Figure S1. Reversal of SNI induced tactile allodynia (pain-like behavior) was similar between male and female rats, and for ipsilateral, contralateral, or bilateral glutamate injections in the dorsal hippocampus. A. Temporal response profile for relief from tactile allodynia after microinjection of glutamate (21.2 pmol in 1µl volume) into dorsal hippocampus (DH) ipsilateral to the SNI injury in female rats and contralateral and bilateral to SNI injury in males and rats. In the female rats the glutamate injection was repeated at day 11 from SNI surgery. Both magnitude of pain relief and its duration were replicated with the second injection. **B, C & D.** Individual rat responses to glutamate injection in DH. Tactile thresholds are shown only for -1 hour prior to glutamate injection and at 2 hours post glutamate injection: for glutamate injected **B.** ipsilateral in female rats at day 7 and day 11 (n=5); **C.** contralateral in male rats at day 7 (n=5); and **D.** bilateral in male rats at day 7 (n=5), relative to the SNI injury. Post-hoc statistical significance of responses from baseline are indicated as **p < 0.001 (1-way ANOVA, post-hoc comparison with -1h) and ##p < 0.005 (compared with -1h paired t-test). For detailed statistics, see **Table S1**.

<u>Methods for Suppl. Figure S1</u>:

For these experiments we used Sprague Dawley rats weighing 200-250 g. The animals were group-housed and had free access to standard chow and water. These experiments were done in Sun Yat-Sen University, Guangzhou, China. These animals were kept at 21±2°C temperature and 30-60% humidity, under a 12/12 h light/dark cycle. Handling and testing were performed during the light period. To minimize stress, they were handled regularly before surgery and behavioral testing. SNI surgeries were performed as described in the main manuscript.



Supp. Figure S2. Time response curve to PSEM activation. A-B. Tactile thresholds of SNI rats, tested at 5 time-intervals relative to i.p. injection of PSEM^{89s} (30 mg/kg). The PSEM^{89s} unmasks Na+ channels on the membrane surface of DH neurons, previously infected with PSAM-5HT3. Pain relief peaked at 1 hour after injection, lasting for less than 2 hours (n=4). ## p< 0.005 (1-way ANOVA post-hoc comparison with -1 h), # p<0.05 (post hoc comparison with -1 h, and with +2h), ns p> 0.05 (post hoc comparison between -1h and +2h). This time course of observed analgesia closely matches previous reports of the duration of unmasking Na+ channels with PSEM^{89s}, see (Aldrin-Kirk and Bjorklund 2019).



Supp. Figure S3. Projections from dorsal and ventral hippocampus in 2 healthy mice. Anterograde tracing was performed by injecting AAV8-mCherry (UNC, 0.5µl/site) into the dorsal (anteroposterior -3mm; mediolateral 1 mm; ventrodorsal 2.25mm; blue arrow, box) or ventral (anteroposterior -1.8mm; mediolateral 2.25 mm; ventrodorsal 3mm; blue arrow, box) hippocampus. Immunostaining was performed using anti-mCherry antibodies (1:1,000, Abcam, ab167453) and visualized using diaminobenzidine. DH = terminations seen from dorsal hippocampus injection; VH = terminations from ventral hippocampus injection. mPFC = medial prefrontal cortex; BLA = basolateral amygdala; RSC = retrosplenial cortex; MM = mammillary bodies; PAG = periaqueductal grey.

Methods for Supp. Figure S3:

Two 9-week-old male C57BL/CN mice obtained from a commercial supplier (Harlan) were used for these experiments. Mice were individually housed and allowed ad libitum access to food and water. Immunostaining was performed using anti-mCherry antibodies (1:1,000, Abcam, ab167453) and visualized using diamino benzidine, as described previously (Corcoran KA et al., 2011).

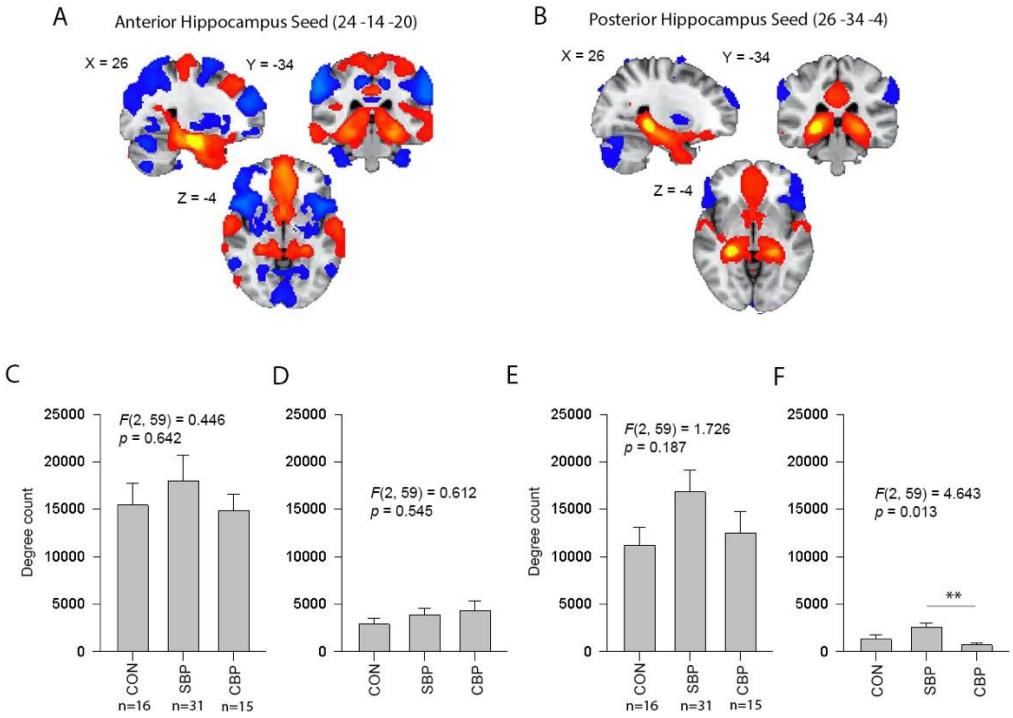


Figure S4: Human hippocampal anterior (AH) and posterior (PH) subdivisions show distinct negative functional connectivity (FC) in back pain patients. A & B Functional connections (FC) (red/yellow are positive; blue negative) for AH and PH seeds respectively (coordinates derived from study Qin, Duan et al. 2016) were used in meta-analysis website (Neurosynth, Yarkoni, Poldrack et al. 2011) and based on n=1000 healthy subjects. Negative FC from AH extends to many more brain regions and shows stronger connectivity than from PH. Degree count for positive **(C & E)** and negative **(D & F)** FC for AH and PH seeds in healthy controls (CON), subacute backpain (SBP) and chronic backpain (CBP) subjects. **C.** There was no statistically significant degree count differences between the three groups (1-way ANOVA, F(2,59) = 0.446, p = 0.642) for positive FC degree counts for the AH seed. **D.** There was no statistically significant degree count differences between the three groups (F(2,59) = 0.612, p = 0.545) for negative FC degree counts for the AH seed. **E.** There was no statistically significant degree count differences between the three groups (F(2,59) = 1.726, p = 0.187) for positive FC degree counts for the PH seed. **F.** Degree count for PH negative FC showed significant differences between groups (F(2,59) = 4.643, p = 0.013).

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Methods for Supp. Figure S4:

Participants: The human study included 16 healthy control (CON) subjects (6/10 women/men; age: 36.69±7.08 years old, mean±SD), 15 sex- and age-matched patients with chronic back pain (CBP) (5/10 women/men; age: 42.67±5.34 years), and 31 matched patients with subacute back pain (SBP) (15/16 women/men; age: 40.19±11.02 years), all of whom were previously studied in (Baliki M et al., 2012;Mutso AA et al., 2014). All participants were right-handed, and all patients were diagnosed by a clinician for back pain and had pain intensity greater than 40/100 on the visual analog scale (VAS 0, no pain; 100, "worst pain imaginable"). SBP patients had pain duration of 4–16 weeks, and CBP patients had pain duration of more than 16 weeks. Participants were excluded if they reported any other chronic painful conditions, systemic disease, history of head injury, psychiatric diseases, or more than mild depression [score > 19, according to Beck's Depression Inventory (BDI)].
MRI scanning parameters: Subjects were scanned on a 3 Tesla Siemens Skyra scanner at Northwestern University, Chicago, USA. T1-anatomical brain images were acquired with following parameters: voxel size 1 × 1 × 1 mm^3; TR/TE = 2500/3.36 ms; flip angle = 9°; in-plane resolution = 256 × 256; slices per volume = 160; field of view = 256 mm. fMRI images were acquired on the same day with the following parameters: TR/TE = 2500/30 ms; flip angle = 90°; voxel size = 3.4375 x 3.4375 x 3 mm^3; in-plane resolution = 64×64; number of volumes = 244; number of slices = 36, which covers the whole brain from the cerebellum to the vertex.

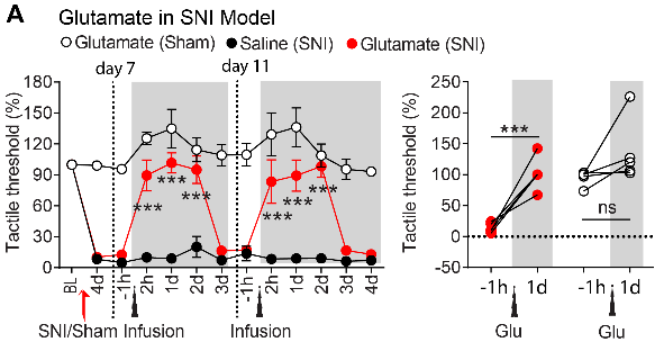
fMRI data acquisition, preprocessing and registration: During fMRI scan, subjects used a finger span device to perform a standardized visual task. All subjects underwent an initial training phase before scanning to learn the use of the finger span device and ensure adequate task performance (defined as r > 0.8 for correlation of stimulus time course with subject feedback). During imaging sessions, stimulus onset coincided with fMRI acquisition onset and frame rate was synchronized with fMRI TEs.

A scrubbing-based preprocessing pipeline (Power JD et al., 2014) was applied to all fMRI data, including the following procedures: discard of first 4 volumes, motion correction, slice-time correction, intensity normalization, regression of six motion vectors and cerebrospinal fluid and white matter signals, motion-volume censoring and band-pass filtering (0.008-0.1 Hz). The details of each step were described in (Huang S et al., 2019).

All pre-processed MRI data were registered to MNI152 2mm template by using a two-step FNIRT [https://www.fmrib.ox.ac.uk/datasets/techrep/tr07ja2/tr07ja2.pdf]. Each preprocessed fMRI volume was registered with a 7 degrees of freedom affine transformation to its corresponding T1 brain. Transformation parameters were also computed by nonlinearly registering all T1 brains to the MNI152 template. Combining the two transformations by matrix multiplication yielded transformation parameters normalizing fMRI data to standard space.

Functional connectivity between subregions of hippocampus and cortex: To examine and distinguish the extent of functional connectivity (FC) between two subregions (anterior and posterior) of hippocampus and cortex, "degree count" (Sporns O, 2013) was computed. Firstly, the anterior and posterior hippocampus BOLD signals averaged overall voxels of their ROIs were extracted, respectively. Both anterior and posterior ROIs consisted of 27 voxels. The BOLD signal correlations between each ROI and each voxel of cortex region defined in (Qin S et al., 2016) were calculated, generating individual anterior and posterior hippocampus FC correlation maps. The range of

correlation is between -1 and 1 and negative value represents inverse relationship between two BOLD signals. Finally, a ROI region and a voxel were considered functionally connected if their BOLD signals correlated with r > threshold or r < -threshold. Two values (degrees) were assigned to each ROI after counting the number of connections between the ROI and the cortex when the threshold was set, corresponding to the extent of positive and negative functional connectivity. For each subject, there were 4 degree counts, representing positive/negative functional connectivity between anterior/posterior hippocampus and cortex, respectively. In this study, the threshold was set as 0.3, guaranteeing functional connections had a false positive rate less than 0.001 (Mutso AA et al. 2014).

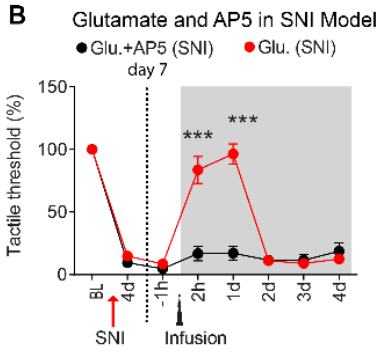


Supp. Table S1-1 (Figure 1A)

Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 1A (Left)	Saline (SNI, N = 6), Glu (SNI, N = 6), Glu (Sham, N = 6)	Group	2, 195	352.2	< 0.0001
		Test-session	12, 195	16.21	< 0.0001
		Interaction	24, 195	7.387	< 0.0001

Fig. 1A: Tukey's post-hoc multiple comparisons test					
Within group comparison			***Between group comparison***		
Group	*Test*	*P*	*Group*	*Test*	*P*
Saline (SNI)	-1h, 2h (1st infu.)	> 0.9999	**Saline (SNI), Glutamate (SNI)**	-1h (post 1st infu.)	0.8210
	-1h, 1d (1st infu.)	> 0.9999		2h (post 1st infu.)	< 0.0001
	-1h, 2d (1st infu.)	0.9939		1d (post 1st infu.)	< 0.0001
	-1h, 2h (2nd infu.)	> 0.9999		2d (post 1st infu.)	< 0.0001
	-1h, 1d (2nd infu.)	> 0.9999		-1 (post 2nd infu.)	0.9670
	-1h, 2d (2nd infu.)	> 0.9999		2h (post 2nd infu.)	< 0.0001
Glutamate (SNI)	-1h, 2h (1st infu.)	< 0.0001		1d (post 2nd infu.)	< 0.0001
	-1h, 1d (1st infu.)	< 0.0001		2d (post 2nd infu.)	< 0.0001
	-1h, 2d (1st infu.)	< 0.0001	**Glutamate (SNI), Glutamate (Sham)**	-1h (post 1st infu.)	< 0.0001
	-1h, 2h (2nd infu.)	< 0.0001		2h (post 1st infu.)	0.0168
	-1h, 1d (2nd infu.)	< 0.0001		1d (post 1st infu.)	0.0294
	-1h, 2d (2nd infu.)	< 0.0001		2d (post 1st infu.)	0.2981
Glutamate (Sham)	-1h, 2h (1st infu.)	0.5176		-1h (post 2nd infu.)	< 0.0001
	-1h, 1d (1st infu.)	0.1208		2h (post 2nd infu.)	0.0015
	-1h, 2d (1st infu.)	0.9644		1d (post 2nd infu.)	0.0011

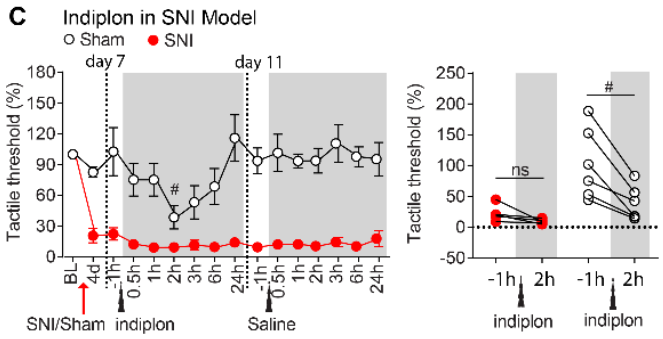
	-1h, 2h (2nd infu.)	0.9510		2d (post 2nd infu.)	0.7013
	-1h, 1d (2nd infu.)	0.6951			
	-1h, 2d (2nd infu.)	> 0.9999			
Paired t test comparison					
Figure	*Group / N*	*P*			
Fig. 1A (Left)	SNI, N = 6	0.0007			
	Sham, N = 6	0.0875			



Supp. Table S1-2 (Figure 1B)

Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 1B	Glu+AP5 (SNI, N = 9) Glu (SNI, N = 9)	Group	1, 128	59.20	< 0.0001
		Test-session	7, 128	98.61	< 0.0001
		Interaction	7, 128	25.98	< 0.0001

Fig. 1B: Tukey's post-hoc multiple comparisons test					
Within group comparison			***Between group comparison***		
Group	*Test*	*P*	*Group*	*Test*	*P*
Glutamate+ AP5 (SNI)	-1h, 2h	0.6241	**Glutamate+AP 5 (SNI), Glutamate (SNI)**	-1h (post infu.)	> 0.9999
	-1h, 1d	0.5999		2h (post infu.)	< 0.0001
	-1h, 2d	0.9701		1d (post infu.)	< 0.0001
Glutamate (SNI)	-1h, 2h	< 0.0001		2d (post infu.)	> 0.9999
	-1h, 1d	< 0.0001			
	-1h, 2d	> 0.9999			

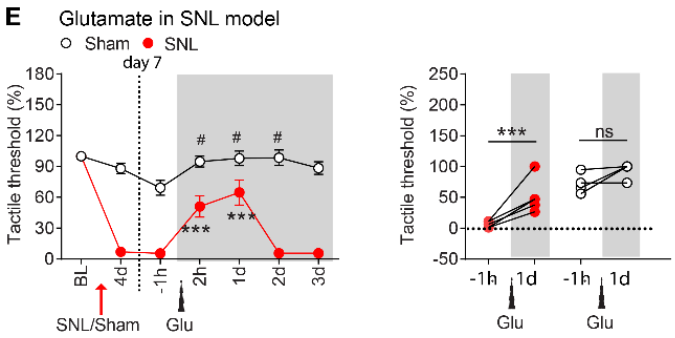


Supp. Table S1-3 (Figure 1C)

Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 1C (Left)	infusion (SNI, N = 5), infusion (Sham, N =6)	Group	1, 144	257.1	< 0.0001
		Test-session	15, 144	3.823	< 0.0001
		Interaction	15, 144	2.394	< 0.0001

Fig. 1C: Tukey's post-hoc multiple comparisons test					
Within group comparison			***Between group comparison***		
Group	*Test*	*P*	*Group*	*Test*	*P*
Infusion (SNI)	-1h, 2h (post indiplon)	> 0.9999	**Infusion (SNI), Infusion (Sham)**	-1h (post indiplon)	0.0001
	-1h, 3h (post indiplon)	> 0.9999		0.5h (post indiplon)	0.0056
	-1h, 6h (post indiplon)	> 0.9999		1h (post indiplon)	0.0028
	-1h, 2h (post saline)	> 0.9999		2h (post indiplon)	0.7799
	-1h, 3h (post saline)	> 0.9999		3h (post indiplon)	0.2256
	-1h, 6h (post saline)	> 0.9999		6h (post indiplon)	0.0124
Infusion (Sham)	-1h, 2h (post indiplon)	0.0128		24h (post indiplon)	< 0.0001
	-1h, 3h (post indiplon)	0.1791		-1h (post saline)	< 0.0001
	-1h, 6h (post indiplon)	0.7753		0.5h (post saline)	< 0.0001
	-1h, 2h (post saline)	> 0.9999		1h (post saline)	< 0.0001
	-1h, 3h (post saline)	0.9996		2h (post saline)	< 0.0001
	-1h, 6h (post saline)	> 0.9999		3h (post saline)	< 0.0001
				6h (post saline)	< 0.0001
				24h (post saline)	0.0002

Paired t test comparison		
Figure	*Group / N*	*P*
Fig. 1C (Right)	SNI, N = 5	0.0975
	Sham, N = 6	0.0064

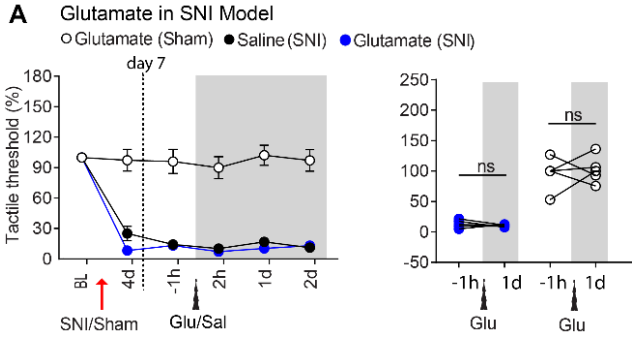


Supp. Table S1-4 (Figure 1E)

Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 1E (Left)	Glu (Sham, N = 5), Glu (SNL, N = 6)	Group	1, 63	286.0	< 0.0001
		Test-session	6, 63	26.01	< 0.0001
		Interaction	6, 63	13.86	< 0.0001

Fig. E (Left): Tukey's post-hoc multiple comparisons test					
Within group comparison			***Between group comparison***		
Group	*Test*	*P*	*Group*	*Test*	*P*
Glutamate (Sham)	-1h, 2h	0.0463	**Glutamate (Sham), Glutamate (SNL)**	-1h (post infu.)	< 0.0001
	-1h, 1d	0.0175		2h (post infu.)	< 0.0001
	-1h, 2d	0.0144		1d (post infu.)	0.0028
Glutamate (SNL)	-1h, 2h	< 0.0001		2d (post infu.)	< 0.0001
	-1h, 1d	< 0.0001			
	-1h, 2d	> 0.9999			

Paired t test comparison		
Figure	*Group / N*	*P*
Fig. 1E (Right)	SNI, N = 6	0.0045
	Sham, N = 5	0.0559



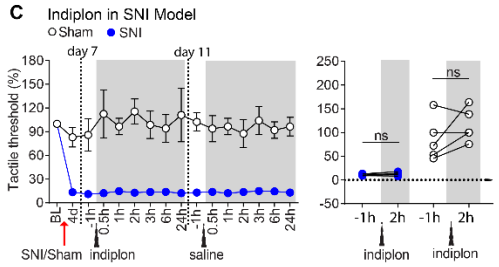
Supp. Table S1-5 (Fig. 2A)

Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 2A (Left)	Glu (SNI, N = 5), Saline (SNI, N = 5), Glu (Sham, N = 5)	Group	2, 72	259.0	< 0.0001
		Test-session	5, 72	47.27	< 0.0001
		Interaction	10, 72	10.72	< 0.0001

Fig. 2 (Left): Tukey's post-hoc multiple comparisons test

Within group comparison			*Between group comparison*		
Group	*Test*	*P*	*Group*	*Test*	*P*
Glutamate (SNI)	-1h, 2h	0.9826	**Saline (SNI), Glutamate (SNI)**	-1h (post infu.)	0.9923
	-1h, 1d	0.9995		2h (post infu.)	0.9334
	-1h, 2d	> 0.9999		1d (post infu.)	0.7415
Glutamate (Sham)	-1h, 2h	0.9815		2d (post infu.)	0.9740
	-1h, 1d	0.9784			
	-1h, 2d	> 0.9999			
Paired t test comparison					
Figure	*Group / N*	*P*			
Fig. 2A (Right)	SNI, N = 5	0.4496			
	Sham, N = 5	0.7150			

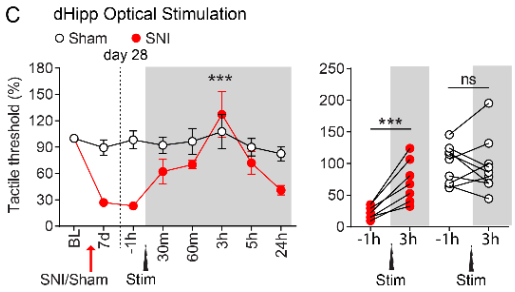
Supp. Table S1-6 (Figure 2C)



Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 2C (Left)	infusion (SNI, N = 5), infusion (Sham, N = 5)	Group	1, 128	317.7	< 0.0001
		Test-session	15, 128	1.820	0.0384
		Interaction	15, 128	1.697	0.0591

Fig. 2C (Left): Tukey's post-hoc multiple comparisons test					
Within group comparison			*Between group comparison*		
Group	*Test*	*P*	*Group*	*Test*	*P*
Infusion (SNI)	-1h, 2h (post indiplon)	> 0.9999	**Infusion (SNI), Infusion (Sham)**	-1h (post indiplon)	0.0008
	-1h, 3h (post indiplon)	> 0.9999		0.5h (post indiplon)	< 0.0001
	-1h, 6h (post indiplon)	> 0.9999		1h (post indiplon)	0.0002
	-1h, 2h (post saline)	> 0.9999		2h (post indiplon)	< 0.0001
	-1h, 3h (post saline)	> 0.9999		3h (post indiplon)	< 0.0001
	-1h, 6h (post saline)	> 0.9999		6h (post indiplon)	0.0002
Infusion (Sham)	-1h, 2h (post indiplon)	0.7860		24h (post indiplon)	< 0.0001
	-1h, 3h (post indiplon)	> 0.9999		-1h (post saline)	< 0.0001
	-1h, 6h (post indiplon)	> 0.9999		0.5h (post saline)	0.0003

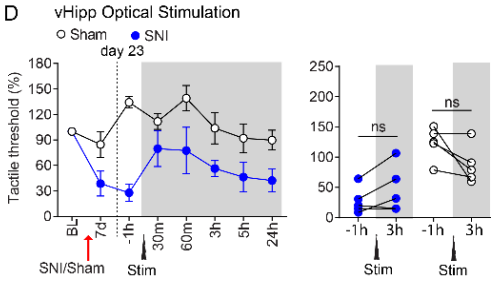
	-1h, 2h (post saline)	> 0.9999		1h (post saline)	< 0.0001
	-1h, 3h (post saline)	0.9960		2h (post saline)	0.0010
	-1h, 6h (post saline)	> 0.9999		3h (post saline)	< 0.0001
				6h (post saline)	0.0004
				24h (post saline)	0.0001
Paired t test comparison					
Figure	*Group / N*	*P*			
Fig. 2C (Right)	SNI, N = 5	0.2953			
	Sham, N = 5	0.1974			



Supp. Table S1-7 (Figure 3C)

Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 3C (Left)	Sham (N = 9), SNI (N = 7)	Group	1, 112	24.80	< 0.0001
		Test-session	7, 112	6.238	< 0.0001
		Interaction	7, 112	3.513	0.0019

Fig. 3C (Left): Tukey's post-hoc multiple comparisons test					
Within group comparison			***Between group comparison***		
Group	*Test*	*P*	*Group*	*Test*	*P*
Sham	-1h, 30min	> 0.9999	**Sham, SNI**	-1h (post optical stim.)	0.0001
	-1h, 60min	> 0.9999		30min (post optical stim.)	0.5917
	-1h, 3h	> 0.9999		60min (post optical stim.)	0.9255
	-1h, 5h	> 0.9999		3h (post optical stim.)	> 0.9999
	-1h, 24h	> 0.9999		5h (post optical stim.)	> 0.9999
SNI	-1h, 30min	0.8001		24h (post optical stim.)	0.1095
	-1h, 60min	0.2429			
	-1h, 3h	< 0.0001			
	-1h, 5h	0.1785			
	-1h, 24h	> 0.9999			
Paired t test comparison					
Figure	Group / N	P			
Fig. 3C (Right)	SNI, N = 7	0.0022			
	Sham, N = 9	0.8741			

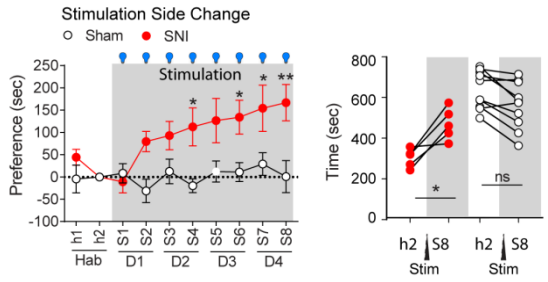


Supp. Table S1-8 (Figure 3D)

Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 3D (Left)	Sham (N = 5), SNI (N = 5)	Group	1, 64	43.53	< 0.0001
		Test-session	7, 64	2.705	0.0161
		Interaction	7, 64	2.051	0.0620

Fig. 3D (Left) : Tukey's post-hoc multiple comparisons test					
Within group comparison			***Between group comparison***		
Group	*Test*	*P*	*Group*	*Test*	*P*
Sham	-1h, 30min	> 0.9999	**Sham, SNI**	-1h (post optical stim.)	< 0.0001
	-1h, 60min	> 0.9999		30min (post optical stim.)	> 0.9999
	-1h, 3h	> 0.9999		60min (post optical stim.)	0.0333
	-1h, 5h	> 0.9999		3h (post optical stim.)	0.2030
	-1h, 24h	0.9988		5h (post optical stim.)	0.2477
SNI	-1h, 30min	0.4190		24h (post optical stim.)	0.2007
	-1h, 60min	0.5500			
	-1h, 3h	> 0.9999			
	-1h, 5h	> 0.9999			
	-1h, 24h	> 0.9999			

Paired t test comparison		
Figure	*Group / N*	*P*
Fig. 3D (Right)	SNI, N = 5	0.1217
	Sham, N = 5	0.0858



Supp. Table S1-9 (Figure 4C)

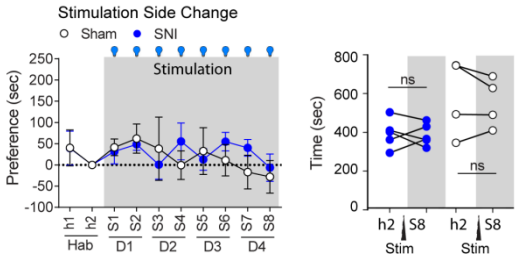
Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 4C (Left)	Sham (N = 5), SNI (N = 9)	Group	1, 120	43.40	< 0.0001
		Test-session	9, 120	2.558	0.0100

		Interaction	9, 120	2.031	0.0415

Fig. 4C (Left): Tukey's post-hoc multiple comparisons test

Within group comparison			***Between group comparison***		
Group	*Test*	*P*	*Group*	*Test*	*P*
Sham	h2, S1	> 0.9999	**Sham, SNI**	h2	> 0.9999
	h2, S2	> 0.9999		S1 (post optical stim.)	> 0.9999
	h2, S3	> 0.9999		S2 (post optical stim.)	0.0998
	h2, S4	> 0.9999		S3 (post optical stim.)	0.5957
	h2, S5	> 0.9999		S4 (post optical stim.)	0.0224
	h2, S6	> 0.9999		S5 (post optical stim.)	0.0786
	h2, S7	> 0.9999		S6 (post optical stim.)	0.0430
	h2, S8	> 0.9999		S7 (post optical stim.)	0.0371
SNI	h2, S1	> 0.9999		S8 (post optical stim.)	0.0015
	h2, S2	> 0.9999			
	h2, S3	> 0.9999			
	h2, S4	0.9258			
	h2, S5	0.4243			
	h2, S6	0.2737			
	h2, S7	0.0757			
	h2, S8	0.0319			

Paired t test comparison		
Figure	*Group / N*	*P*
Fig. 4C (Right)	SNI, N = 5	0.0415
	Sham, N = 9	0.9823



Supp. Table S1-10 (Figure 4E)

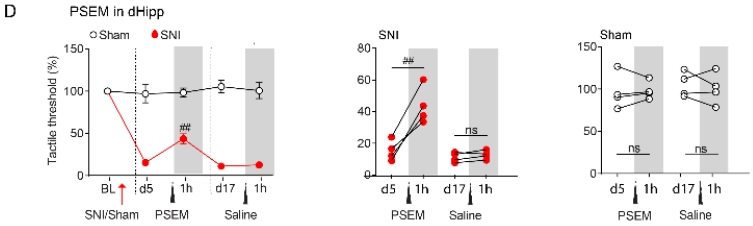
Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 4E (Left)	Sham (N = 4), SNI (N = 5)	Group	1, 70	0.3848	0.5370
		Test-session	9, 70	0.7193	0.6896
		Interaction	9, 70	0.4581	0.5370

Fig. 4E (Left): Tukey's post-hoc multiple comparisons test

Within group comparison			***Between group comparison***		
Group	*Test*	*P*	*Group*	*Test*	*P*
Sham	h2, S1	> 0.9999	**Sham, SNI**	h2	> 0.9999
	h2, S2	> 0.9999		S1 (post optical stim.)	> 0.9999
	h2, S3	> 0.9999		S2 (post optical stim.)	> 0.9999
	h2, S4	> 0.9999		S3 (post optical stim.)	> 0.9999

	h2, S5	> 0.9999		S4 (post optical stim.)	> 0.9999
	h2, S6	> 0.9999		S5 (post optical stim.)	> 0.9999
	h2, S7	> 0.9999		S6 (post optical stim.)	> 0.9999
	h2, S8	> 0.9999		S7 (post optical stim.)	> 0.9999
SNI	h2, S1	> 0.9999		S8 (post optical stim.)	> 0.9999
	h2, S2	> 0.9999			
	h2, S3	> 0.9999			
	h2, S4	> 0.9999			
	h2, S5	> 0.9999			
	h2, S6	> 0.9999			
	h2, S7	> 0.9999			
	h2, S8	> 0.9999			

Paired t test comparison		
Figure	*Group / N*	*P*
Fig. 4E (Right):	SNI, N = 5	0.9797
	Sham, N = 4	0.5209



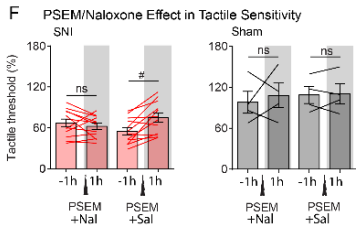
Supp.Table S1-11 (Figure 5D)

Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. 5D (Left)	Sham (N = 4), SNI (N = 4)	Group	1, 30	302.7	< 0.0001
		Test-session	4, 30	20.81	< 0.0001
		Interaction	4, 30	22.30	< 0.0001

Fig. 5D (Left): Tukey's post-hoc multiple comparisons test					
Within group comparison			***Between group comparison***		
Group	*Test*	*P*	*Group*	*Test*	*P*
Sham	-1h, 1h (post PSEM)	> 0.9999	**Sham, SNI**	-1d	> 0.9999
	-1h, 1h (post saline)	> 0.9999		-1h (post PSEM)	< 0.0001
SNI	-1h, 1h (post PSEM)	0.0167		1h (post PSEM)	< 0.0001
	-1h, 1h (post saline)	> 0.0009		-1h (post Saline)	< 0.0001
				1h (post Saline)	< 0.0001

Fig. 5D (Middle, Right): Paired t test comparisons					
Figure	*Group / N*	*P*	***Figure***	*Group / N*	*P*
Fig. 5D (Middle)	PSEM, N = 4	0.0080	**Fig. 5D (Right)**	PSEM, N = 4	0.7914
	Saline, N = 4	0.1884		Saline, N = 4	0.5585

Supp. Table S1-12 (Figure 5F)



Paired t test comparisons					
Figures	*Group / N*	*P*	*Figures*	*Group /N*	*P*
Fig. 5F	PSEM+Nal, N = 12	0.3653	**Fig. 5F**	PSEM+Nal, N = 4	0.6597
	PSEM+Sal, N = 12	0.0037		PSEM+Sal, N = 4	0.8331

Supp. Table S1-13 (Supp. Figure S1)

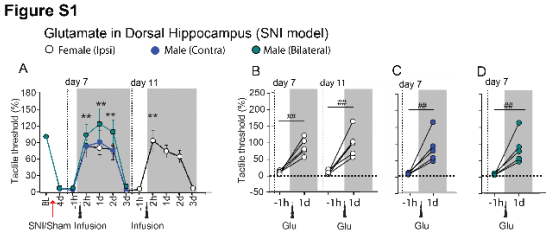
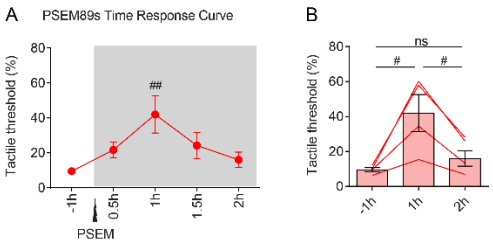


Fig 1S.A Repeated measures ANOVA					
Figure	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. S1. A	Fem. Ipsi (N = 5), Male Contra (N = 5) Male Bi (N = 5)	Group	2,104	0.746	0.495
		Test-session	6,104	88.956	<0.001
		Interaction	12,104	0.663	0.780

Tukey's multiple comparisons test					
Group	*Test comp. with BL*	*P*	*Group*	*Test comp. with -1h*	*P*
Female (Ipsi), Male (Contra), Male (Bilateral)	-1h	<0.001	**Female (Ipsi), Male (Contra), Male (Bilateral)**	2h (post Glu)	<0.001
	2h (post Glu)	0.190		1d (post Glu)	<0.001
	1d (post Glu)	0.763		2d (post Glu)	<0.001
	2d (post Glu)	0.079		3d (post Glu)	1.000
	3d (post Glu)	<0.001			

Fig. 1S B, C & D: Paired t test comparisons					
Figure	*Group / N*	*P*	***Figure***	*Group / N*	*P*
Fig. 1S.B (Female Ipsi)	-1h, 2h (day7)	0.0019	**Fig. 1S.C (Male Contra)**	-1h, 2h (day7)	0.0023
	-1h, 2h (day11)	0.0017	**Fig. 1S.D (Male Bilateral)**	-1h, 2h (day7)	0.0016



Supp. Table S1-14 (Supp. Figure S2)

Repeated measures one-way ANOVA					
Figures	*Test / N*	*Effect*	*DF*	*F*	*P*
Fig. S1A	Time course, N = 4	Test session	4, 12	5.992	0.0069
		Individuals	2, 12	5.133	0.0163
Fig. S1B	Time course, N = 4	Test session	2, 6	12.11	0.0078
		Individuals	3, 6	4.008	0.0698
Tukey's post-hoc multiple comparisons test					
Figure	*Comparison*	*Test*	*DF*		*P*
Fig. S1A	Between test sessions comparison	-1h, 0.5h	12		0.4052
		-1h, 1h	12		0.0020
		-1h, 1.5h	12		0.2137
		-1h, 2h	12		0.8523
Tukey's post-hoc multiple comparisons test					
Figure	*Comparison*	*Test*	*DF*		*P*
Fig. S1B	Between test sessions comparison	-1h, 1h	6		0.0074
		-1h, 2h	6		0.4328
		1h, 2h	6		0.0318

Supp. Table S2. Functional connectivity changes between PSEM^{89S} and saline conditions, based on network analysis after parceling the brain into 96 regions.

	Target ROI				
Source ROI	Increased r	Decreased r	Mean Delta r	Standard Error	P-Value
45 – Anterodorsal L	16 - Cingulate Cortex R		0.12	0.0504	0.0234
45 – Anterodorsal L	21 - Insular Cortex L		0.08	0.0449	0.0297
45 – Anterodorsal L	23 - Medial Prefrontal Cortex L		0.07	0.0502	0.0487
45 – Anterodorsal L	25 - Motor Cortex L		0.09	0.0537	0.0481
45 – Anterodorsal L	35 - Somatosensory Cortex L		0.09	0.0556	0.0457
45 – Anterodorsal L	38 - Temporal Association Cortex R		0.09	0.0445	0.0154
45 – Anterodorsal L	46 - Hippocampus Anterodorsal R		0.13	0.0596	0.0396
45 – Anterodorsal L	65 - Mesencephalic Region L		0.09	0.0481	0.0386
45 – Anterodorsal L	71 - Periaqueductal Grey L		0.14	0.0405	0.0026*
45 – Anterodorsal L	95 - Zona Incerta L		0.09	0.0341	0.0191
46 – Anterodorsal R	33 - Retrosplenial Cortex L		0.13	0.0359	0.0043*
46 – Anterodorsal R	44 - Globus Pallidus R		0.12	0.0432	0.006*
46 – Anterodorsal R		51 - Subiculum L	-0.12	0.0519	0.0037*
46 – Anterodorsal R	85 - Thalamus Dorsolateral L		0.10	0.0402	0.0262
46 – Anterodorsal R	86 - Thalamus Dorsolateral R		0.12	0.0527	0.0294
46 – Anterodorsal R	87 - Thalamus Midline Dorsal L		0.11	0.0446	0.0145
46 – Anterodorsal R		91 - Ventral Pallidum L	-0.09	0.0449	0.0432
46 – Anterodorsal R	94 - Ventral Tegmental Area R		0.12	0.0395	0.0041*
49 – Posterodorsal L	13 - Auditory Cortex L		0.13	0.0689	0.0235
49 – Posterodorsal L		51 - Subiculum L	-0.10	0.0375	0.0497
49 – Posterodorsal L		79 - Substantia Innominata L	-0.11	0.0471	0.031
49 – Posterodorsal L		90 - Thalamus ventromedial R	-0.11	0.0402	0.0042*
50 – Posterodorsal R		5 - Amygdala L	-0.11	0.0504	0.0275
50 – Posterodorsal R	16 - Cingulate Cortex R		0.10	0.0557	0.0153
50 – Posterodorsal R	22 - Insular Cortex R		0.11	0.0367	0.0074*
50 – Posterodorsal R	23 - Medial Prefrontal Cortex L		0.12	0.0612	0.024
50 – Posterodorsal R	24 - Medial Prefrontal Cortex R		0.12	0.0663	0.0344
50 – Posterodorsal R	29 - Parietal Association Cortex L		0.11	0.0607	0.0269
50 – Posterodorsal R	30 - Parietal Association Cortex R		0.12	0.0616	0.016
50 – Posterodorsal R	36 - Somatosensory Cortex R		0.09	0.0562	0.0475
50 – Posterodorsal R		48 - Hippocampus Posterior R	-0.11	0.0696	0.0376
50 – Posterodorsal R		55 - Hypothalamus Lateral L	-0.07	0.0435	0.0435
50 – Posterodorsal R	60 - Internal Capsule R		0.11	0.0573	0.0227
50 – Posterodorsal R	85 - Thalamus Dorsolateral L		0.13	0.0491	0.0072*

Brain regions with increased and decreased connectivity to the 4 dorsal hippocampus regions are shown, together with mean change in correlation coefficient, standard error, and permutation-based p values. We corrected for false discovery rate (FDR) using Benjamini-Hochberg procedure. The corrected p-values for a false discovery rate of 0.25 divided by the number of tests performed = 34, resulting in a FDR p-value cut-off = 0.25/34= 0.0075. Only 8 of the identified connections survive, highlighted and indicated by * next to their p-values. ROI numbers are listed in (Baliki MN et al., 2014).

Increased functional connectivity is observed between DH and: periaqueductal grey, retrosplenial cortex, globus pallidus, ventral tegmental area, insula, and lateral thalamus. In contrast, decreased functional connectivity is observed between DH and subiculum, and between DH and medial thalamus. Given that the PSAM-5HT3 virus was injected bilaterally in the DH, the laterality of observed functional connections is not important.

Supp. Table S3. Brain regions where PSEM^{89s}-dependent changes in functional connectivity with the DH were correlated with changes in tactile allodynia.

Positive covariance with VF thresholds change

Anatomical structure		Size (Vox)	p value	z value	Coordinates (mm)		
					x	y	z
L	Primary Somatosensory Cortex (limb)	259	1.65E-21	3.82	0.58	-2.52	2.20
L	Thalamus Dorsolateral	121	4.98E-12	3.76	-1.55	-4.65	-2.00
R	Primary Motor Cortex	88	2.76E-09	3.41	2.33	-1.94	2.60
R	Superior Colliculus / Retrosplenial Cortex	64	4.77E-07	4.17	0.78	-3.49	-7.00
L	Medial Prefrontal Cortex (Infralimbic, IL)	47	2.54E-05	3.63	-0.39	-6.01	3.40
L	Superior Colliculus / Retrosplenial Cortex	43	6.99E-05	3.00	0.00	-3.68	-6.00
R	Posterior Hippocampus / Subiculum	41	1.17E-04	2.98	4.07	-2.91	-6.40
R	Medial Prefrontal Cortex	40	1.53E-04	3.86	1.55	-4.46	3.40
L	Retrosplenial Cortex	38	2.59E-04	3.55	-1.55	-2.52	-6.80
R	Cortex Insular	34	7.72E-04	2.84	3.30	-4.65	2.60

Negative covariance with VF thresholds change

Anatomical structure		Size (Vox)	p value	z value	Coordinates (mm)		
					x	y	z
L	Zona Incerta	126	2.02E-12	3.75	-1.55	-7.17	-3.60
R	Primary Somatosensory Cortex (barrel field, S1BF)	111	3.16E-11	4.07	4.46	-2.71	-3.00
L	Primary Somatosensory Cortex (barrel field, S1BF)	96	5.60E-10	3.29	-3.68	-2.71	-2.80
R	Posterior Hippocampus (CA1)	93	1.01E-09	3.67	3.10	-1.94	-4.00
L	Caudate Putamen	57	2.26E-06	3.53	-2.33	-3.30	0.40
R	Substantia Nigra / Peripeduncular nucleus	57	2.26E-06	3.13	2.52	-7.56	-5.20
L	Ventral Pallidum	42	9.05E-05	3.00	-2.52	-7.75	-0.60
L	Caudate Putamen / Insula	40	1.53E-04	3.48	-5.04	-6.98	-0.80

Anatomical structures (R=right, L=left hemisphere), cluster sizes, p-values, peak z-values, and peak coordinates are labeled. Cluster and intensity corrected for multiple comparisons for maps identified in the discovery data. Only brain regions where p was <0.0001 are shown. Three brain regions which survived replication are in grey (circled in yellow in **Figure 7A**).

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