Cell Reports, Volume *40*

Supplemental information

Activation of VIP interneurons in the prefrontal cortex ameliorates neuropathic pain aversiveness

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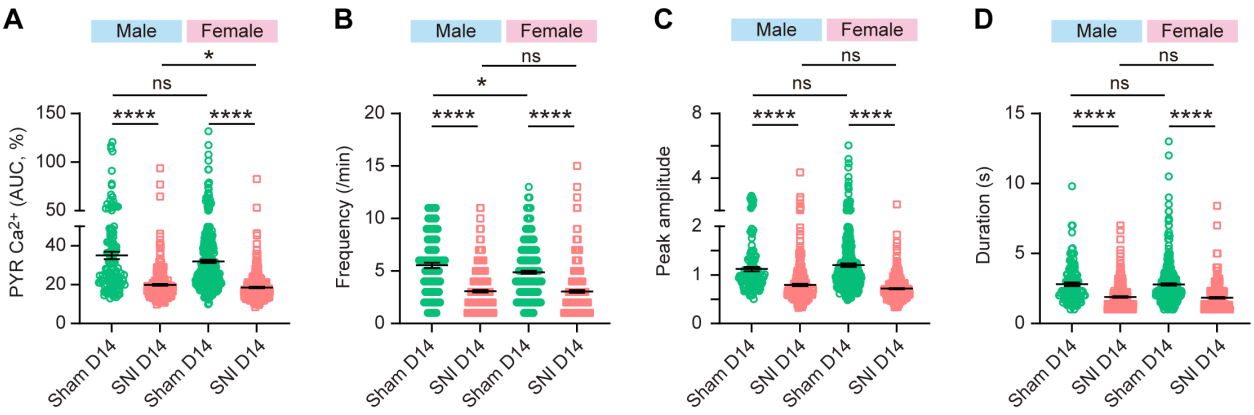

Figure S1. Neuropathic pain decreases pyramidal neuron Ca^{2+} activity in PL of male and female mice, related to Figure 1

(**A**) SNI decreases pyramidal neuron (PYR) Ca^{2+} activity in both male ($P < 0.0001$) and female ($P < 0.0001$) mice. 14 days after SNI, AUC is slightly higher in males than females ($P = 0.0171$). Within the sham group, no significant difference between males and females ($P = 0.6358$).

(**B**) SNI decreases PYR Ca^{2+} frequency in both male ($P < 0.0001$) and female ($P < 0.0001$) mice. Within the sham group, frequency is slightly higher in males than females ($P = 0.0279$). Within the SNI group, no significant difference between males and females ($P = 0.3833$).

(**C**) SNI deceases peak amplitude of PYR Ca^{2+} transients in both male ($P < 0.0001$) and female ($P < 0.0001$) mice. No difference between males and females within the sham ($P = 0.2165$) and SNI ($P = 0.1464$) group.

(**D**) SNI deceases Ca^{2+} transient duration in both male ($P < 0.0001$) and female ($P < 0.0001$) mice. No difference between males and females within the sham ($P = 0.7705$) and SNI ($P = 0.5056$) group.

In (**A**–**D**), $n = 121, 292$ cells from 2, 3 males; $n = 365, 341$ cells from 3, 4 females; sham and SNI respectively.

Individual dots represent data from a single cell. Summary data are presented as mean ± S.E.M. *$P < 0.05$, ****$P < 0.0001$; ns, not significant; by Mann-Whitney test.

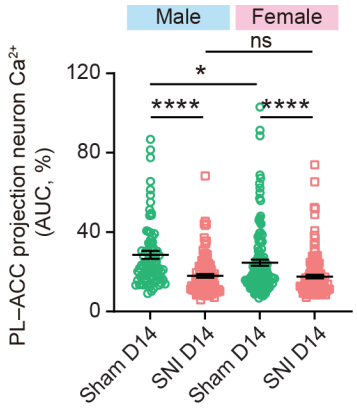

Figure S2. Neuropathic pain decreases the Ca^{2+} activity of PL neurons projecting to ACC, regardless of the sex of animals, related to Figure 2

SNI decreases Ca^{2+} activity in PL–ACC projection neurons in both male ($P < 0.0001$; sham, n = 88 cells from 3 mice; SNI, n = 113 cells from 4 mice) and female mice ($P < 0.0001$; sham, n = 126 cells from 4 mice; SNI, n = 136 cells from 4 mice). No difference between males and females within the SNI group ($P = 0.6981$).

Individual dots represent data from a single cell. Summary data are presented as mean ± S.E.M. *$P < 0.05$, ****$P < 0.0001$; ns, not significant; by Mann-Whitney test.

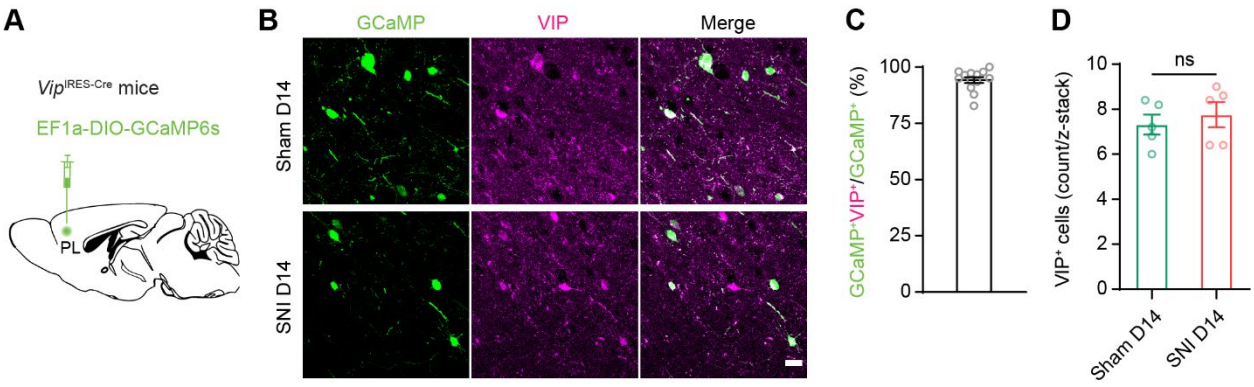

Figure S3. Neuropathic pain has no effects on VIP interneuron density in PL, related to Figure 3

(**A**) Experimental design to specifically express GCaMP6s in VIP interneurons in the mouse PL.

(**B**) Representative fluorescence images of PL neurons showing the colocalization of GCaMP fluorescence and anti-VIP immunoreactivity. Scale bar, 20 μm.

(**C**) Percentage of colocalized somas in PL to estimate the specificity of Cre-mediated GCaMP expression (n = 13 sections from 4 mice). 94.26 ± 1.30% of GCaMP-expressing cells are colocalized with anti-VIP immunoreactivity.

(**D**) The number of VIP-expressing interneurons (per 200 μm × 200 μm × 20 μm) in PL 14 days after sham or SNI surgery (P = 0.4603, n = 5 sections from 2 mice per group).

Individual dots represent data from a single section. Summary data are mean ± S.E.M. ns, not significant; by Mann-Whitney test.

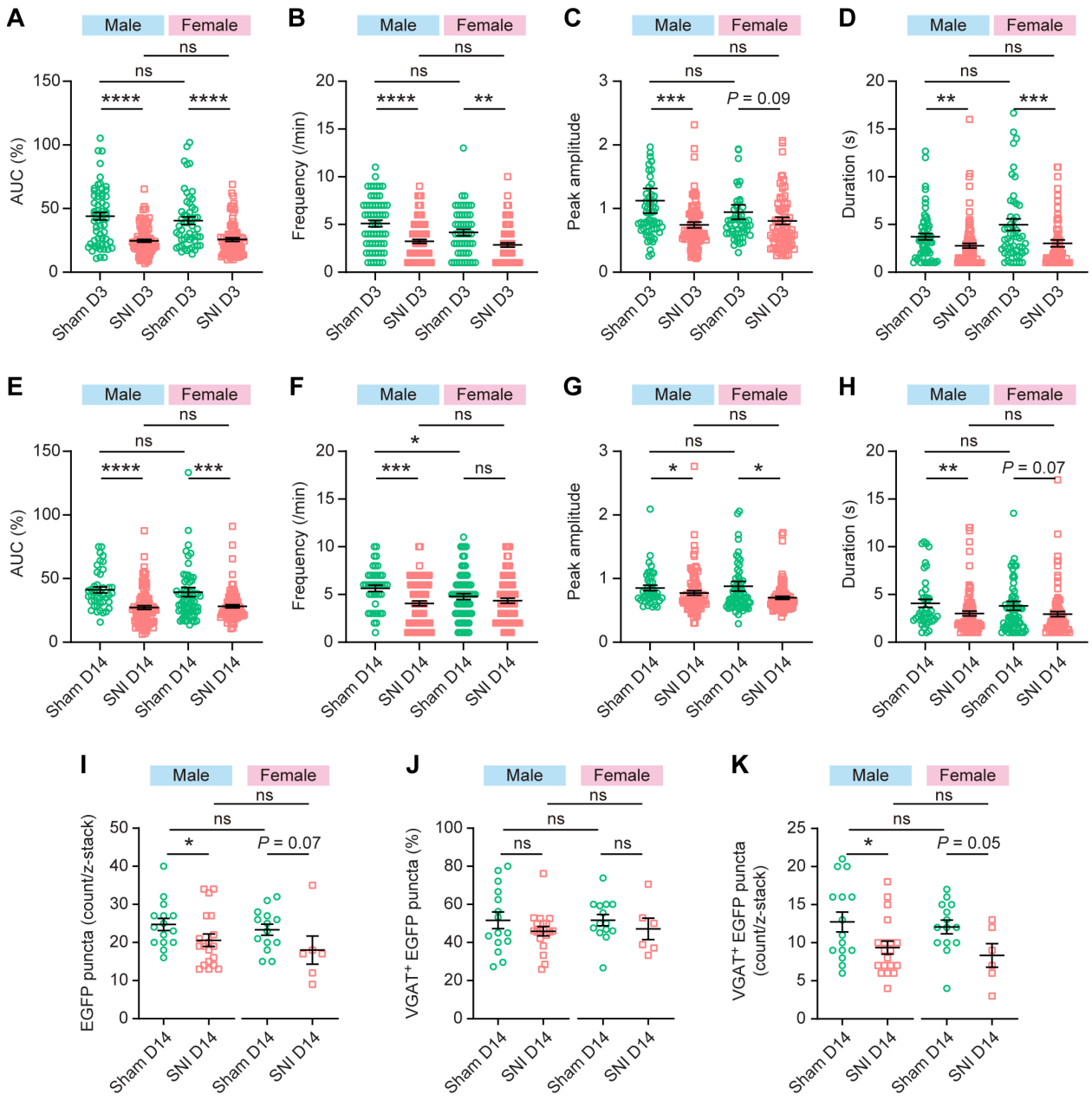

Figure S4. Neuropathic pain decreases Ca^{2+} activity and presynaptic terminal number in VIP interneurons, regardless of the sex of animals, related to Figure 3

(**A**) SNI decreases VIP Ca^{2+} activity in male ($P < 0.0001$) and female ($P < 0.0001$) mice 3 days after surgery. No difference between males and females within the sham or SNI group (sham: $P = 0.3195$, SNI: $P = 0.9287$).

(**B**) SNI decreases VIP Ca^{2+} frequency in male ($P < 0.0001$) and female ($P = 0.0011$) mice 3 days after surgery. No difference between males and females within the sham or SNI group (sham: $P = 0.0547$, SNI: $P = 0.1549$).

(**C**) SNI decreased VIP Ca^{2+} peak amplitude in male ($P = 0.0001$) and female ($P = 0.0900$, a trend) mice 3 days after surgery. No difference between males and females within the sham or SNI group (sham: $P = 0.1761$, SNI: $P = 0.4860$).

(**D**) SNI decreases VIP Ca^{2+} duration in male ($P = 0.0024$) and female ($P = 0.0002$) mice 3 days after surgery. No difference between males and females within the sham or SNI group (sham: $P = 0.3238$, SNI: $P = 0.8011$).

In (**A**–**D**), $n = 60, 94$ cells from 3, 6 males, $n = 53, 83$ cells from 3, 6 females.

(**E**) SNI decreases VIP neuron Ca^{2+} activity in male ($P < 0.0001$) and female ($P = 0.0004$) mice 14 days after surgery. No difference between males and females within the sham or SNI group (sham: $P = 0.0523$, SNI: $P = 0.3467$).

(**F**) SNI decreases VIP Ca^{2+} frequency in male ($P = 0.0006$) but not female ($P = 0.2679$) mice 14 days after surgery. Within the sham group, the frequency is slightly lower in females ($P = 0.0315$). No difference between males and females within the SNI group ($P = 0.4537$).

(**G**) SNI decreases VIP Ca^{2+} peak amplitude in male ($P = 0.0176$) and female ($P = 0.0292$) mice 14 days after surgery. No difference between males and females within the sham or SNI group (sham: $P = 0.1122$, SNI: $P = 0.0975$).

(**H**) SNI decreases VIP Ca^{2+} duration in male ($P = 0.0022$) and female ($P = 0.0729$, a trend) mice 14 days after surgery. No difference between males and females within the sham or SNI group (sham: $P = 0.1811$, SNI: $P = 0.7159$).

In (**E**–**H**), $n = 42, 91$ cells from 3, 4 males, $n = 70, 91$ cells from 3, 4 females.

(**I**) The count of VIP presynaptic EGFP puncta (per 30 μm × 30 μm × 20 μm) in PL of male ($P = 0.0453$) and female ($P = 0.0790$, a trend) mice 14 days after sham or SNI. No difference between males and females (sham: $P = 0.6894$, SNI: $P = 0.3089$).

(**J**) Percentage of VIP EGFP puncta colocalized with VGAT in male ($P = 0.5774$) and female ($P = 0.3956$) mice 14 days after sham or SNI. No difference between males and females (sham: $P = 0.6749$, SNI: $P = 0.9386$).

(**K**) The count of $VGAT^{+}$ EGFP puncta (per 30 μm × 30 μm × 20 μm) in PL of male ($P = 0.0446$) and female ($P = 0.0521$, a trend) mice 14 days after sham or SNI. No difference between males and females (sham: $P = 0.9742$, SNI: $P = 0.6270$).

In (**I**–**K**), $n = 15, 19$ sections from 2, 3 males, $n = 14, 6$ sections from 2, 2 females.

Throughout, individual dots represent data from a single cell or section. Summary data are mean ± S.E.M. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; ns, not significant; by Mann-Whitney test.

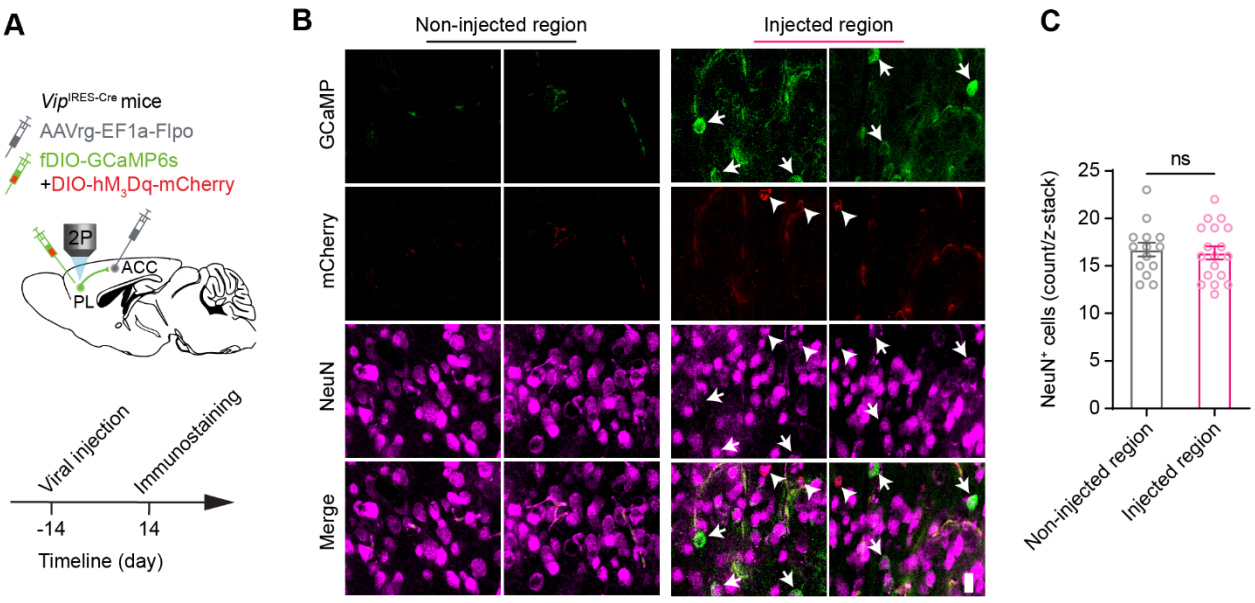

Figure S5. Injection of multiple viruses has no detectable effects on neuronal density in the injected regions, related to Figure 4

(**A**) Experimental design to virally express GCaMP6s in PL–ACC projection neurons and hM$_3$Dq-mCherry in PL VIP interneurons. Four weeks after viral infection, cortical sections were immunostained for NeuN, a neuronal marker.

(**B**) Immunofluorescence images showing NeuN$^+$ cells in the cortical regions with or without viral injections. Arrows indicate virus targeted PL–ACC neurons expressing GCaMP6. Arrowheads indicate virus targeted VIP cells expressing hM$_3$Dq-mCherry. Scale bar, 20 μm.

(**C**) The number of NeuN$^+$ cells (per 100 μm × 100 μm × 20 μm) in the cortical regions with or without viral injections (P = 0.7560, n = 14, 18 fields of view from 3 mice). Individual dots represent data from a single field of view. Summary data are mean ± S.E.M. ns, not significant; by Mann-Whitney test.

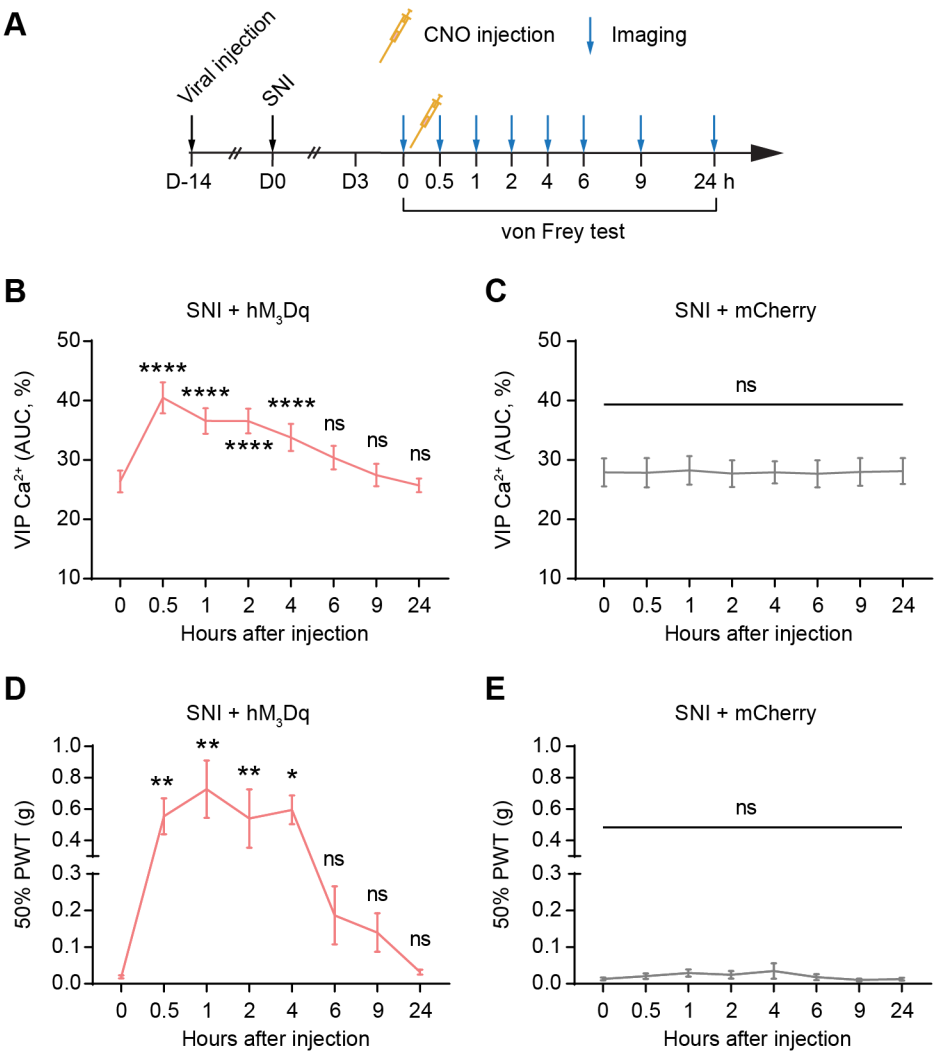

Figure S6. A single injection of CNO transiently suppresses VIP interneuron activity in PL and mechanical allodynia in mice, related to Figure 5

(**A**) Experimental design to examine the acute effects of hM$_3$Dq activation on VIP interneuron activity in PL and mechanical pain thresholds (von Frey test) in mice at day 3 post-SNI.

(**B**) VIP Ca^{2+} activity before and after a single CNO injection in SNI mice expressing hM$_3$Dq ($P < 0.0001$ for 0.5, 1, 2, 4 h *vs.* baseline, $P = 0.0828$, > 0.9999, > 0.9999 for 6, 9, 24 h *vs.* baseline; $n = 145$ cells from 4 mice).

(**C**) VIP Ca^{2+} activity before and after a single CNO injection in SNI mice expressing mCherry ($P > 0.9999$, > 0.9999, > 0.9999, $= 0.1443$, > 0.9999, > 0.9999, > 0.9999 for 0.5, 1, 2, 4, 6, 9, 24 h *vs.* baseline; $n = 115$ cells from 3 mice).

(**D**) Time course of mechanical pain thresholds before and after a single CNO injection in SNI mice expressing hM$_3$Dq in PL VIP neurons ($P = 0.0087$, 0.0055, 0.0087, 0.0109 for 0.5, 1, 2, 4 h *vs.* baseline, $P = 0.9635$, > 0.9999, > 0.9999 for 6, 9, 24 h *vs.* baseline; $n = 5$ mice). PWT, paw withdrawal threshold.

(**E**) Time course of mechanical pain thresholds before and after a single CNO injection in SNI mice expressing mCherry in PL VIP neurons ($P > 0.9999$, $= 0.9850$, $= 0.9850$, > 0.9999, > 0.9999, > 0.9999, > 0.9999 for 0.5, 1, 2, 4, 6, 9, 24 h *vs*. baseline; $n = 6$ mice).

Data are presented as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$; ns, not significant; by Friedman test followed by Dunn's multiple comparisons test.

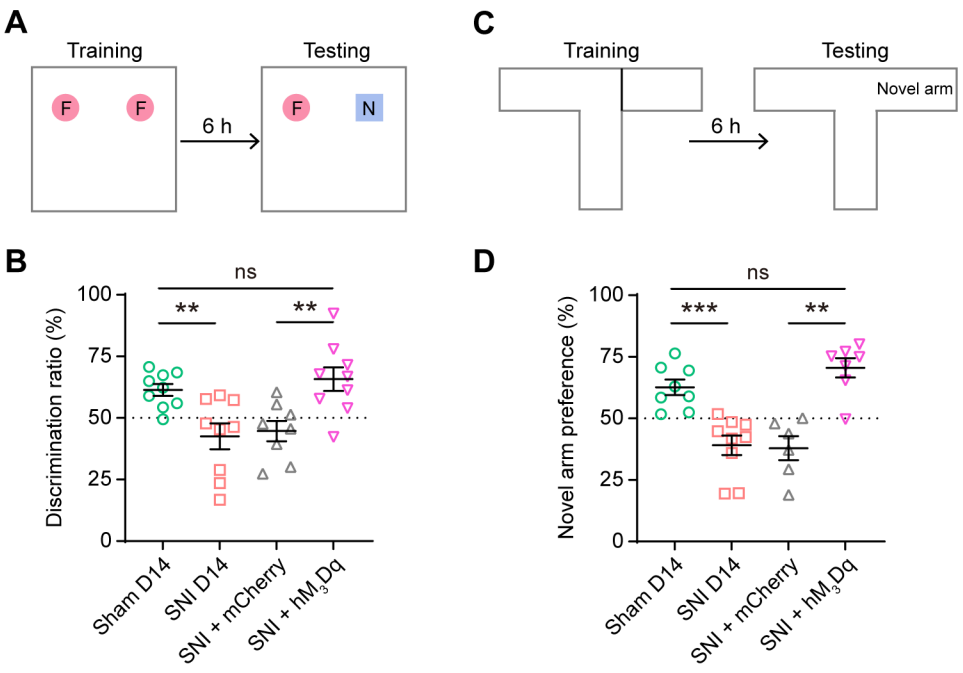

Figure S7. Daily activation of VIP interneurons following nerve injury ameliorates cognitive impairments associated with neuropathic pain, related to Figure 5

(**A**) Schematic and timeline for the novel object recognition (NOR) test, which measures the animal's recognition memory, based on the willingness of rodents to explore a novel object. F, familiar object; N, novel object.

(**B**) Discrimination ratio of sham and SNI mice 14 days after surgery ($P = 0.0056$). Daily activation of PL VIP interneurons prevents recognition memory impairment after SNI ($P = 0.0055$ *vs.* mCherry; $P = 0.5457$ *vs.* sham). $n = 9, 9, 8, 9$ mice.

(**C**) Schematic and timeline for the T maze test, which measures the animal's spatial memory, based on the willingness of rodents to explore a new environment.

(**D**) Percentage of time spent in the novel arm during a T maze test 14 days after sham or SNI surgery ($P = 0.0002$). Daily activation of PL VIP interneurons prevents spatial memory impairment after SNI ($P = 0.0023$ *vs.* mCherry; $P = 0.1206$ *vs.* sham). $n = 8, 9, 6, 7$ mice.

Individual dots represent data from a single mouse. Summary data are mean ± S.E.M. $^{**}P < 0.01$, $^{***}P < 0.001$; ns, not significant; by Mann-Whitney test.