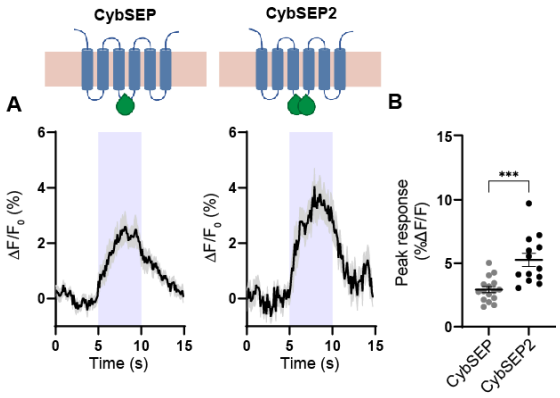



**Figure S1. Comparison of fluorescence levels of CybSEP and CybSEP2 evoked by electrical stimulation**

(A and B) Schematic and average traces showing comparison between CybSEP (n = 18) and CybSEP2 (n = 13) expressing cells (duplicated from Figures 1G) during electrical stimulation at 100 Hz and quantification of percent $\Delta F/F_0$ peak intensity in (A) (***$p < 0.001$ via unpaired t test). Data are represented as mean ± SEM.

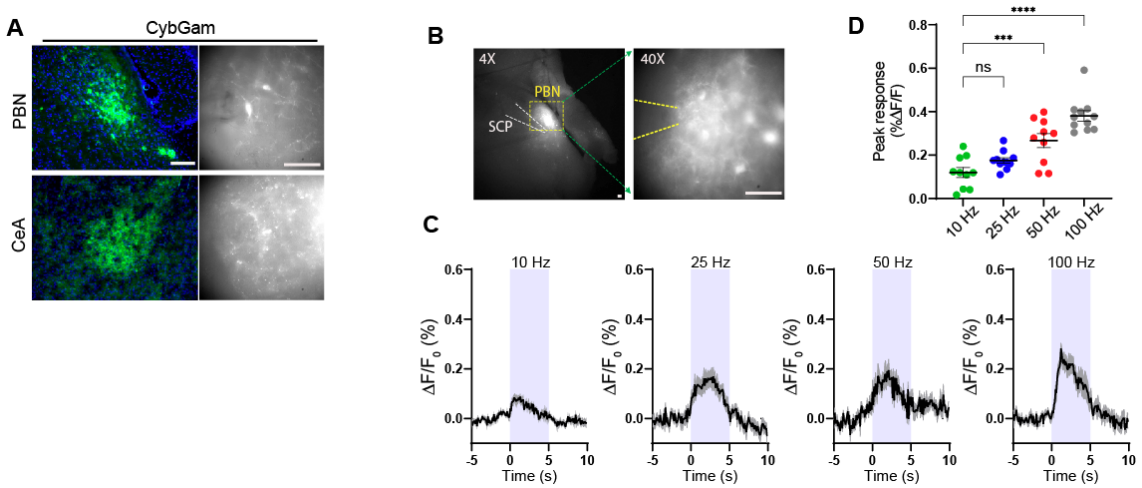



**Figure S2. Expression of CybGam and imaging CybSEP2 in acute brain slice containing the PBN**

(A) Schematic and images showing expression of CybGam in the PBN and the CeA of *Calca*$^{Cre/+}$.

(B) Images of PBN showing CybSEP2 expression for slice imaging. SCP indicates superior cerebellar peduncle (Scale bar, 100 μm).

(C and D) Average traces of fluorescence change in response to various electrical stimulation and quantification of data in (C) (10-12 traces from 18 slices prepared from 3 mice; ***p < 0.001, ****p < 0.0001 via one way ANOVA followed by Tukey's multiple comparisons to the 10 Hz). Data are represented as mean ± SEM.

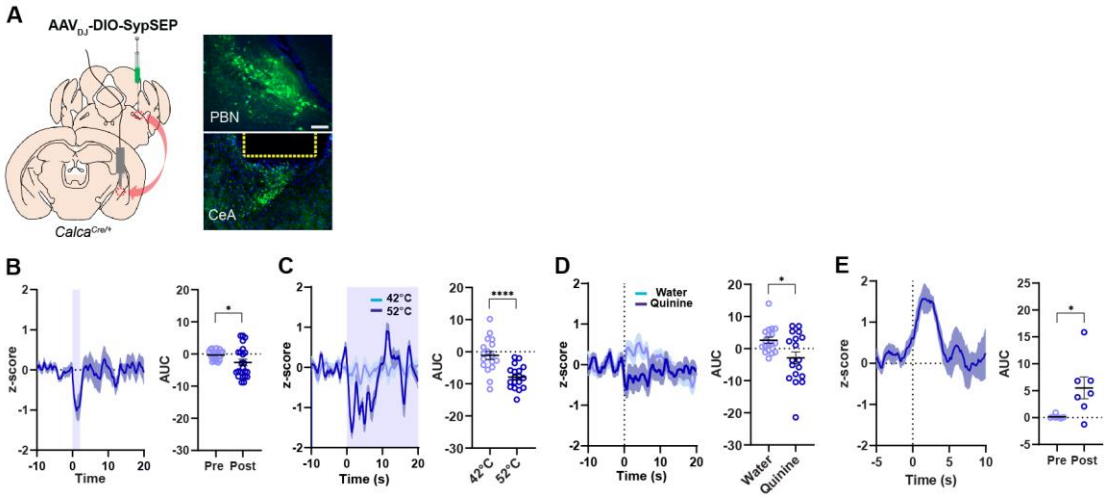



**Figure S3. Deep brain recording of the SV sensor in the synaptic terminals of freely moving mice**

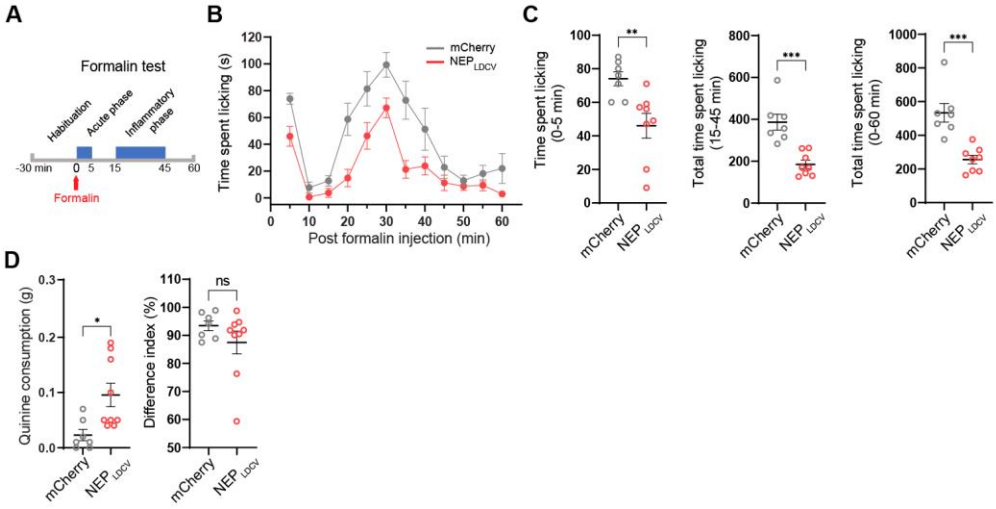
(A) Schematic illustration of viral injection and images showing expression of SypSEP in the PBN and in the CeA of *Calca*$^{Cre/+}$. Yellow dot line represents the location of optic fiber (Scale bar, 100 μm).

(B) Average trace of fluorescence change on footshock and quantification of data 10 s before and after footshock (23 traces from 4 mice; *p < 0.05 via paired t test).

(C) Average traces of fluorescence change during thermal stimulus and quantification of data for 0-10 s (18 traces from 4 mice; ****p < 0.0001 via unpaired t test).

(D) Average traces of fluorescence change during quinine intake and quantification of data for 0-10 s (18 traces from 4 mice *p < 0.05 via unpaired t test). Data are represented as mean ± SEM.

(E) Average traces of fluorescence change during Ensure intake and quantification of data for 0-10 s (7 traces from 3 mice *p < 0.05 via unpaired t test). Data are represented as mean ± SEM.




880

881 **Figure S4. The effect of the $NEP_{LDCV}$ on pain behavior by sensory stimulus and formalin injection.**

882 (A) Schematic of formalin assay for acute and inflammatory pain tests.

883 (B) Time course of formalin-induced nociceptive responses in the mice expressing mCherry (n=7 for

884 mCherry, n=9 for $NEP_{LDCV}$).

885 (C) Quantification of acute phase (0-5 min, left), inflammatory phase (15-45 min, middle), and a total spent

886 time for locking (0-60 min). $^{**}P<0.01$, $^{***}P<0.001$ via unpaired t-test comparisons to mCherry. Data are

887 represented as mean ± SEM.

888 (D) Quinine consumption (left) and two bottle (right) tests. in the mice expressing mCherry (n = 7) and

889 $NEP_{LDCV}$ (n = 9). $^{*}P<0.05$, ns via unpaired t-test comparisons to mCherry group.

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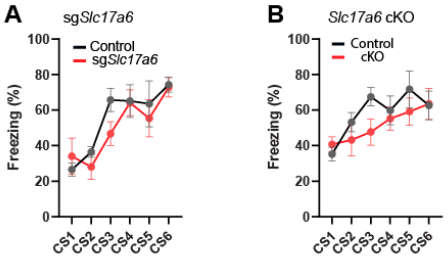


**Figure S5** Learning curves during fear conditioning.

(A) Freezing during tone (CS) in learning sessions (n = 4 for control, n = 6 for *sgSlc17a6*).

(B) Freezing during tone (CS) in learning sessions (n = 7 for control, n = 6 for cKO).