

# **Paraventricular hypothalamic vasopressin neurons induce self-grooming in mice**

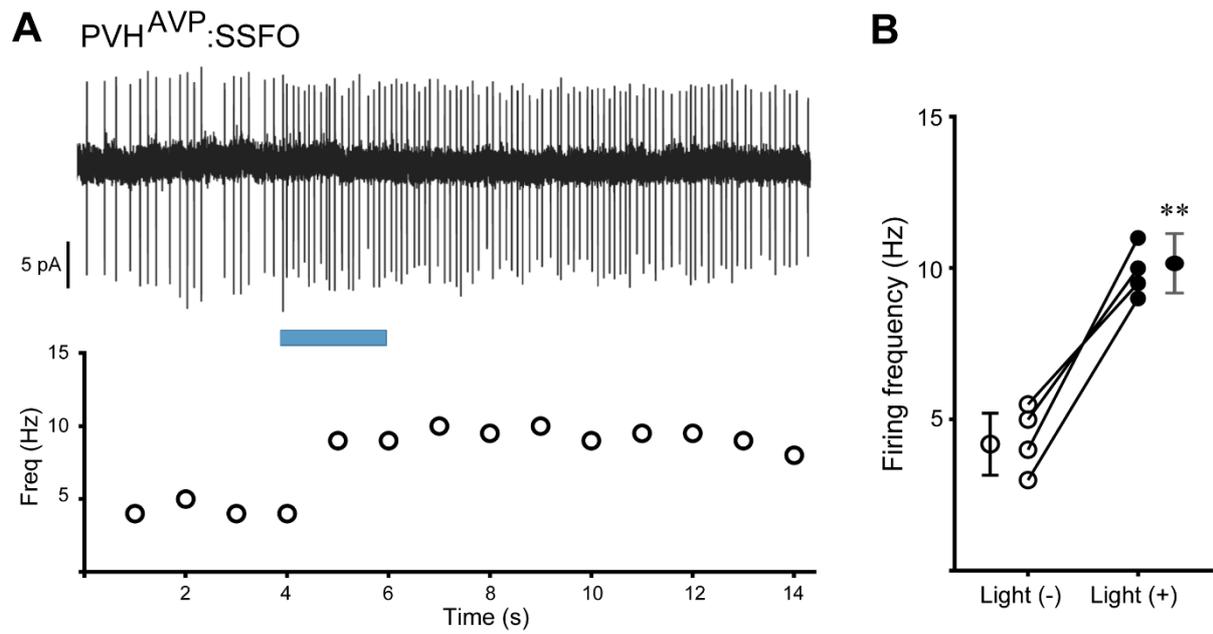
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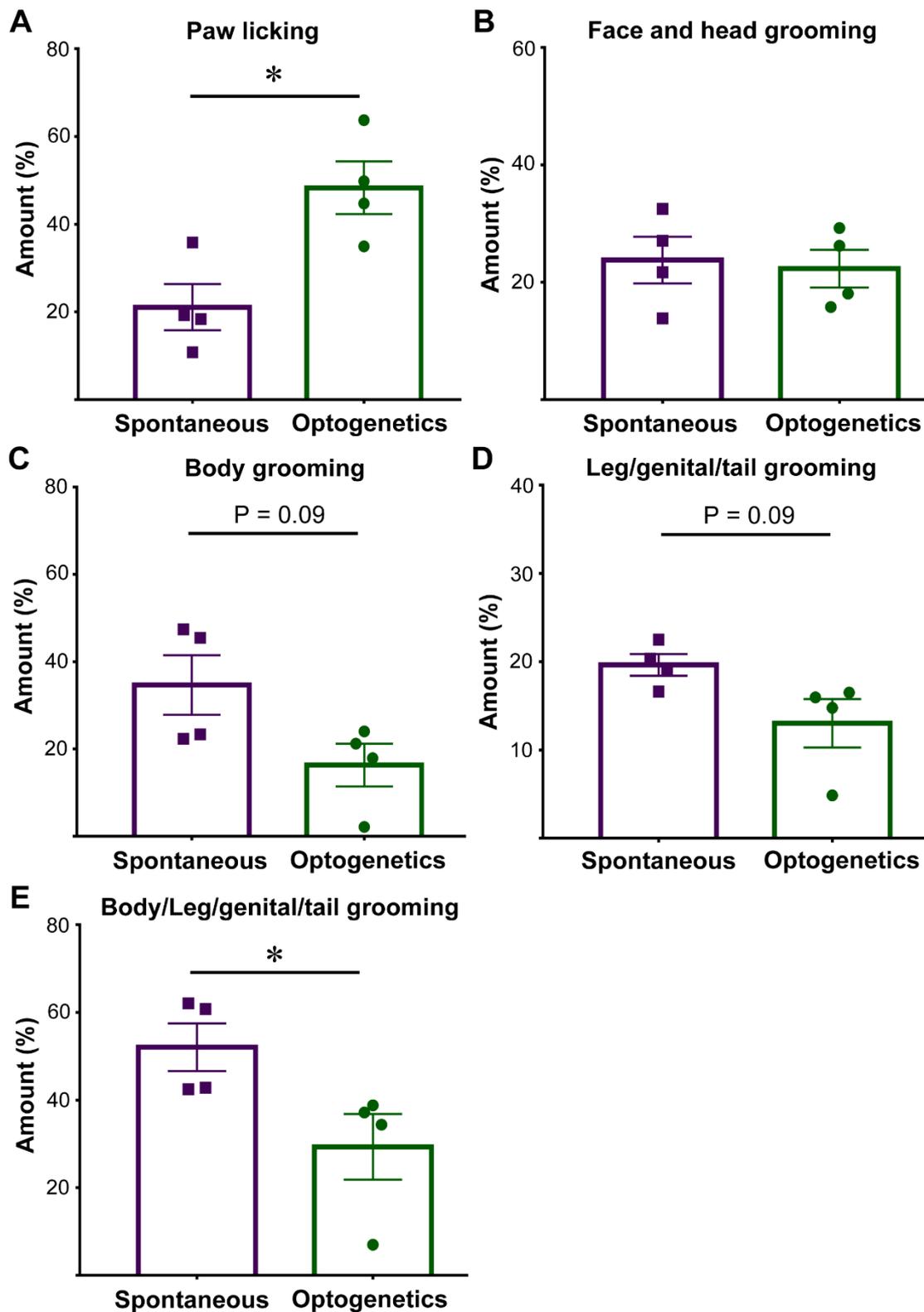
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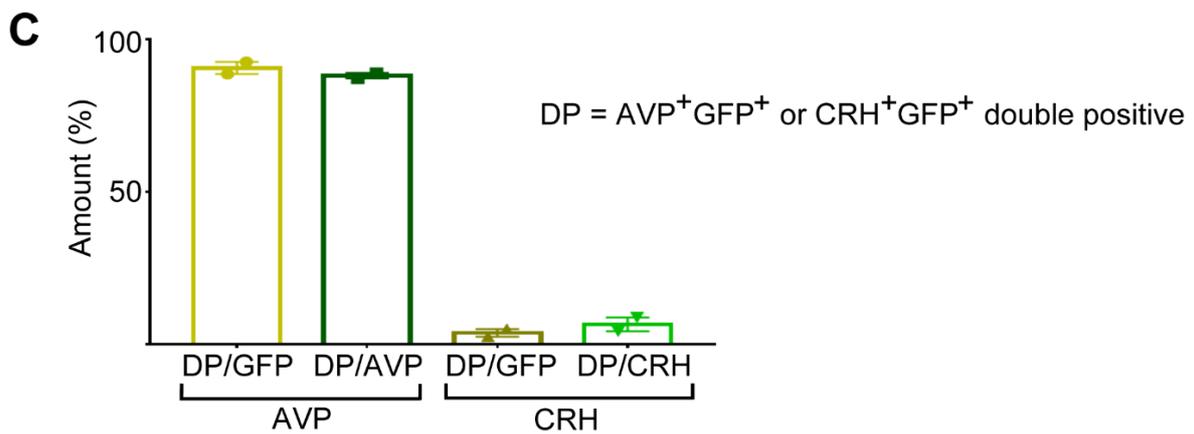
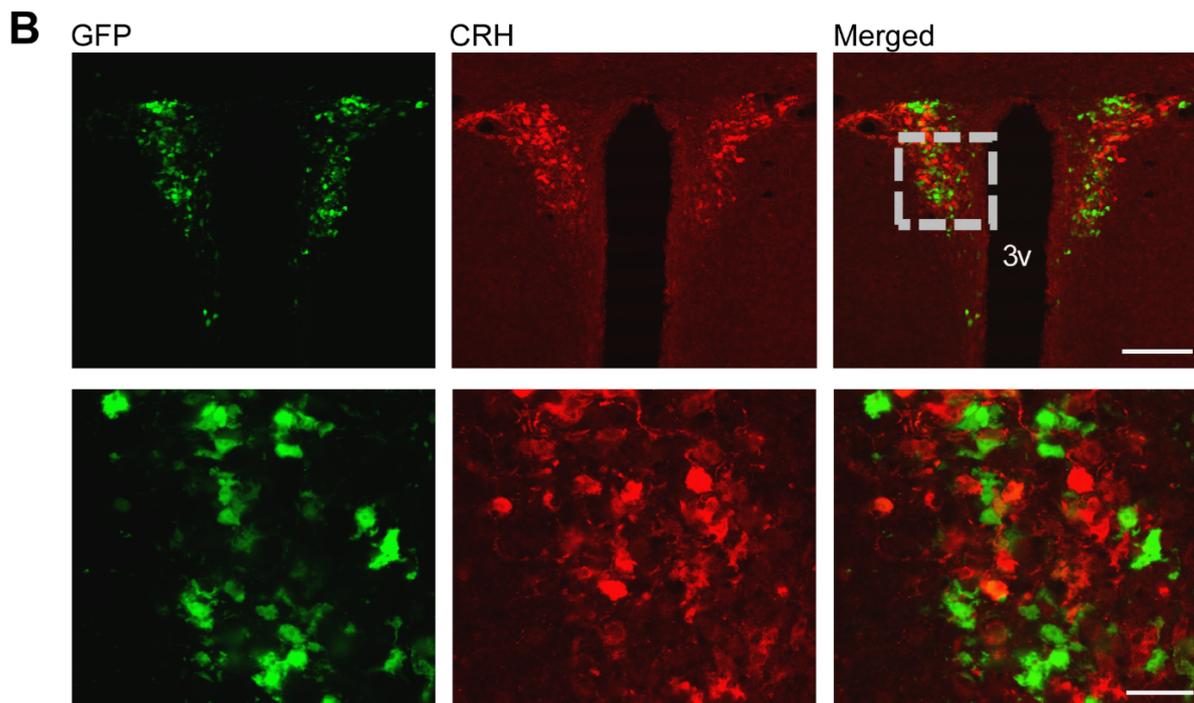
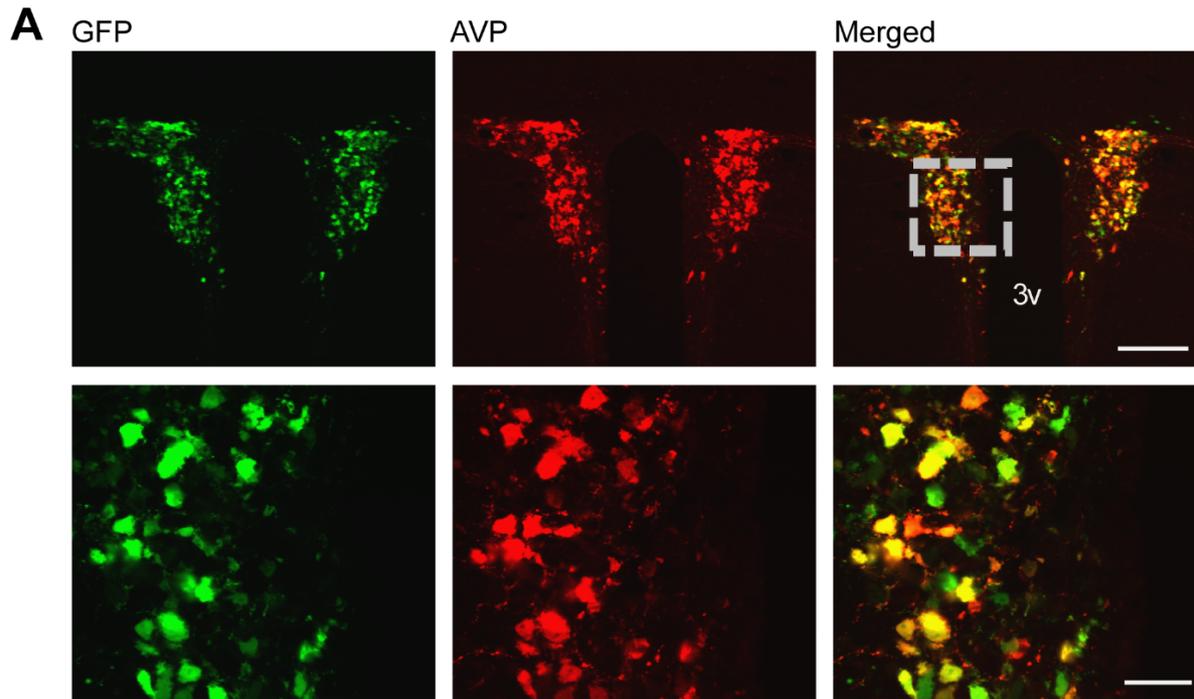
Figures S1-S4



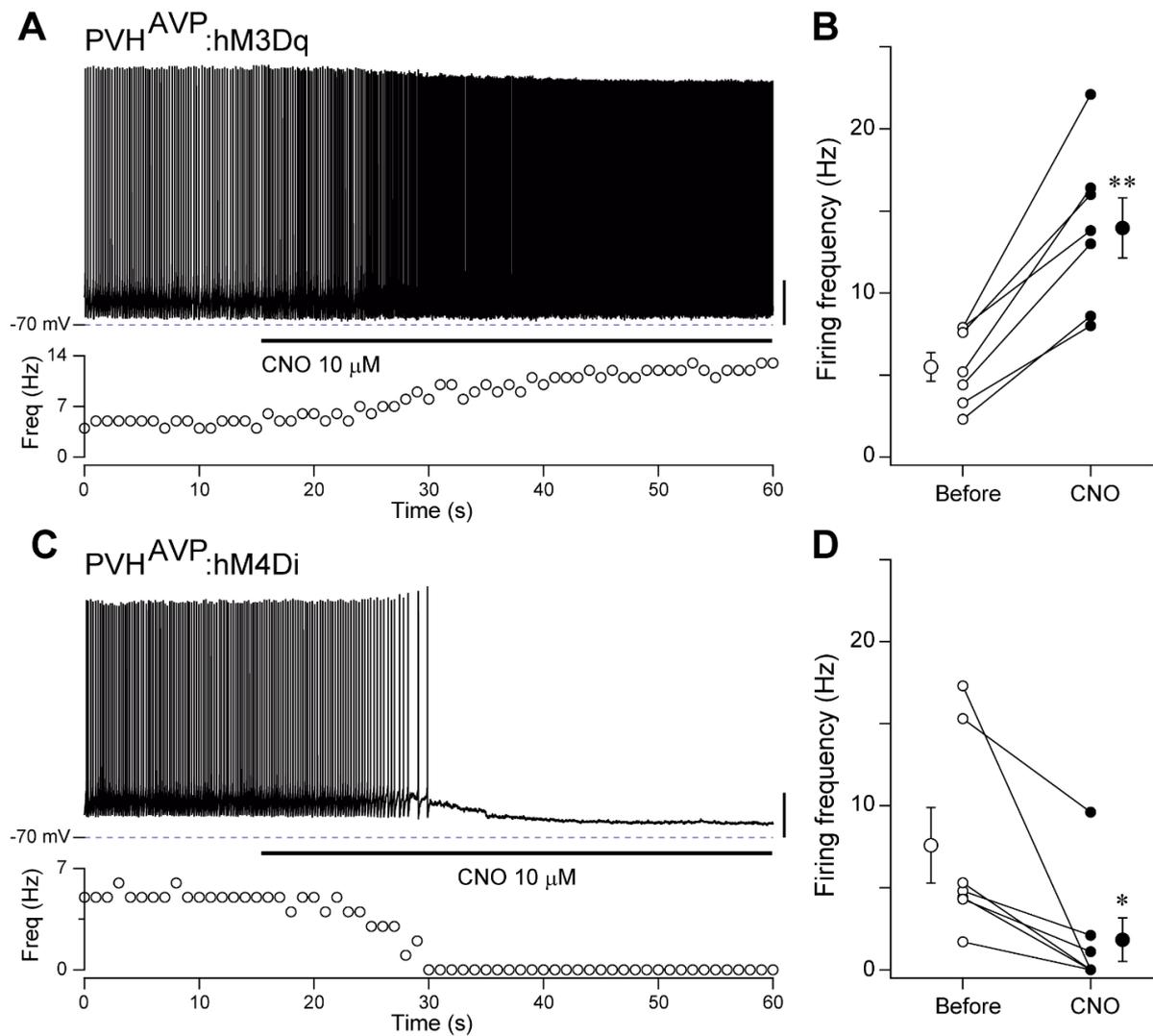
**Figure S1. Stimulation of SSFO in PVH<sup>AVP</sup> neurons increases their firing frequency in slices.** (A) Cell-attached recording from an SSFO-EYFP-expressing PVH<sup>AVP</sup> neuron. Blue light illumination (2 s) increased its firing frequency. The lower graph shows the change in firing frequency. (B) Summary plots showing the blue light effects on the firing frequency of PVH<sup>AVP</sup> neurons. Welch's t-test; \*\* $p < 0.005$ .



**Figure S2. Patterns of spontaneous and optogenetically-induced self-grooming.** The proportion of paw licking (A), face/head grooming (B), body grooming (C), leg/genital/tail grooming (D), or body/leg/genital/tail grooming (E, sum of C and D) during spontaneous and PVH<sup>AVP</sup> neuron-induced self-grooming. Welch's t-test; n = 4; \* $p < 0.05$ .



**Figure S3. Targeted PVH<sup>AVP</sup> neurons constitute a population distinct from PVH<sup>CRH</sup> neurons.** (A, B) Representative coronal brain sections containing the PVH prepared from *Avp-Cre* mice with a focal injection of AAV-*CAG-FLEX-EGFP* in the PVH. Sections were double-stained with anti-AVP (red) and anti-GFP (green) antibodies (A), or anti-CRH (red) and anti-GFP (green) antibodies (B). 3v, third ventricle. (Scale bar: 200  $\mu$ m for upper panels or 50  $\mu$ m for lower panels). (C) Proportions of AVP<sup>+</sup>:EGFP<sup>+</sup> cells to EGFP<sup>+</sup> cells [DP/EGFP<sup>+</sup>] or to AVP<sup>+</sup> cells [DP/AVP<sup>+</sup>], and those of CRH<sup>+</sup>:EGFP<sup>+</sup> cells to EGFP<sup>+</sup> cells [DP/EGFP<sup>+</sup>] or to CRH<sup>+</sup> cells [DP/CRH<sup>+</sup>]. DP: AVP<sup>+</sup>GFP<sup>+</sup> or CRH<sup>+</sup>GFP<sup>+</sup> double positive. n = 2.



**Figure S4. Stimulation of hM3Dq or hM4Di in PVH<sup>AVP</sup> neurons increases or decreases their firing frequency in slices.** (A) Whole-cell current-clamp recording from an hM3Dq-mCherry-expressing PVH<sup>AVP</sup> neuron. Bath application of 10  $\mu$ M CNO increased its firing frequency. The lower graph shows the change in firing frequency. (B) Summary plots showing the CNO effects on the firing rate in the hM3Dq-expressing PVH<sup>AVP</sup> neurons. (C) Representative trace showing that the 10  $\mu$ M CNO application stopped the firing in an hM4Di-mCherry expressing PVH<sup>AVP</sup> neuron. (D) Summary plots showing the CNO effects on the firing rate in the hM4Di-expressing PVH<sup>AVP</sup> neurons. (A, C) Scale bars, 20 mV. (B, D) Summary values are mean  $\pm$  SEM. Paired t-test; \* $p$ <0.05, \*\* $p$ <0.005.