

## Supplementary Information

### Chemogenetic silencing of GABAergic dorsal horn interneurons induces morphine-resistant spontaneous nocifensive behaviours

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#### Supplementary Figure 1

##### mCherry and PAX2 immunofluorescence in the SDH of *Vgat-Cre*;AAV-hM4Di<sup>FLEX</sup> mice

mCherry (red) and immunofluorescence of PAX2 (green) and NeuN (blue) in spinal sections of *Vgat-Cre*;AAV-hM4Di<sup>FLEX</sup> mice in the presence (upper panels) or absence (lower panels) of PAX2 primary antibody. PAX2 immunofluorescence with high and low intensity were indicated by arrow and arrowhead, respectively in upper panels.

#### Supplementary Figure 2

##### Whole-cell patch-clamp recording from *Vgat-Cre*<sup>neg</sup> lamina II neurons

(a) Schematic illustration of whole-cell patch-clamp recording. A suction electrode was used to stimulate dorsal root to evoke synaptic responses in recording neurons. (b) Representative morphology of *Vgat-Cre*<sup>neg</sup> lamina II neurons and the bottom images showed separated images of top merge image in white dotted open square (green, neurobiotin; red, mCherry fluorescence).

### **Supplementary Figure 3**

#### **CNO-induced facilitation of polysynaptic EPSCs in *Vgat-Cre*<sup>neg</sup> lamina II neurons receiving mono- and poly-synaptic A fibre inputs**

Representative mono- and polysynaptic excitatory postsynaptic currents (EPSCs) evoked by A fibre stimulation (100  $\mu$ A for 100  $\mu$ s) in *Vgat-Cre*<sup>neg</sup> lamina II neurons before (top) and after bath application of CNO (10  $\mu$ M; middle) and CNO with D-AP5 (50  $\mu$ M; bottom).

### **Supplementary Video 1**

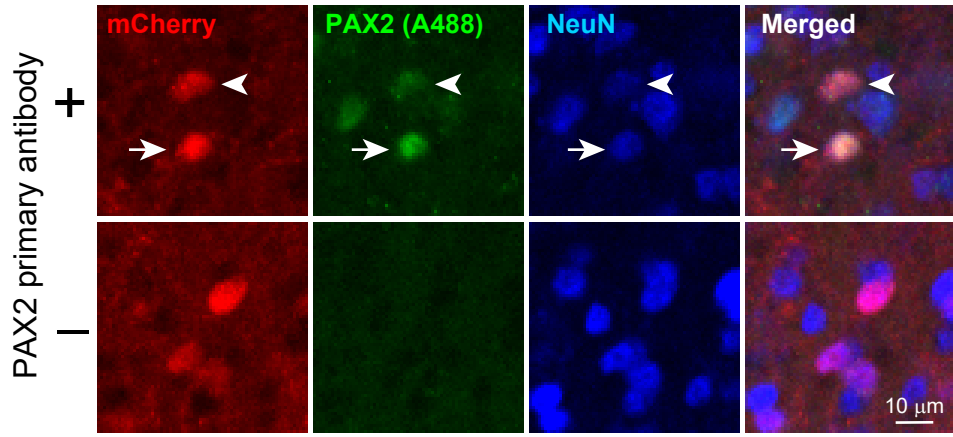
#### **Nocifensive behaviours in *Vgat-Cre*;hM4Di<sup>FLEX</sup> mouse with saline or CNO (during 10–20 min post-injection)**

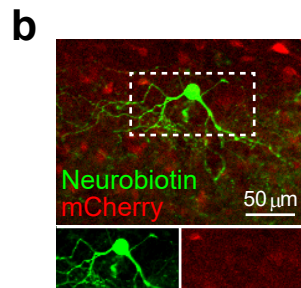
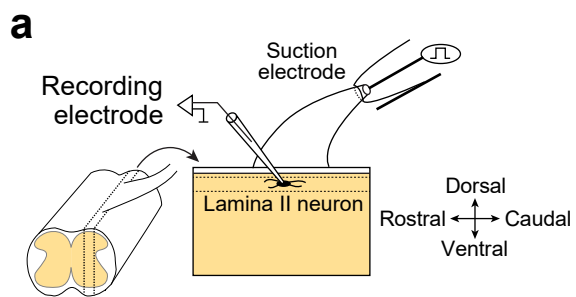
Note: saline-injected *Vgat-Cre*;hM4Di<sup>FLEX</sup> mouse showed normal behaviours, but CNO-injected *Vgat-Cre*;hM4Di<sup>FLEX</sup> mouse displayed robust spontaneous licking, biting and flinching of the hindpaw ipsilateral to the hM4Di expression (left side).

### **Supplementary Video 2**

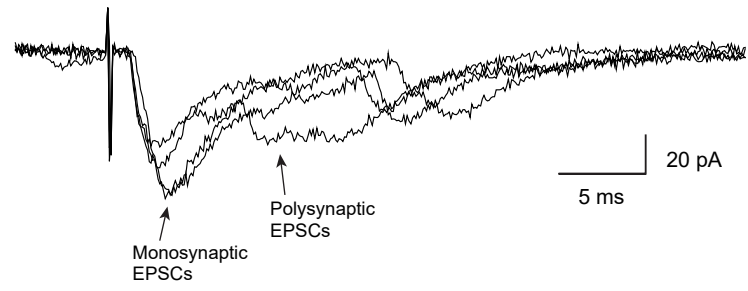
#### **CNO-induced nocifensive behaviours in *Vgat-Cre*;hM4Di<sup>FLEX</sup> mouse pretreated with saline or morphine (during 40–50 min post-CNO injection)**

Note: the CNO-induced flinching behaviour was increased in morphine-pretreated *Vgat-Cre*;hM4Di<sup>FLEX</sup> mouse.

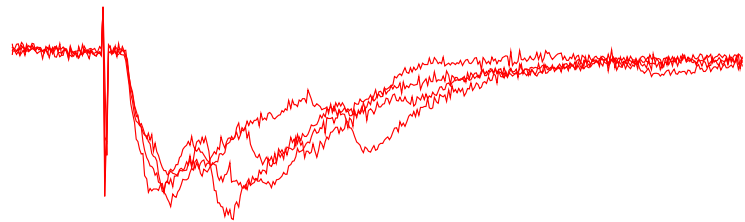




Control



CNO



CNO + D-AP5

