Supplementary Information

Supplementary Tables

Table S1. Stereotaxic kainic acid (KA), tracer and virus injection.

Item	Nuclei	Purpose	Mice	Volume	Manufacture
KA (1μg/μl)	dmPFC	Nucleus Lesion	C57BL/6J	0.2 μl	K0250, Sigma, St. Louis, MO, USA
10% BDA	dmPFC	Anterograde Tracing	C57BL/6J	0.1 μl	D1956, Invitrogen, Eugene, OR, USA
AAV2/9-DIO-EGFP-TVA and AAV2/9-DIO-RG, RV-EnvA-ΔG-dsRed	vlPAG	Retrograde Transsynaptic Tracing	VGLUT2-ires-Cre	0.3 μl	BrainVTA, Wuhan, China
AAV2/2-CaMKIIα-hChR2-EYFP or AAV2/2-CaMKIIα-EYFP)	dmPFC	Optogenetic Test	C57BL/6J	0.3 μl	BrainVTA, Wuhan, China
AAV2/4-hSyn-DIO-HA-hM3Dq-mCitrin or AAV2/4-hSyn-DIO-HA-mCitrin	dmPFC	Chemogenetic Test	Vgat-ires-Cre	0.3 μl	BrainVTA, Wuhan, China
4% FG	vlPAG, RVM, SDH	Retrograde Tracing	C57BL/6J	0.04 μl	80014; Biotium; Hayward, CA, USA
retroBeads	vlPAG	Retrograde Tracing	C57BL/6J	0.1 μl	Lumafluor; New York, NY, USA
scAAV2/1-hSyn-FLEX-EGFP- WPRE-pA	dmPFC	Anterograde Transsynaptic Tracing	C57BL/6J	0.3 μl	Taitool, Shanghai, China
AAV2/2Retro-hSyn-Cre-WPRE-pA	vlPAG	Retrograde Tracing	C57BL/6J	0.3 μl	BrainVTA, Wuhan, China
AAV2/9-hSyn-DIO-hM3Dq-eGFP-WPRE-pA and AAV2/9-mDlx-mCherry-WPRE-pA	dmPFC	Optogenetic and Chemogenetic Test	C57BL/6J	0.3 μl	BrainVTA, Wuhan, China

Table S2. Antibodies used in each group.

Antigen	Primary Antibodies	Secondary Antibodies
CaMKII/FG	Rabbit anti-CaMKII (1:200; ab34703, Abcam, Cambridge, MA, USA)/Gp anti-FG (1:200)	Alexa488-conjugated donkey anti-rabbit IgG (1:500; A-21206, Invitrogen, Carlsbad, CA, USA)/ Alexa594-conjugated goat anti-Gp IgG (1:500; A-11076, Invitrogen)
GAD67/FG	Mouse anti-GAD67 (1:500; MAB5406, Millipore)/Gp anti-FG (1:200)	Alexa488-conjugated donkey anti-mouse IgG (1:500; A-21202, Invitrogen)/ Alexa594-conjugated goat anti-Gp IgG (1:500)
NeuN/FG	Mouse anti-NeuN (1:500; MAB377, Millipore)/ Gp anti-FG (1:200)	Alexa488-conjugated donkey anti-mouse IgG (1:500)/ Alexa594-conjugated goat anti-Gp IgG (1:500)
NeuN/GFAP	Rabbit anti-NeuN (1:200; 12943, Cell Signaling Technology)/ Mouse anti-GFAP (1:2,000; MAB3402, Millipore)	Alexa488-conjugated donkey anti-mouse IgG (1:500)/ Alexa594-conjugated donkey anti-rabbit IgG (1:500; A-21207, Invitrogen)
Fos/DAPI	Rabbit anti-Fos (1:500; ab209794, Abcam)	Alexa594-conjugated donkey anti-rabbit IgG (1:500)/ DAPI (1:5,000; D1306, Molecular Probes, Eugene, OR, USA)
mCitrin/GAD67/Fos	Chicken anti-GFP (1:200; GFP-1020, Aves Labs, Tigard, OR, USA)/ Mouse anti-GAD67 (1:500)/Rabbit anti-Fos (1:500)	FITC-conjugated goat anti-chicken IgY (1:500; A16055, Invitrogen)/Alexa594-conjugated donkey anti-mouse IgG (1:500; A-21203, Invitrogen)/Alexa647-conjugated donkey anti-rabbit IgG (1:500; A-31573, Invitrogen)
EYFP/FG/Synapsin	Chicken anti-GFP (1:200)/Gp anti-FG (1:200)/ Rabbit anti-Synapsin (1:500; A-6442, Invitrogen)	FITC-conjugated goat anti-chicken IgY (1:500)/Alexa594-conjugated goat anti-Gp IgG (1:500) /Alexa647-conjugated donkey anti-rabbit IgG (1:500)
EYFP/FG	Chicken anti-GFP (1:200)/Gp anti-FG (1:200)	FITC-conjugated goat anti-chicken IgY (1:500)/Alexa594-conjugated goat anti-Gp IgG (1:500)
5-HT/FG/VGLUT2	Goat anti-5-HT (1:200; 20079, ImmunoStar, Houston, Texas, USA)/ Gp anti-FG (1:200)/Rabbit anti-VGLUT2 (1:500; ab2251, Abcam)	Alexa488-conjugated donkey anti-rabbit IgG (1:500)/Alexa594-conjugated donkey anti-Goat IgG (1:500; A-11058, Invitrogen) /Alexa647-conjugated donkey anti-Gp IgG (1:500; AP193SA6, Millipore)
Biocytin/DAPI	NA	FITC-conjugated avidin (1:1,000; A-2001, Vector Laboratories) / DAPI (1:5,000)
EYFP/Fos	Chicken anti-GFP (1:200)/Rabbit anti-Fos (1:500)	FITC-conjugated goat anti-chicken IgY (1:500)/Alexa594-conjugated donkey anti-rabbit IgG (1:500)

Supplementary Figures

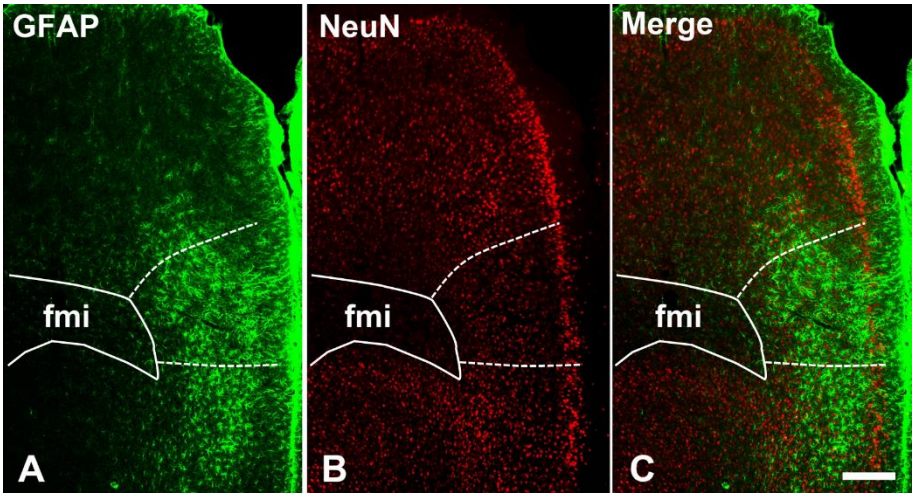

Figure S1. Injection of KA into the dmPFC induces the neuronal loss and astrocytic proliferation in the injection site.

The double fluorescence immunostaining of GFAP (**A**) and NeuN (**B**) after the injection of KA into the dmPFC. (**C**) Merged image from **A**-**B**. fmi: forceps minor of the corpus callosum, the area between the two dash line is dmPFC. Scale bars=200 μm in **C** (applied in **A**-**B**).

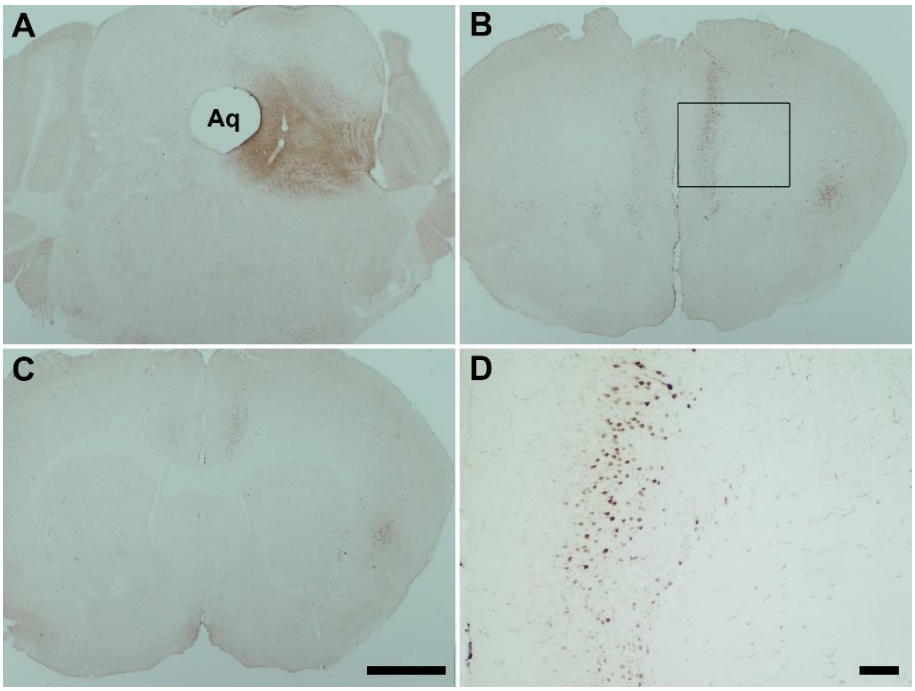

Figure S2. Distribution of retrogradely labeled neurons in the dmPFC after FG was injected into the vlPAG.

Photomicrographs showing FG injection sites in the vlPAG (**A**) and the resultant of FG-labeled neurons in the dmPFC (**B**) and ACC (**C**). The area in the black frame in **B** was magnified in **D**. (**D**) The retrogradely FG-labeled neurons were mainly distributed in the layer V of the dmPFC. Scale bars=300 μm in **C** (applied in **A**-**B**), 100 μm in **D**.

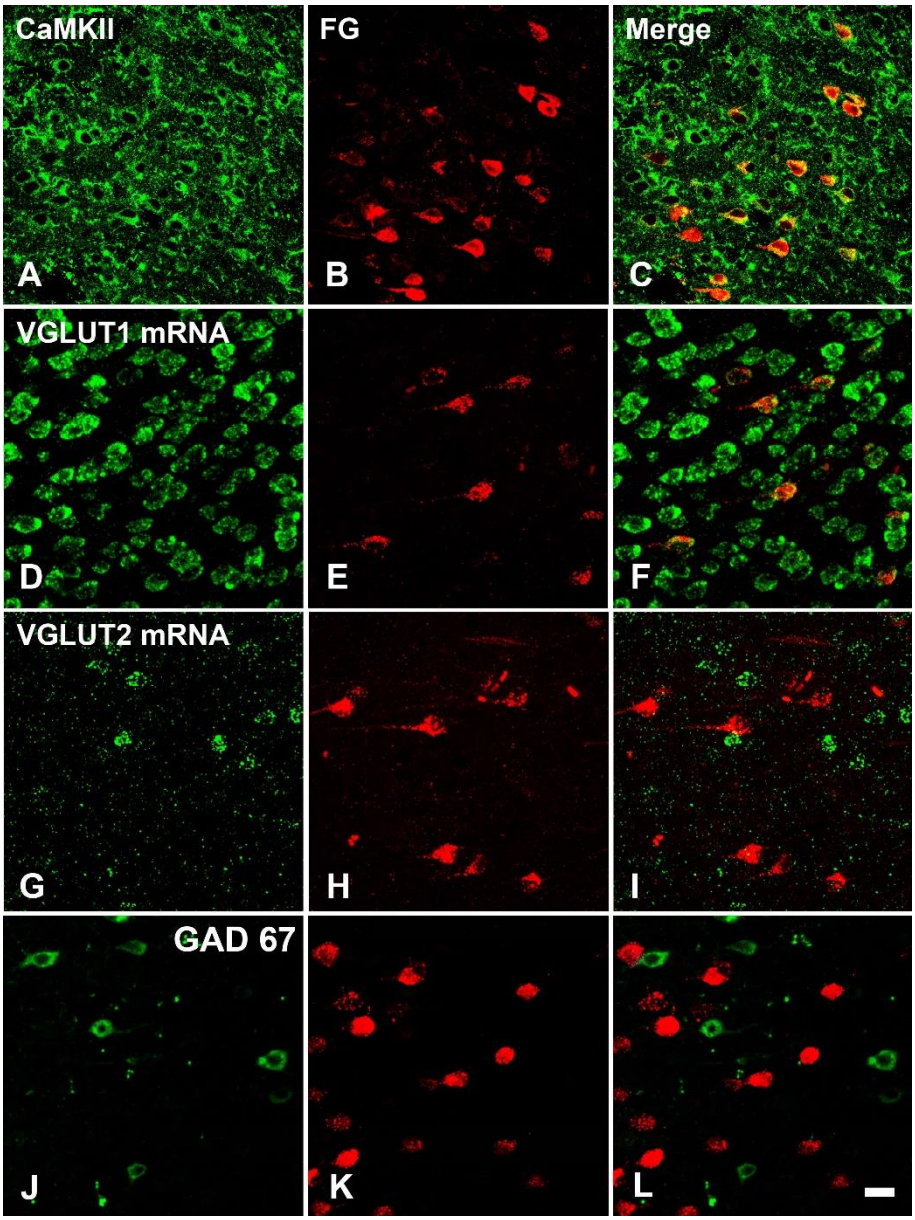

Figure S3. Fluorescent photomicrographs showing the distributions of CaMKII-ir neurons, VGLUT1 or VGLUT2 mRNA-containing neurons, GAD67-ir neurons and FG labeled neurons in the dmPFC after FG was injected into the vlPAG.

(**A**-**C**) All the FG-labeled neurons (red) in the dmPFC expressed CaMKII (green). (**D**-**F**) All the FG-labeled neurons (red) in the dmPFC contained VGLUT1 mRNA (green). The FG-labeled neurons (red) in the dmPFC did not contain VGLUT2 mRNA (green) (**G**-**I**) express GAD67 (**J**-**L**)**.** Scale bars=20 μm in **L** (applied in **A**-**K**).

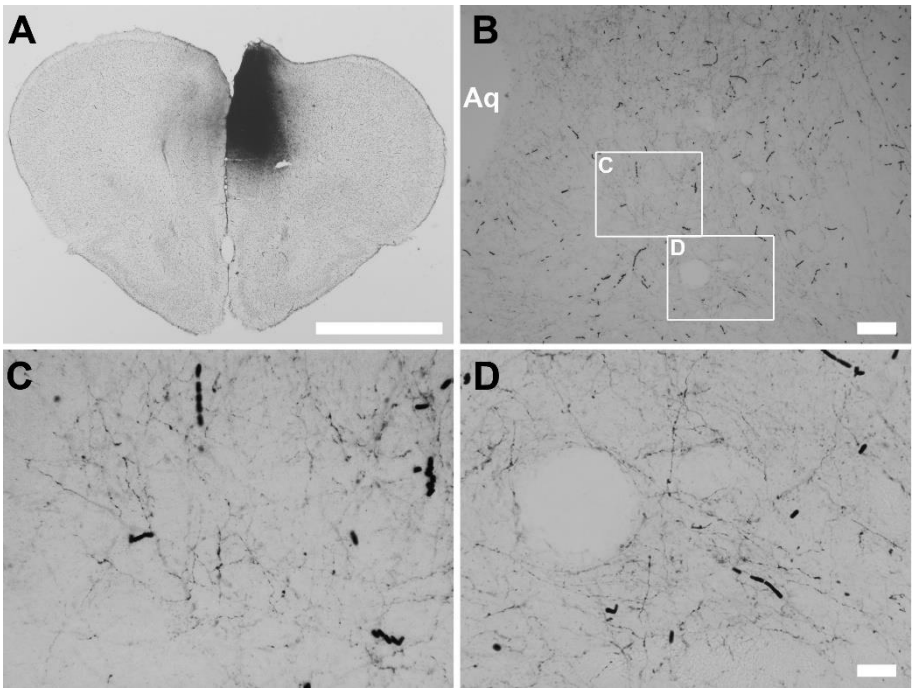

Figure S4. The projections from the dmPFC to the vlPAG.

(**A**) Photomicrographs showing the BDA injection site in the dmPFC. (**B**) Anterogradely BDA-labeled axonal fibers and terminals originated from the dmPFC were mainly observed on the ipsilateral side of the lPAG and vlPAG. (**C**-**D**) The rectangular areas in (**B**) were enlarged and displayed in (**C**) and (**D**). Scale bars=500 μm in **A**, 100 μm in **B**, 25 μm in **D** (applied in **C**).

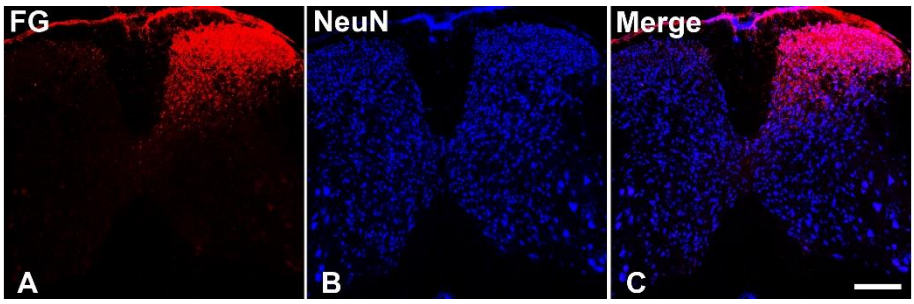

Figure S5. Fluorescence photomicrographs showing the FG injection site in the spinal cord.

(**A-C**) Fluorescence photomicrographs showing the double staining of FG (red) and NeuN (blue) in the spinal cord. Scale bars=200 μm in **C** (applied in **A-B**).

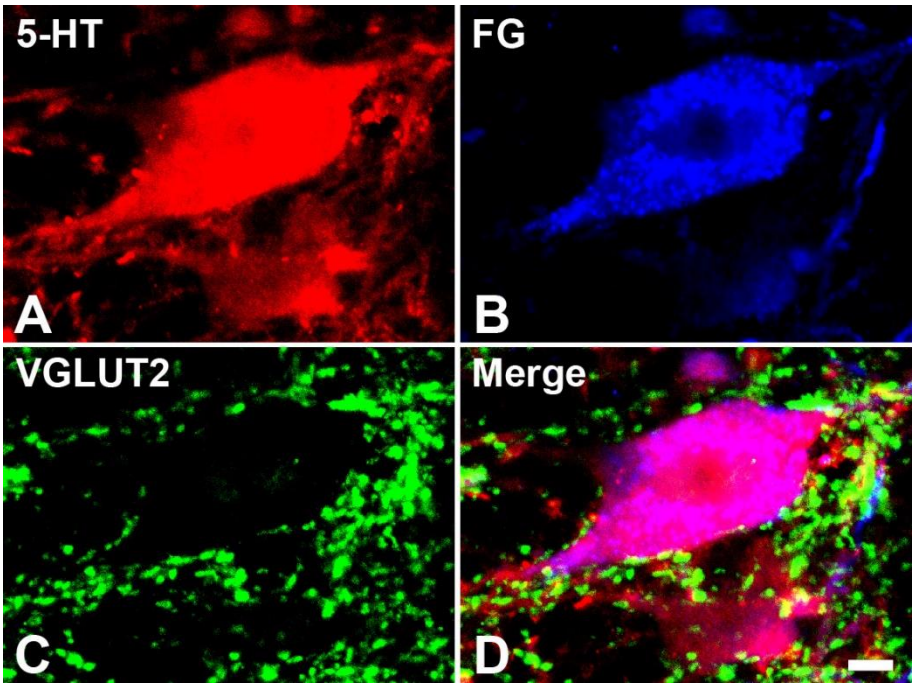

Figure S6. The connection between VGLUT2-ir terminals and 5-HT- and/or FG-ir neurons in the RVM.

(**A**-**D**) Fluorescence photomicrographs showing that VGLUT2-ir axon terminals (**C**, green) made close connections with a 5-HT-ir (**A**, red) neuronal cell body projecting to the spinal cord (**B**, FG-ir, blue) in the RVM. Scale bars=5 μm in **D** (applied in **A**-**C**).

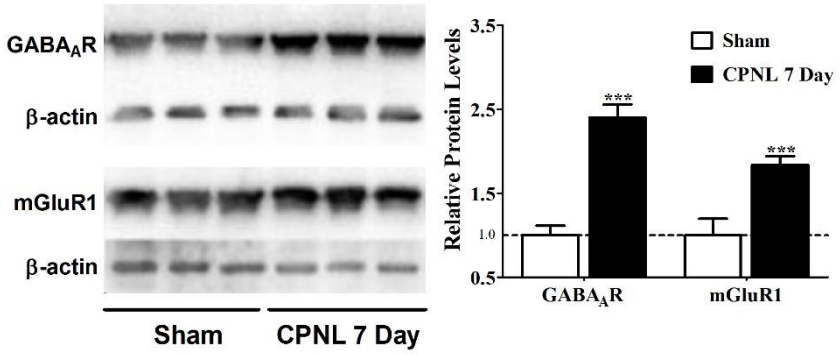

Figure S7. The expressions of GABA$_A$R and mGluR1 were remarkably increased in the dmPFC after CPNL 7 days.

The expressions of GABA$_A$R and mGluR1 in the dmPFC of each mice in different groups, were revealed by Western blot. The *bar graphs* (right) demonstrated the mean levels of GABA$_A$R and mGluR1 normalized to β-actin. The asterisk was used, when CPNL 7 Day group was compared with sham group. ****P*<0.001, Student's *t*-test, n=3 in each group.

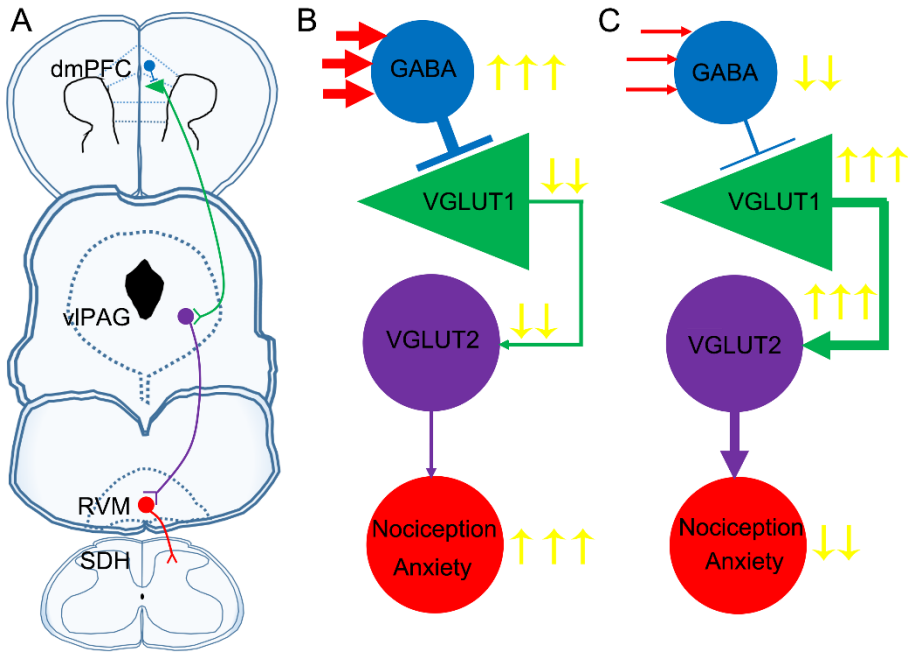

Figure S8. Graphical abstract

(**A**) Schematic diagram of putative 'Top-Down' descending neural pathway, dmPFC-vlPAG-RVM-SDH. We hypothesize that periphery nerve injury induces increased excitatory inputs to the inhibitory neurons in the dmPFC, which then deactivates the descending dmPFC-vlPAG neural pathway. These in turn lead to mechanical hyperalgesia and anxiety-like behaviors (**B**). The deactivation of inhibitory neurons in the dmPFC or activating dmPFC-vlPAG neural pathway produces analgesic and anxiolytic effects (**C**).