PNAS
www.pnas.org

Supplementary Information for

Reversal of hyperactive subthalamic circuits differentially mitigates pain hypersensitivity phenotypes in parkinsonian mice

Yiwen Luan, Dongliang Tang, Haichuan Wu, Weixin Gu, Yuqing Wu, Junli Cao*, Cheng Xiao*, Chunyi Zhou*

* Corresponding authors
Chunyi Zhou, MD, PhD
Email: 100002016033@xzhmu.edu.cn
Cheng Xiao, MD, PhD
Email: xchengxj@xzhmu.edu.cn
Junli Cao, MD, PhD
Email: caojl0310@aliyun.com

This PDF file includes:

Supplementary text： 4 pages
Figures S1 to S12
Tables S1
SI References: 7

www.pnas.org/cgi/doi/10.1073/pnas.1916263117

Supplementary Information Text

Materials and Methods

Animals The care and use of animals and the experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee and the Office of Laboratory Animal Resources of Xuzhou Medical University. C57BL/6 mice were purchased from Jinan Pengyue Laboratory Animal Breeding Co. Ltd, and were group-housed (≤ 4 per cage) on a 12-hour light/dark cycle. The mice had free access to water and food. Efforts were made to minimize animal suffering and to reduce the numbers of animals used.

Adenovirus associated virus (AAV) vectors The viral vectors, including AAV-CaMKIIα-EGFP, AAV-CaMKII-hChR2 (H134R)-YFP, and AAV-CaMKII-NpHR3.0 -eYFP (serotypes 2, 1 × 1012 to 5 × 1012 vg/ml), were purchased from OBIO Technology (Shanghai, China) or Brain VTA (Wuhan, China).

Surgical procedure for unilateral 6-hydroxydopamine (OHDA) lesion of SNc DA neurons. Individual male mice (12 weeks old) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg) and stabilized on a stereotaxic frame (RWD Life Science Co., Ltd). A small craniotomy was made above the medial forebrain bundle (MFB) of the right hemisphere. 0.3 μl 6-OHDA (12 μg/μl in 0.2% ascorbic acid, Tocris, USA) was stereotaxically injected into the MFB (1 mm anterior to bregma in the anterior–posterior dimension (AP); 1.2 mm lateral to the midline (ML); 4.8 mm in depth (DV)) with a microinjection pump (KD Scientific, USA) at a rate of 0.1 μl/min. Sham mice were prepared by injecting 0.3 μl 0.2% ascorbic acid into the MFB. Half an hour before 6-OHDA injection, the mice were intraperitoneally injected with 25 mg/kg desipramine to protect adrenergic neurons from being damaged by 6-OHDA. Post-surgery care was similar for both sham and 6-OHDA injected mice.

Viral delivery and optical fiber implantation Male mice (8–12 weeks old) were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg) and stabilized on the stereotaxic frame. A midline incision was made on the skin of the head and small holes were drilled in the skull above the regions of interest. Viral vectors (0.3 μl, at a rate of 50 nl/min) were microinjected into the STN of the right hemisphere (AP, −1.48 mm; ML, 1.77 mm; DV, 4.7 mm). The optical fibers (200 μm in diameter, NA 0.37, Inper (Hangzhou)) were implanted above the STN (AP, −1.48 mm; ML, 1.77 mm; DV, 4.5 mm), SNr (AP, −3.1 mm; ML, 1.5 mm; DV, 4.5 mm), GPi (AP, −1.3 mm; ML, 1.75 mm; DV, 4.3 mm), and VP (AP, 0.3 mm; ML, 1.5 mm； DV, 4.5 mm) in the right hemisphere. The optical fiber implants were adhered to the skull with dental cement and the incision was sutured. The mice were maintained for at least 3 weeks for viral expression before the behavioral, electrophysiological, and morphological tests.

Transsynaptic neuronal tracing For trans-synaptic tracing of STN neurons, Alexa-488 conjugated wheat germ agglutinin (WGA, 0.1μl, 1μg/μl in saline, Thermo Fisher Scientific, USA) was injected into the STN of anesthetized mice. 48 h later, the mice were sacrificed with CO_2.

After overnight fixation, the brain was sliced into 50-μm-thick sections with a vibratome, and the sections were mounted onto glass slides before imaging with a confocal microscope (Zeiss LSM880).

Patch-clamp recordings To verify that the virus was functional, we performed patch-clamp recordings on brain slices using a modified version of a protocol described previously (1-3). In brief, the mice were sacrificed with CO_2 and then decapitated. The brain was removed and cut into 300 μm thick parasagittal slices with a vibratome (VT-1200S, Leica), while immersed in ice-cold modified sucrose-based artificial cerebral spinal fluid (sACSF), saturated with 95% O2 / 5% CO2 (carbogen) containing (in mM) 85 NaCl, 75 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 4.0 $MgCl_2$, 0.5 $CaCl_2$, 24 $NaHCO_3$, and 25 glucose . Brain slices containing the regions of interest were allowed to recover at 32 ± 1°C in a holding chamber filled with carbogenated sACSF. One hour later, the brain slices were transferred into carbogenated normal ACSF, containing (mM) 125 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 26 $NaHCO_3$, and 11 glucose, and maintained at room temperature. Individual slices were transferred to the recording chamber and superfused (1.5–2.0 ml/min) with carbogenated ACSF at 32 ± 0.5°C. Three to four slices per mouse were used each day for recordings.

Neurons in brain slices were visualized with an upright microscope (FN-1, Nikon) equipped with a CCD-camera (Flash 4.0 LTE, Hamamatsu) and near-infrared illumination. Whole-cell patch-clamp signals were recorded with MultiClamp 700B amplifiers (Molecular Devices, CA), Digidata 1550B analog-to-digital converters (Molecular Devices), and pClamp 10.7 software (Molecular Devices). The patch electrodes had resistances of 4 – 6 MΩ when filled with an intrapipette solution containing (in mM) 135 K gluconate, 5 KCl, 0.2 EGTA, 0.5 $CaCl_2$, 10 HEPES, 2 Mg-ATP, and 0.1 GTP. The pH was adjusted to 7.2 with Tris-base and the osmolarity was adjusted to 300 mOsm with sucrose. The junction potential between the patch pipette and the bath solution was nulled just before gigaseal formation. Series resistance was monitored without compensation throughout the experiment by the MultiClamp 700B. The data were discarded if the series resistance (11–20 MΩ) changed by > 20% during whole-cell recordings. Data were sampled at 10 kHz and filtered at 2 kHz.

In vivo optogenetic stimulation Optical implants in individual mice were connected to a 473 nm or 589 nm laser (Newdoon, Hangzhou) through an optical patch cable. Blue light (473 nm, 5 ms pulse width, 4 mW) and yellow light (598 nm, constant, 3 mW) were used to activate ChR2 or NpHR3.0, respectively, expressed in STN neurons or their axonal terminals *in vivo* (1).

Behavioral tests

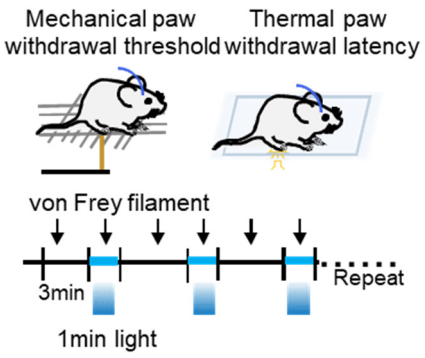
Mobility test Mice were placed in the chamber used for pain threshold tests and their motor behavior was recorded for 17 min with a video camera controlled by Ethovision XT 9 software (1, 4). After a baseline measurement was obtained between the 5th and 10th minutes, the mice were subjected to 2 min optogenetic stimulation or inhibition of STN neuronal somata or terminals in downstream nuclei. The mobility rate during optogenetic modulation was compared with the baseline mobility rate to address whether motor functions were altered by the optogenetic modulation.

Adhesive tape removal Mice were habituated for at least 30 min in a transparent chamber (7.5 cm long, 7.5 cm wide, and 15 cm high). An 8-mm-diameter circle of adhesive tape was attached to the plantar surface of the right hindpaw (5). After a latency period, the mouse tried to remove the tape. Time latencies for mice to contact the tape (sense time) and to remove the tape were recorded. This test was performed once per day for 5 consecutive days (Fig. S2).

Von Frey filament test Mice were placed in a test chamber (7.5 cm long, 7.5 cm wide, and 15 cm high, without a bottom and a cover) on a wide gauge wire mesh supported by an elevated platform. Mechanical pain thresholds were measured with von Frey filaments using the up–down method (6). In brief, the first von Frey filament (0.41 g) was applied to the plantar surface of the hindpaw. If a withdrawal response was observed within 2 s, the next filament with a lower force was applied. Conversely, if the first filament failed to elicit a withdrawal response, the next filament with a higher force was applied. After the first withdrawal response, this paradigm was repeated until a total of six readings were recorded, starting from the one before the first change in response. The 50% paw withdrawal threshold (PWT) was determined from these readings, as described previously (7).

Thermal pain test Thermal paw withdrawal latencies of individual mice were measured with a plantar anesthesia tester (Boerni, Tianjin, China) on an elevated platform. Individual mice were placed in a chamber (7.5 cm long, 7.5 cm wide, and 15 cm high, without a bottom and a cover) on the glass surface of the tester (6). A heat light source was positioned under the glass, pointing to the plantar surface of the hindpaw. The time between the initiation of heating and the mouse removing its hindpaw from the heating spot was recorded. The cut-off time was set to 20 s to prevent tissue damage. The results from three tests were averaged to represent the paw withdrawal latency (PWL) for each mouse.

Pain threshold tests before and during optogenetic modulation of STN circuitry After habituating the mice to the chamber, baseline pain threshold tests were performed and, 3 min later, either blue (1 min, 20 Hz (10 ms), 4 mW) or yellow (1 min constant, 3 mW) light was delivered through the optical fiber implant for photo-stimulation or photo-inhibition of STN circuits. A mechanical pain threshold measurement (application of one von Frey filament) was made 30 s after the initiation of light delivery. Then, the mice were allowed to recover for at least 3 min before the next round of testing. The procedure was repeated until enough data were acquired to accurately calculate pain thresholds (see the following diagram). Thermal pain thresholds were examined at least three times before and during light application.

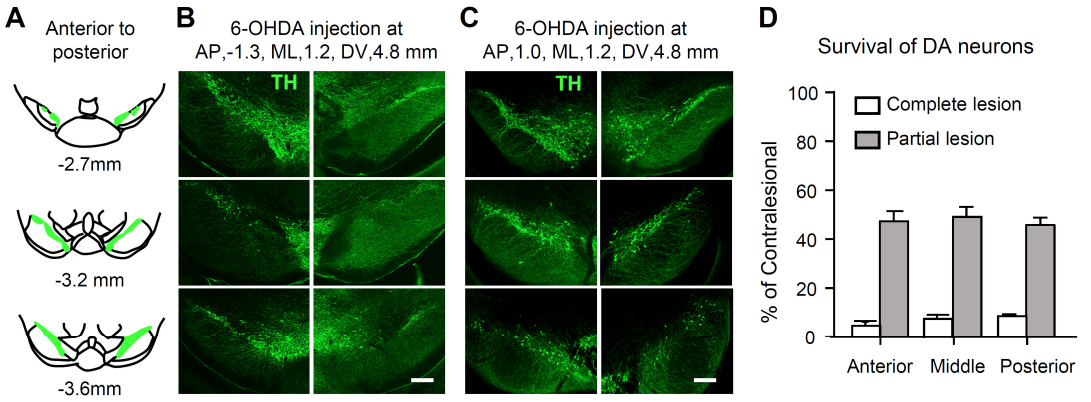


Immunohistochemistry After being sacrificed in a CO_2 chamber, mice were subjected to cardiac perfusion with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in PBS. Mouse brains were removed and post-fixed in PFA for 12 h at 4°C. Brain sections (50 μm) were cut on a vibratome and mounted onto glass slides. For immunostaining, brain sections were sequentially washed in PBS for 10 min, blocked in 5% donkey serum and 0.1% Triton in PBS for 90 min, incubated in primary antibody (rabbit anti-c-fos, Cell Signaling Technology, 1:2000; mouse anti-TH, Santa-Cruz, 1:500) in 1% donkey serum and 0.1% Triton in PBS for 24 hours at 4°C, washed three times (10 min each) in PBS, incubated with secondary antibodies (donkey anti-rabbit Cy2 or Cy3, donkey anti-mouse Cy2 or Cy3, Jackson ImmunoResearch, 1:500) for 90 min at room temperature, washed three times (10 min each) in PBS, dried in the dark, and then cover-slipped in mounting medium.

Confocal microscopy Low- and high-magnification images were acquired with a Zeiss LSM 880 confocal microscope equipped with a 10× Plan Apochromat objective (NA, 0.45), a 20× Plan Apochromat objective (NA, 0.8), and five laser lines (405 nm, 458 nm, 488 nm, 561 nm, and 633 nm). The microscope was controlled by Zen2 acquisition software (Zeiss), enabling automatic tiling and z-stacking. The images were processed with Image J (Schneider et al., 2012).

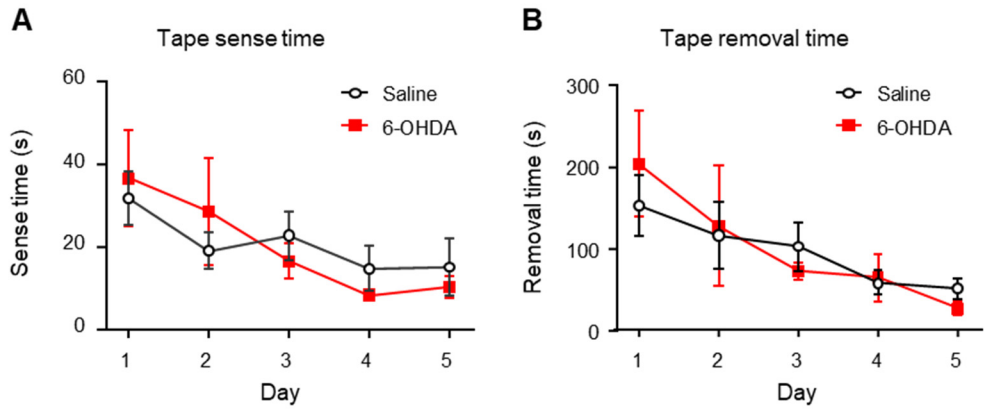
Statistical analysis SigmaPlot (version 14.0, SPSS Inc.) was used for all statistical analyses. Data are expressed as the mean ± S.E.M in all graphs. Two-way ANOVAs were used to analyze differences in the numbers of SNc DA neurons, movement distances, and mechanical and thermal pain thresholds between saline and 6-OHDA injected mice at different time points after surgery, and to compare the frequencies of firing evoked by a series of depolarizing current steps between saline and 6-OHDA injected mice. A one-way ANOVA was used to compare the numbers of c-fos-positive neurons among multiple groups and was followed by the Holm–Sidak method test for pairwise comparison. Two-tailed paired t-tests were used to compare pain thresholds before and during optogenetic modulation. In each experiment, we used 5-10 mice in control groups to calculate the mean and the standard deviation of the mean for a parameter, and calculated the sample sizes required for other groups using SigmaPlot 14.0 by setting the power and alpha to 0.9 and 0.05, respectively. For statistical comparisons, $P < 0.05$ was considered significant.

Figure S1



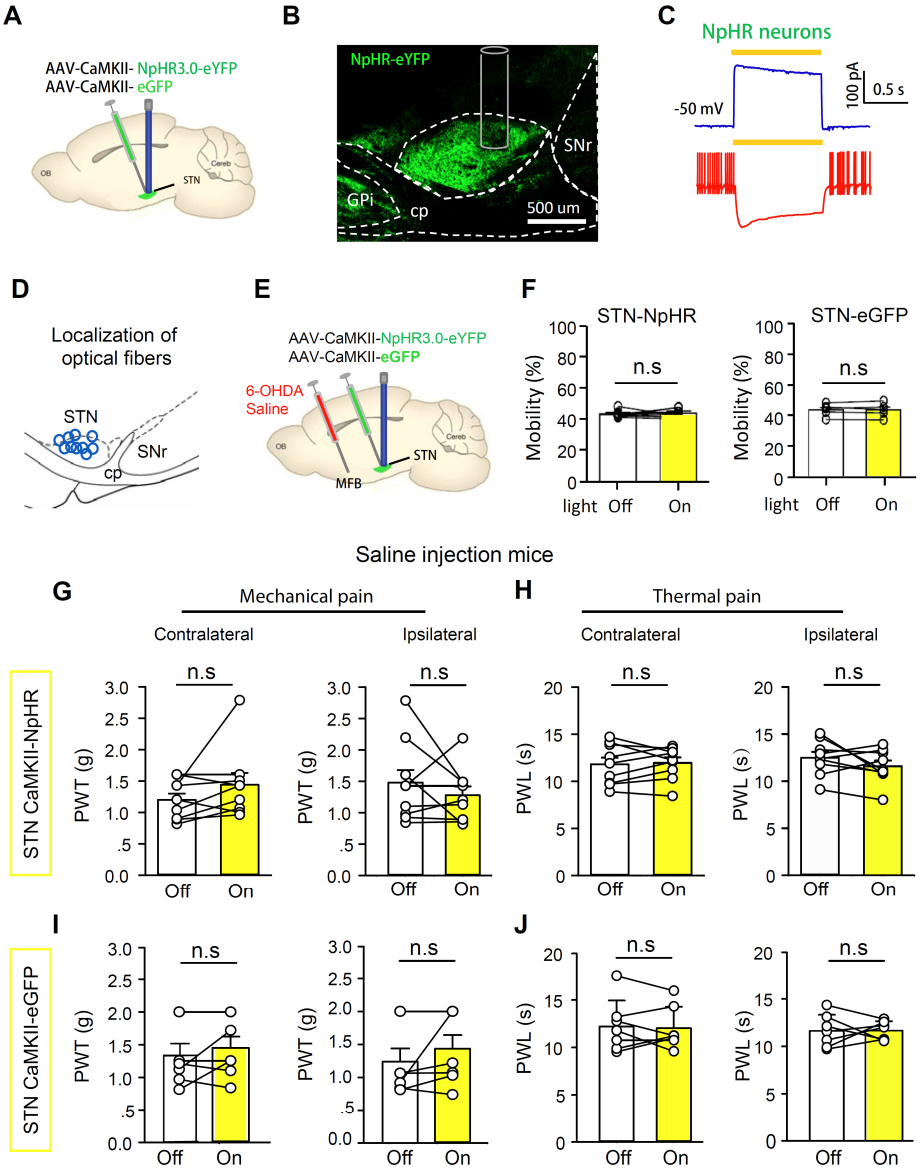
Lesion of SNc DA neurons after 6-OHDA injection in the medial forebrain bundle in mice. 3.6 μg 6-OHDA in 0.3 μL saline (0.2% ascorbate) was injected into two coordinates in the medial forebrain bundle of the right hemisphere (site 1: AP -1.3, ML 1.2, DV 4.8 mm; site2: AP 1.0, ML 1.2, DV 4.8 mm). 14 days after injection, TH-staining was performed and images from the anterior, middle, and posterior substantia nigra **(A)** were acquired under a 10X objective **(B, C)**. Injection into site 1 **(B)** and site 2 **(C)** induced nearly complete and partial lesion of SNc DA neurons, respectively. **(D)** DA neuron lesions after 6-OHDA injection at either site 1 or site 2 were similar across the anterior, middle, and posterior SNc (site 1: $F = 2.26$, $n = 5$, $P = 0.15$, one-way ANOVA; site 2: $F = 1.05$, $n = 5$, $P = 0.38$, one-way ANOVA). Scale bar: 100 μm.

Figure S2



Partial lesion of unilateral SNc DA neurons does not affect hindpaw withdrawal in the tape-removal test. The tape-removal test was performed to quantify the ability of mice to withdraw the hindpaw. After partial lesion of SNc DA neurons (shown in Figure S1C, S1D), mice performed as well as control mice (saline injection in the medial forebrain bundle) in terms of both the tape sense time **(A)** ($F(4,50) = 0.54$, $P = 0.71$, $n = 5$, two-way ANOVA) and the tape removal time **(B)** ($F(4.47) = 0.37$, $P = 0.83$, $n = 5$, two-way ANOVA).

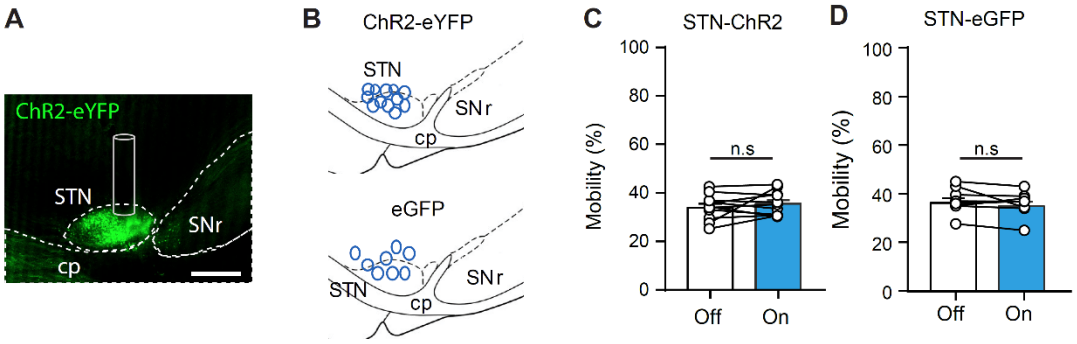
Figure S3



Optogenetic inhibition of STN neurons does not regulate pain thresholds in control mice. **(A)** AAV-CaMKII-NpHR3.0-eYFP or AAV-CaMKII-eGFP was injected into the STN. **(B)** Image of the STN showing eYFP-labeled neurons and damage caused by the optical fiber. Scale bar: 500 µm. **(C)** STN neurons containing NpHR3.0-eYFP responded to yellow light stimulation (yellow lines) with an outward current in voltage-clamp mode (blue trace) and with a hyperpolarization in current-clamp mode (red trace). **(D)** The locations (blue circles) of optical implants for optogenetic inhibition. **(E)** Mice receiving AAV-CaMKII-NpHR3.0-eYFP or AAV-CaMKII-eGFP injection in the STN were subjected to 6-OHDA or saline injection in the medial forebrain bundle (MFB). **(F)** In 6-OHDA-lesioned mice, yellow light illumination of STN neurons did not alter mobility in mice with either NpHR ($t = 0.54$, $P = 0.62$, $n = 9$, paired t-test) or eGFP ($t = 1.94$, $P = 0.12$, $n = 7$, paired t-test) viral injection. Mice receiving a saline injection in the MFB were divided into two groups: those injected in the STN with AAV-CaMKII-NpHR3.0-eYFP (STN CaMKII-NpHR) and those injected in the STN with AAV-CaMKII-eGFP (STN CaMKII-eGFP). In STN CaMKII-NpHR mice, yellow light stimulation in the STN (yellow bars) changed

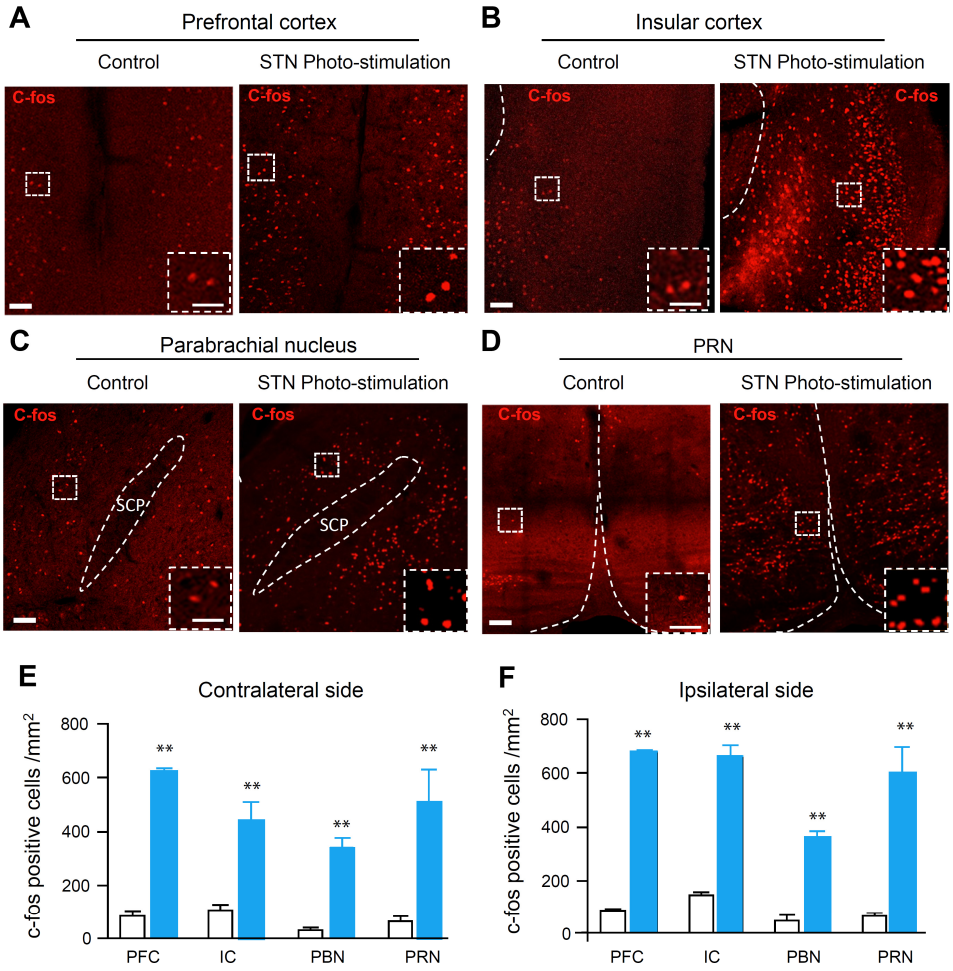
neither the mechanical (**G**; Contralateral: n = 9, t = -1.48, P = 0.18, paired t-test; Ipsilateral: n = 9, t = 0.97, P = 0.38, paired t-test) nor the thermal (**H**; Contralateral: n = 9, t = -0.37, P = 0.72, paired t-test; Ipsilateral: n = 9, t = 1.35, P = 0.22, paired t-test) pain threshold. In STN CaMKII-eYFP mice, yellow light stimulation in the STN changed neither the mechanical **(I**; Contralateral: n = 7, t = -1.1, P = 0.33, paired t-test; Ipsilateral: n = 9, t = -1.27, P = 0.25, paired t-test) nor the thermal (**J**; Contralateral: n = 7, t = 0.33, P = 0.75, paired t-test; Ipsilateral: n = 7, t = -0.06, P = 0.95, paired t-test) pain threshold. n.s, not significant.

Figure S4



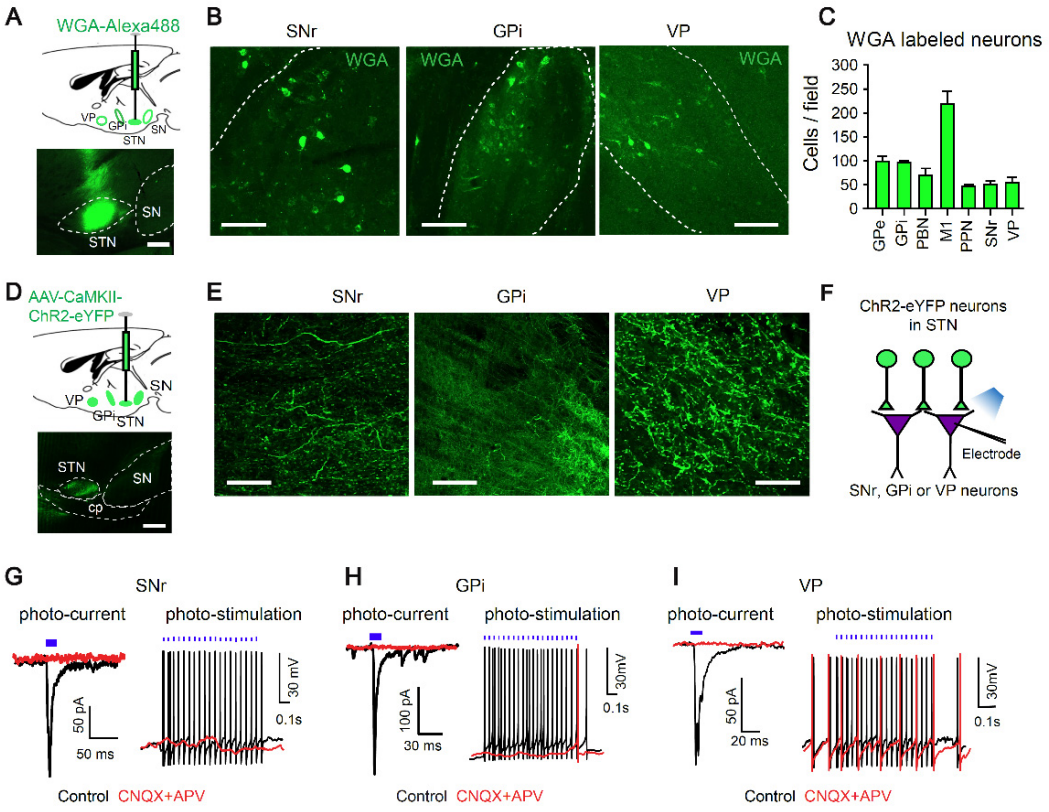
Optogenetic stimulation of the STN does not affect mobility. **(A)** AAV2-CaMKII-ChR2-eYFP or AAV2-CaMKII-eGFP was injected into the STN and an optical fiber was implanted in the STN. **(B)** The estimated location of optical fibers in the STN from 12 ChR2 mice and 8 eGFP mice. Blue light illumination of STN neurons (blue bars) did not change the mobility of mice receiving STN injection of AAV2-CaMKII-ChR2-eYFP (STN-ChR2) **(C)** (n = 12, t = 0.96, P = 0.36, paired t-test) or AAV2-CaMKII-eGFP (STN-eGFP) **(D)** (n = 8, t = 0.96, P = 0.36, paired t-test). n.s, not significant.

Figure S5



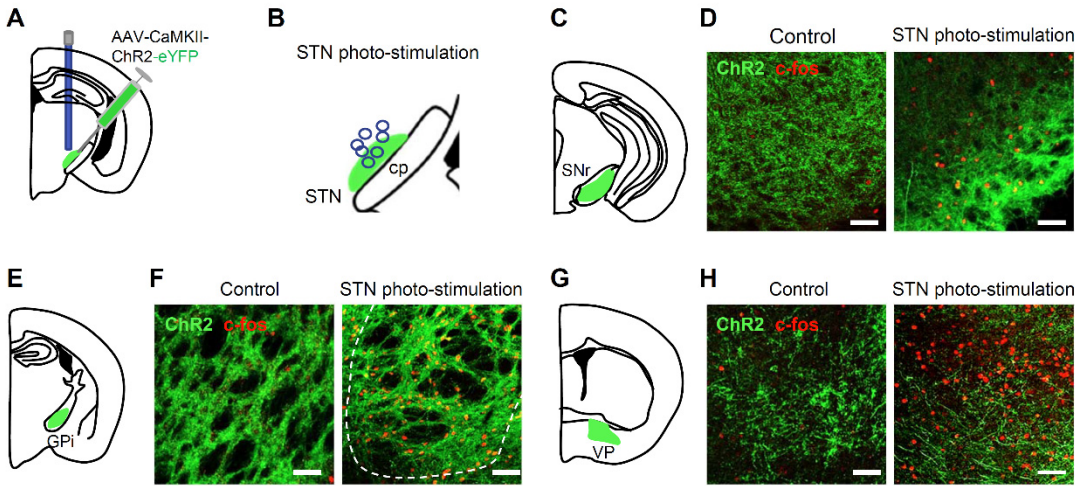
Optogenetic stimulation of the STN causes hyperactivity in pain pathways. Mice receiving an AAV-CaMKII-ChR2-eYFP injection in the STN (ChR2 mice) were divided into two groups: no light stimulation and blue light stimulation in the STN (2 min, 20 Hz, 10 ms light pulses, 4 mW, separated by 2 min, for 30 min). One hour later, mice were sacrificed for c-fos staining. Optogenetic stimulation of the STN increased the number of c-fos-positive neurons in the prefrontal cortex (PFC) **(A)**, the insular cortex (IC) **(B)**, the parabrachial nucleus (PBN) **(C)**, and the pontine reticular nucleus (PRN) **(D)** in both contralateral **(E)** and ipsilateral **(F)** hemispheres. n = 4 mice, ** $P < 0.01$, comparison between ChR2 mice subjected to no light and ChR2 mice subjected to blue light stimulation in the STN. **(E)** (PFC: $t = -65.9$, $P < 0.001$, student's t-test; IC: $t = -5.4$, $P = 0.01$, student's t-test; PBN: $t = -17.5$, $P < 0.001$, student's t-test; PRN: $t = 5.5$, $P = 0.01$, student's t-test); **(F)** (PFC: $t = -120.9$, $P < 0.001$, student's t-test; IC: $t = -13.4$, $P < 0.001$, student's t-test; PBN: $t = -8.7$, $P = 0.003$, student's t-test; PRN: $t = -7.5$, $P = 0.004$, student's t-test). Scale bar: 100 µm in images; 50 µm in insets.

Figure S6



Morphological and electrophysiological verification of synaptic projections from STN neurons to downstream nuclei. **(A)** Alexa-488-conjugated wheat germ agglutinin (WGA) was injected into the STN (upper panel). A representative image showing the location of the injection site (lower panel). **(B)** Alexa-488-conjugated WGA was found in the SNr (left panel), GPi (middle panel), and VP (right panel). **(C)** Summary of WGA-labeled neurons in several brain regions (12 sections from 4 mice, 3 from each). **(D)** AAV-CaMKII-ChR2-eYFP was injected into the STN and the mice were allowed to recover for six weeks. **(E)** ChR2-eYFP-labeled STN axonal fibers were found in the SNr, GPi, and VP. **(F)** Brain slice patch-clamp recordings were performed on neurons in the SNr, GPi, and VP. STN terminals in these regions were optogenetically stimulated with blue light (10 ms, 2.5 mW, single pulse or 1 s pulses at 20 Hz). Blue light stimulation evoked inward currents and increased the firing rate in SNr **(G)**, GPi **(H)**, and VP **(I)** neurons (black traces). These responses were blocked by application of 20 μM CNQX and 50 μM APV (red traces). GPe: globus pallidus externa; GPi: globus pallidus interna; M1: motor cortex; PBN: parabrachial nucleus; PPN: pedunculopontine tegmental nucleus; SNr substantia nigra pars reticulata; VP: ventral pallidum. Scale bar in images: 100 μm.

Figure S7



Optogenetic stimulation of STN neurons increases activity in downstream nuclei. (A) AAV-CaMKII-ChR2-eYFP was injected into the STN (coronal section). **(B)** The location of the tip of the optical implants in mice. Mice received blue light stimulation in the STN (2 min, 20 Hz, 10 ms light pulses, separated by 2 min, for 30 min). One hour later, mice were sacrificed for c-fos staining. In the SNr **(C)**, photo-stimulation of the STN increased the number of c-fos-positive neurons (**D**, right panel) relative to mice not subjected to blue light stimulation (**D**, left panel). Similar results were observed in the GPi **(E, F)** and the VP **(G, H)**. Scale bar in images: 100 µm.

Figure S8

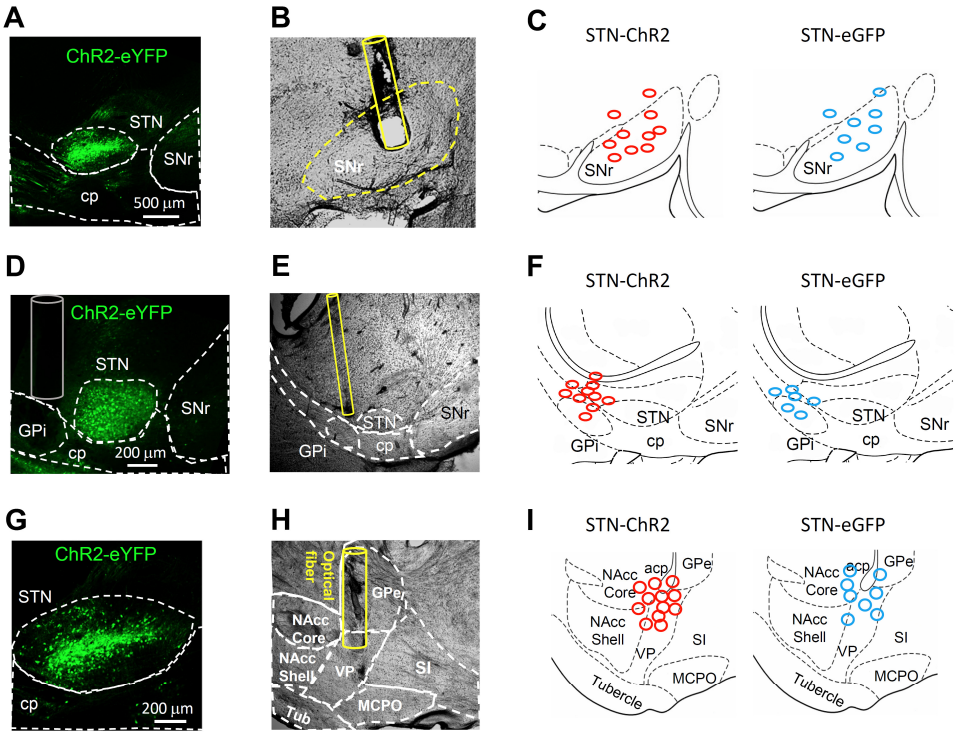


Photo-stimulation of STN projections. **(A, D, G)** AAV-CaMKII-ChR2-eYFP was injected into the STN of the right hemisphere, and an optical implant was inserted above or into the SNr **(B)**, GPi **(E)**, or VP **(H)**. **(C, F, I)** The estimated locations of the tip centers of the optical implants.

Figure S9

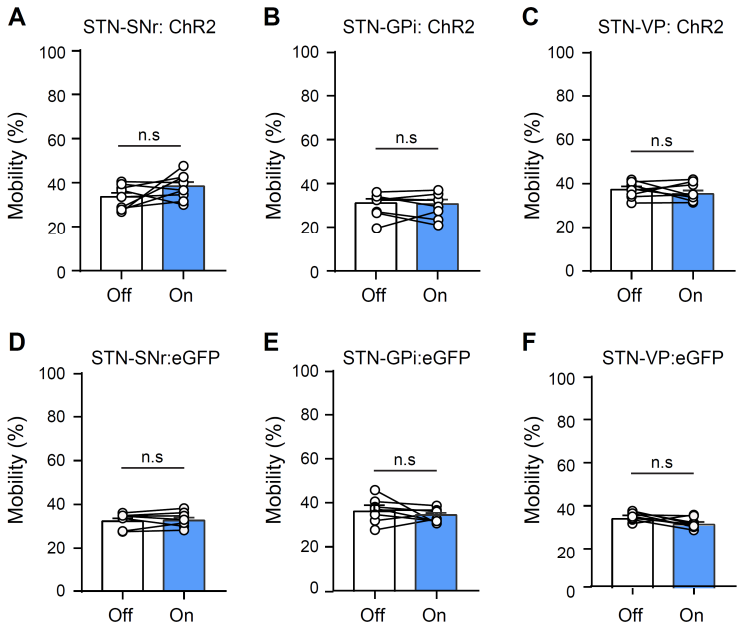
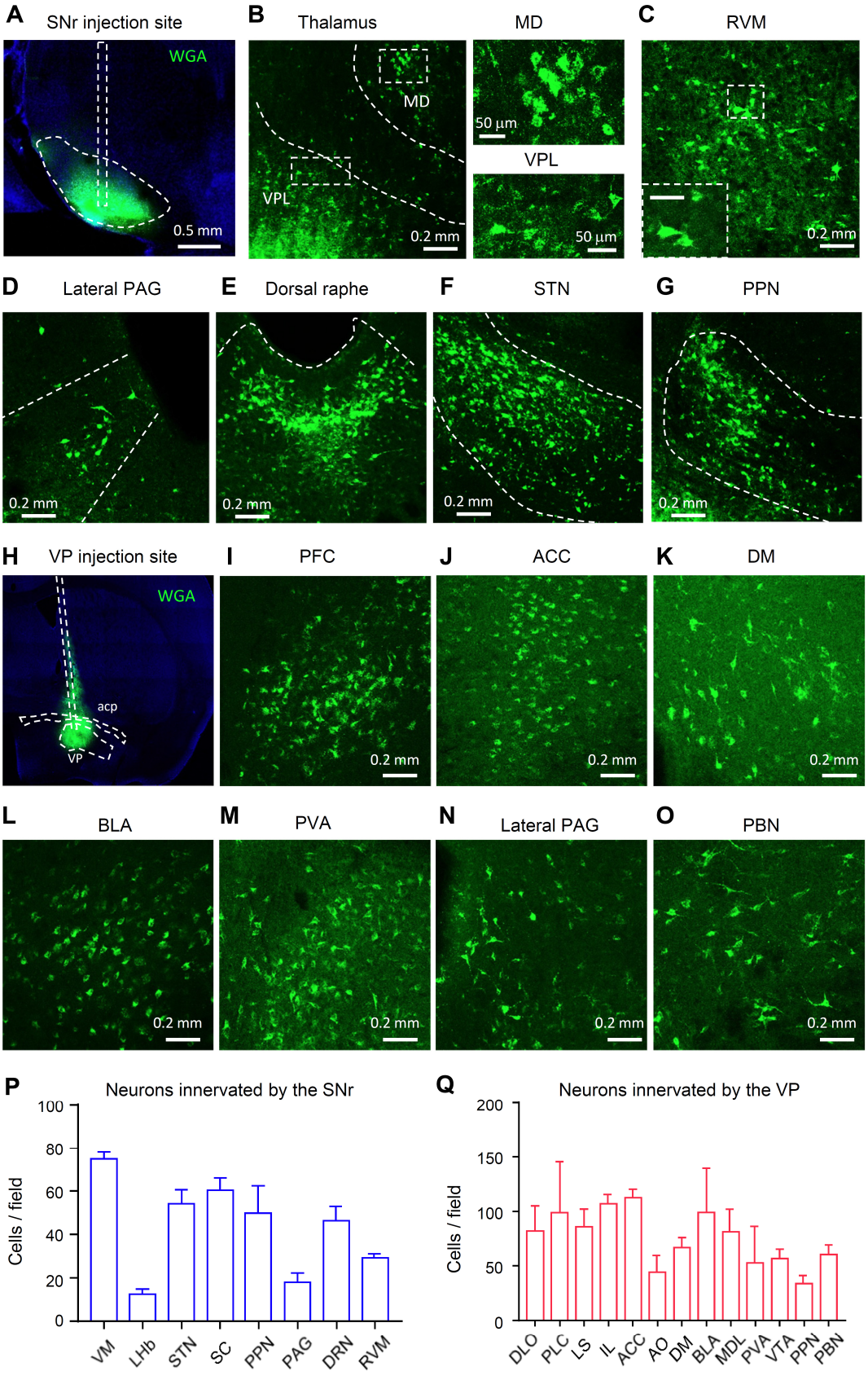


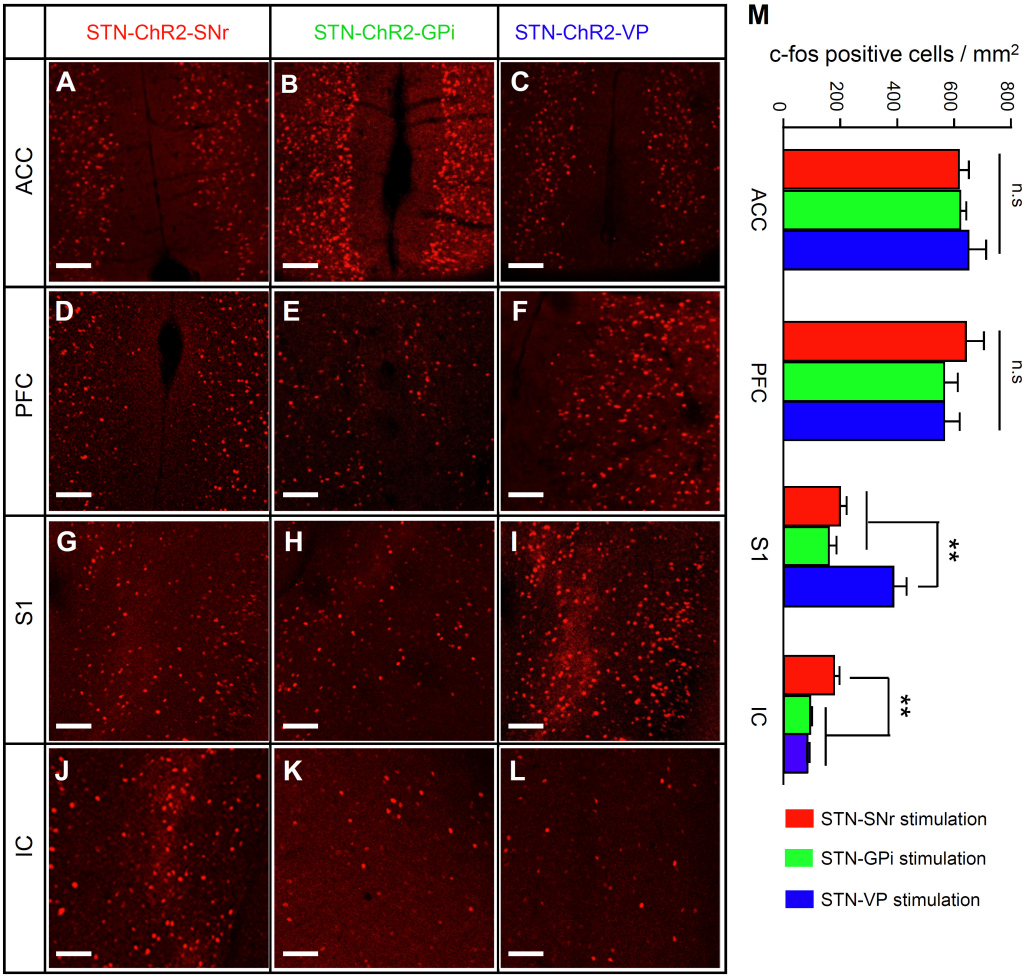
Photo-stimulation of STN projections does not alter mobility. Mobility was measured in the chamber used for testing pain thresholds. ChR2 groups: AAV2-CaMKII-ChR2-eYFP was injected into the STN **(A, B, C)**. eGFP groups: AAV2-CaMKII-eGFP was injected into the STN **(D, E, F)**. STN–SNr, STN–GPi, and STN–VP indicate that optical fibers were implanted in the SNr, GPi, or VP (ipsilateral to virus injection), respectively. Blue bars represent mobility rates measured during light illumination. **(A)** n = 9, t = 1.67, P = 0.13; **(B)** n = 8, t = 0.26, P = 0.8; **(C)** n = 7, t = 1.24, P = 0.26; **(D)** n = 7, t = 0.38, P = 0.72; **(E)** n = 8, t = 1.12, P = 0.3; **(F)** n = 8, t = 2.59, P = 0.06; Paired t-test in **A – F**. n.s, not significant.

Figure S10


A
SNr injection site
WGA
0.5 mm
B
Thalamus
MD
VPL
0.2 mm
MD
50 μm
VPL
50 μm
C
RVM
0.2 mm
D
Lateral PAG
0.2 mm
E
Dorsal raphe
0.2 mm
F
STN
0.2 mm
G
PPN
0.2 mm
H
VP injection site
WGA
acp
VP
I
PFC
0.2 mm
J
ACC
0.2 mm
K
DM
0.2 mm
L
BLA
0.2 mm
M
PVA
0.2 mm
N
Lateral PAG
0.2 mm
O
PBN
0.2 mm
P
Neurons innervated by the SNr
Cells / field
100
80
60
40
20
0
VM
LHb
STN
SC
PPN
PAG
DRN
RVM
Q
Neurons innervated by the VP
Cells / field
200
150
100
50
0
DLO
PLC
LS
IL
ACC
AO
DM
BLA
MDL
PVA
VTA
PPN
PBN

Trans-synaptic tracing of neurons innervated by the STN and VP. WGA, conjugated with Alexa 488, was injected in the SNr **(A)** (n = 3) or the VP **(H)** (n = 3). 48 h later, serial sections were prepared, and WGA-labeled neurons were detected in several downstream nuclei. Typical images show that the SNr neurons form synaptic connections with the thalamus **(B)**, RVM **(C)**, PAG **(D)**, dorsal raphe **(E)**, STN **(F)**, PPN **(G)**, etc; the VP innervates the PFC **(I)**, ACC **(J)**, DM **(K)**, BLA **(L)**, PVA **(M)**, lateral PAG **(N)**, and PBN **(O)**. The numbers of labeled neurons are summarized in **(P)** and **(Q)**. Cells in a field ($1271 \times 1271\ \mu m^2$) taken under a 10X objective were counted. ACC: anterior cingulate cortex; AO, anterior olfactory cortex; BLA: basal lateral amygdala; DLO: dorsolateral orbital cortex; DM: dorsomedial hypothalamus; DRN: dorsal raphe nucleus; IL: infralimbic cortex; LHb: lateral habenula; LS: lateral septum; MDL: mediodorsal lateral thalamic nucleus; PAG: periaqueductal gray; PBN: parabrachial nucleus; PLC: prelimbic cortex; PPN: pedunculopontine tegmental nucleus; PVA: periventricular thalamic nucleus, anterior; RVM: Rostroventral medulla; SC: superior colliculus; STN: subthalamic nucleus; VM: ventromedial thalamic nucleus; VTA: ventral tegmental area.

Figure S11



Optogenetic stimulation of STN terminals causes hyperactivity in pain pathways. In mice receiving AAV-CaMKII-ChR2-eYFP injection in the STN (STN-ChR2 mice), optical implants were inserted into the SNr, GPi, or VP. In each group, 4 mice received no light stimulation, and 4 mice received blue light stimulation (2 min, 20 Hz, 10 ms light pulses, separated by 2 min, for 30 min) through optical implants. One hour later, mice were sacrificed for c-fos staining. Optogenetic stimulation of terminals of STN neurons in the SNr (STN-ChR2-SNr), GPi (STN-ChR2-GPi), and VP (STN-ChR2-VP) increased the numbers of c-fos-positive neurons in the cingulate cortex (ACC) **(A, B, C)**, prefrontal cortex (PFC) **(D, E, F)**, primary somatosensory cortex (S1) **(G, H, I)**, insular cortex (IC) **(J, K, L)**. **(M)** Summary of c-fos staining. For each bar, the number of c-fos-positive neurons was calculated as the number of neurons in mice that received light stimulation (n = 4) minus the number of neurons in mice that did not receive light stimulation (n = 4). The data among groups were compared with a one-way ANOVA followed, if significant, by the Holm–Sidak method for pairwise comparison (ACC: $F = 0.18$, $P = 0.84$) (PFC: $F = 0.59$, $P = 0.57$) (S1: $F = 12.76$, $P = 0.02$; SNr vs. GPi, $P = 0.68$; SNr vs. VP, $P = 0.009$; GPi vs. VP, $P = 0.003$) (IC: $F = 16.90$, $P = 0.02$; SNr vs. GPi, $P = 0.003$; SNr vs. VP, $P = 0.001$; GPi vs. VP, $P = 0.53$). ** $P < 0.01$.

Figure S12

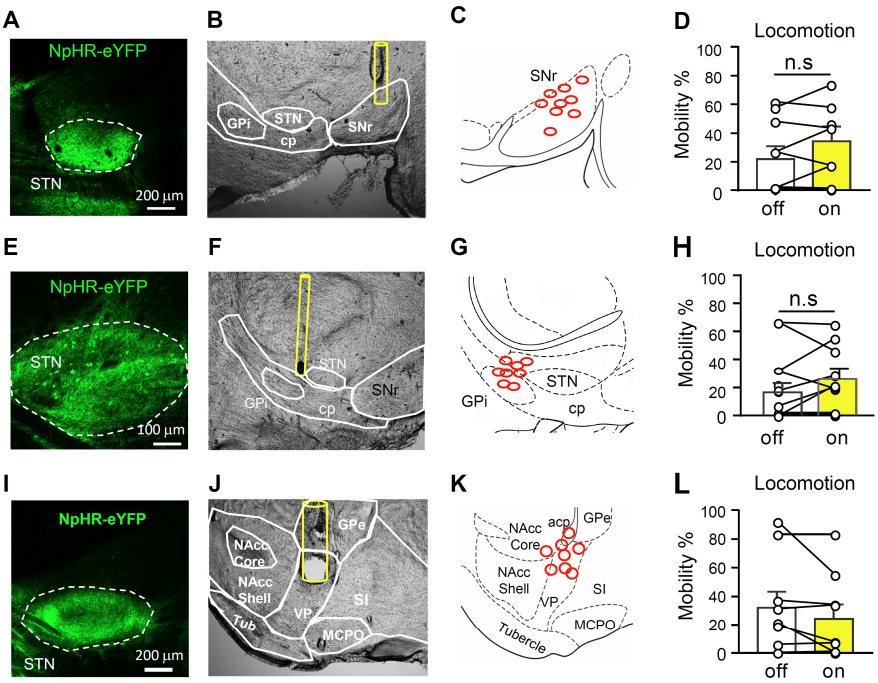


Photo-inhibition of STN projections. (A, E, I) AAV-CaMKII-NpHR3.0-eYFP was injected into the STN, and an optical fiber was implanted in or above the SNr **(B)**, GPi **(F)**, or VP **(J)** (in bright field images). **(D, G, K)** The estimated locations of the tips of the optical implants. Photo-inhibition of STN–SNr **(D)**, STN-GPi **(H)**, and STN-VP **(L)** projections did not alter the mobility of the mice in the apparatus. **(D)** n = 9, t = 0.99, P = 0.34. **(H)** n = 10, t = 0.94, P = 0.36). **(L)** n = 8, t = 0.74, P = 0.47. n.s: not statistically significant, light on vs light off, paired t-test.

Table S1

6-OHDA lesion of SNc DA neurons in experiments

Figure panels	Number of mice	% lesion	Paired t-test (lesion vs non-lesion)
Figure 1D 7 days	n = 7	48.0 ± 3.3	t = 14.4, P < 0.001
14 days	n = 7	53.6 ± 3.4	t = 15.6, P < 0.001
25 days	n = 9	54.3 ± 5.6	t = 9.7, P < 0.001
Figure 1E, F, G 30 days	n = 6	50.9 ± 5.4	t = 6.83, P = 0.01
Figure 1H, 1I	n = 6	52.0 ± 3.4	t = 10.1, P = 0.0002
Fig. 2A, 2B, 2C	n = 3	57.4 ± 7.0	t = 6.12, P = 0.03
Figure 2G, 2H	n = 7	49.1 ± 6.1	t = 7.13, P < 0.001
Figure 2I, 2J	n = 8	56.6 ± 4.3	t = 6.50, P = 0.0002
Figure 3	n = 3	62.3 ± 5.1	t = 26.5, P = 0.001
Figure 6B, 6C, 6D	n = 7	71.2 ± 6.7	t = 2.54, P = 0.02
Figure 6F, 6G, 6H	n = 6	60.2 ± 5.1	t = 4.26, P = 0.008
Figure 6J, 6K, 6L	n = 9	51.9 ± 6.8	t = 4.58, P = 0.004

The table lists % lesion of SNc DA neurons in each experiment mentioned in the main figures. % lesion = 100% * (1 - (TH+ neurons in ipsilesional SNc / TH+ neurons in contralesional SNc)). Paired t-tests were used to compare the number of DA neurons in ipsilesional vs contralesional SNc.

SI References

1. C. Xiao *et al.*, Cholinergic Mesopontine Signals Govern Locomotion and Reward through Dissociable Midbrain Pathways. *Neuron* **90**, 333-347 (2016).
2. C. Xiao *et al.*, Nicotinic Receptor Subtype-Selective Circuit Patterns in the Subthalamic Nucleus. *J Neurosci* **35**, 3734-3746 (2015).
3. C. Zhou *et al.*, Bidirectional dopamine modulation of excitatory and inhibitory synaptic inputs to subthalamic neuron subsets containing alpha4beta2 or alpha7 nAChRs. *Neuropharmacology* **148**, 220-228 (2019).
4. L. P. Noldus, A. J. Spink, R. A. Tegelenbosch, EthoVision: a versatile video tracking system for automation of behavioral experiments. *Behav Res Methods Instrum Comput* **33**, 398-414 (2001).
5. V. Bouet *et al.*, The adhesive removal test: a sensitive method to assess sensorimotor deficits in mice. *Nat Protoc* **4**, 1560-1564 (2009).
6. C. Zhou, Z. D. Luo, Nerve injury-induced calcium channel alpha-2-delta-1 protein dysregulation leads to increased pre-synaptic excitatory input into deep dorsal horn neurons and neuropathic allodynia. *Eur J Pain* **19**, 1267-1276 (2015).
7. W. J. Dixon, Efficient analysis of experimental observations. *Annu Rev Pharmacol Toxicol* **20**, 441-462 (1980).