Supplemental Experimental Procedures

Supplementary Surgical Protocol:

1) Induce a surgical plane of anesthesia, and confirm that the animal remains in this state throughout the procedure through periodic assessment.
2) Place animal in a spinal stereotax (#51690, Stoelting). Secure spinal fixation bars to the stereotax, but retract them to the side of the animal. Prepare the needle/cannula holder, and retract it such that it does not interfere with the procedure.
3) Shave the region surrounding the dorsal hump, and remove all hair from the surgery area. Drape areas of the animal surrounding the shaved region to prevent contamination of the surgical site.
4) Sterilize the incision location.
5) Subcutaneously infiltrate the incision site with local anesthetic prior to the incision.
6) For implantations at spinal segment L3 or L4, make an incision starting slightly caudal of the peak of the dorsal hump, extending approximately 0.5 cm rostral and 0.5 cm caudal from the initial incision site. Larger incisions can be performed initially to allow for greater access. The rostro-caudal position of the incision site can be changed to target different dermatomes (Figure S1a).
7) Make an incision medial to the white tendons running on either side of the vertebral column, spanning slightly more than one vertebral segment, from the outer edge of the caudal transverse process to outer edge of the rostral transverse process. Next, cut the tendon attached to the caudal transverse process, controlling bleeding using absorption spears (Sugi). During this step, clear as much tissue from the transverse processes as possible, while being careful not to damage any nerves entering the spinal cord. Repeat this process on both sides of the spinal column (Figure S1b).
8) Secure one side of the spinal adapter in position. Use curved forceps to grip under the caudal transverse process and gently lift the vertebral column such that the clamp is positioned at the level of the smaller transverse processes in the middle of the vertebra and position the column against the clamp. Use your other hand to move the second clamp into place, and apply moderate pressure to secure the column within the clamps. **It is exceptionally important that the surface of the vertebra be raised from the clamps, as we will later wrap dental cement around the edges of the bone.** Clamping should be done such that minimal tendon and soft tissue is caught between the clamps and the vertebra (Figure S1c).
9) Using a dissecting microscope, make an incision perpendicular to the long axis of the spinal cord, above and below the dorsal process, ensuring that tissue still covers the intervertebral space.

10) Remove superficial tissue from the bone with forceps and spring scissors. Also remove tissue from the raised sides of the vertebra. **The tissue should be completely cleared, and the bone should be dry.** Use absorbent spears (Sugi) to control bleeding (Figure S1d).
11) Use a fine tipped burr drill (~ 0.5 mm in diameter) to flatten the dorsal spinous process, and also lightly abrade the surface of the surrounding bone to remove any remaining soft tissue. It is important to remove all sediment generated from the drilling process to ensure that later applications of glue and dental cement adhere to the bone (Figure S1d).
12) If an intra-spinal viral injection is to be performed in the animal to induce opsin expression, it should be done through this burr hole, prior to implantation. To target dorsal horn neurons, puncture the dura mater, initially insert the needle tip ~ 0.5 mm into the cord, and then retract it to a depth of 0.25 mm. Refer to the Methods section for detailed injection parameters.
13) Once the injection needle is removed and all blood or CSF is cleared from the injection site, prepare a 1.25 mm diameter cannula with a 200 um fiber optic core. Cut the fiber such that only ~ 0.25 mm remains.
14) Coat the bone and area around the burr hole with a small amount of super glue, before lowering the cannula into place. Due to the curvature of the bone, the cannula will not be flush with the vertebra, layer super glue prior to cannula placement to fill this open space, but avoid letting the superglue enter the burr hole (Figure S1e).
15) After lowering the cannula into place, wrap the dental cement around the outside of the vertebra and build up a small mound around the cannula for mechanical stability (Figure S1f).
16) Once the dental cement is dry, remove the spinal fixation bars, apply lidocaine locally, and carprofen and buprenorphine subcutaneously.
17) Suture the skin, and allow the mouse to recover under a heating lamp, under close supervision (Figure S1g).

Supplementary Video 1: Behavioral response to optogenetic activation in mice injected with AAVDJ:CaMKIIa:ChR2-eYFP. Related to Figure 1.
Optogenetic illumination of mice expressing ChR2 in excitatory spinal cord neurons produces a combination of paw retraction and nocifensive behavior.

Supplementary Video 2: Behavioral response to optogenetic activation in mice injected with AAVDJ:ef1a:DIO:ChR2-eYFP. Related to Figure 2.
Optogenetic illumination of SOM-ChR2+ mice results in dermatomally appropriate nocifensive behavior.

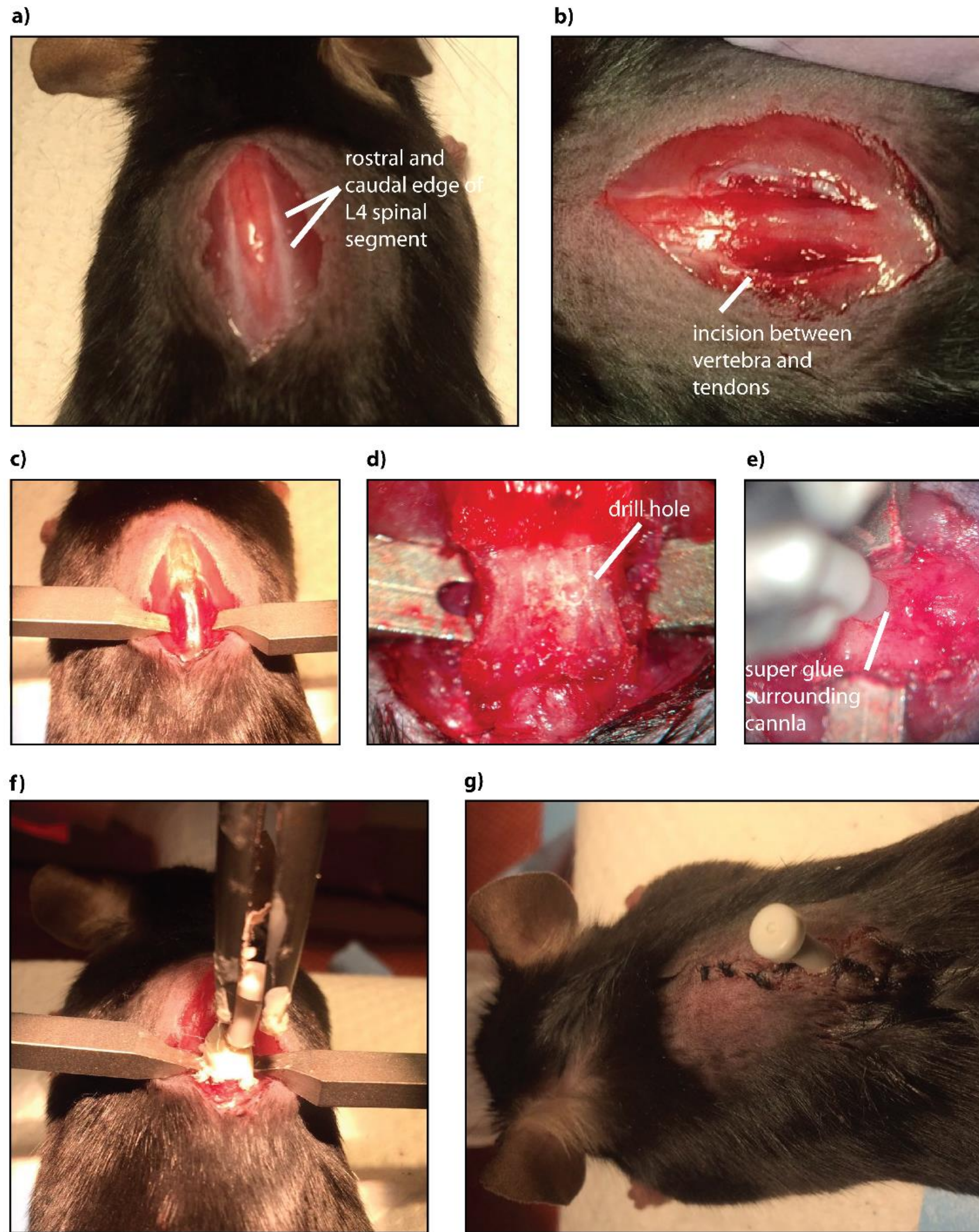

Figure S1: Cannula implantation procedure. Related to Figure 1.
(**a**) The location of incision for an implantation of spinal segment L4. **(b)** Small incisions around vertebral column to allow for spinal fixation bar placement. **(c)** The vertebral column is secured using spinal adapted bars. **(d)** All tissue is cleared from vertebra, and a hole drilled for access to cord. **(e)** The cannula is secured in place with super glue. **(f)** Dental cement is used to stabilize the cannula. **(g)** Incisions are sutured and mouse is removed from the stereotax.

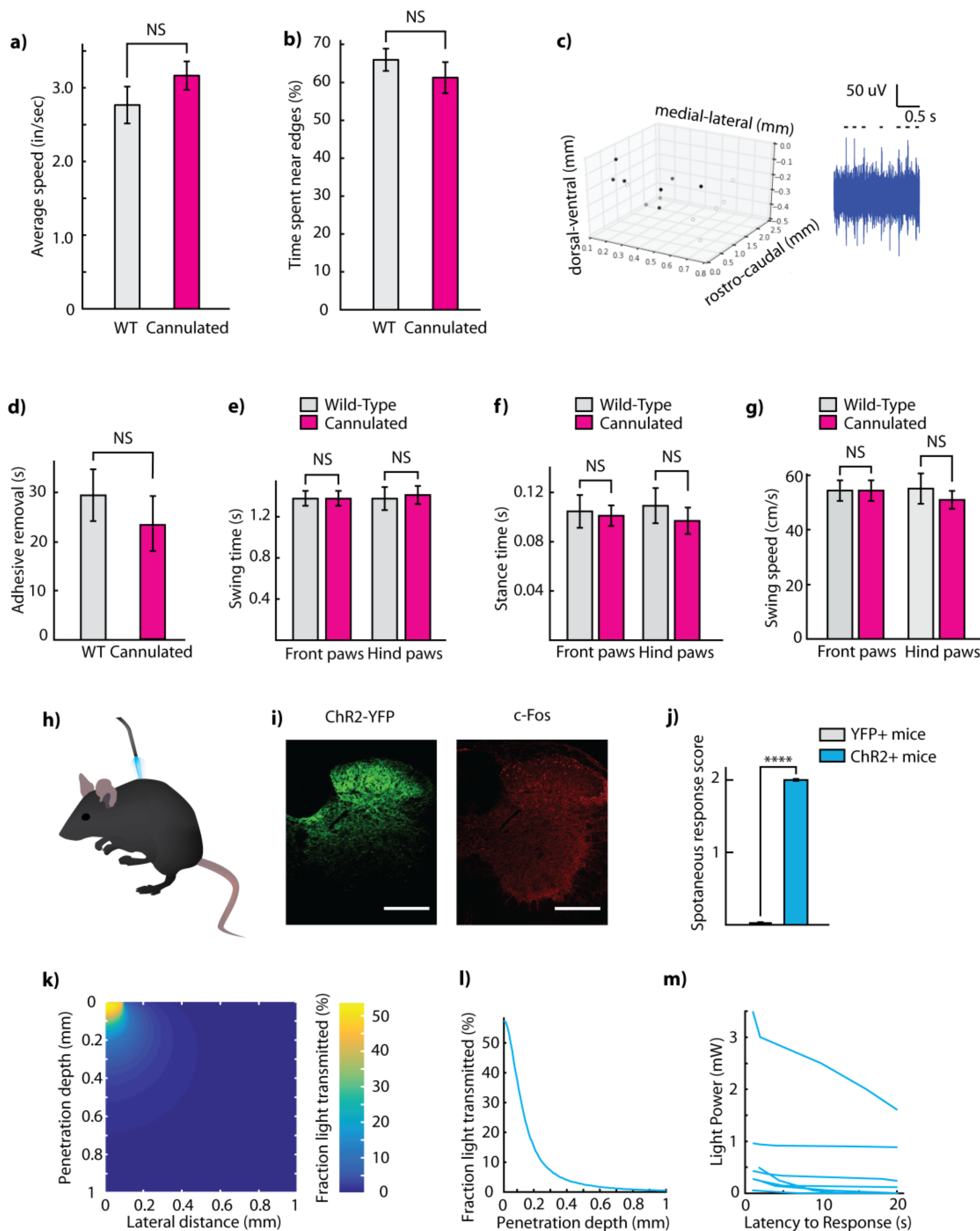

Figure S2: Characterization of cannulated mice. Related to Figure 1.
(a) Average speed on an open field test of wild type and cannulated mice (n = 10 control, 10 implanted. P = 0.108). **(b)** Time spent near the edge of an open field arena of wild type and cannulated mice (n = 10 control, 10 implanted. P = 0.206). **(c)** Location of single units with receptive fields on the plantar surface of the ipsilateral hindpaw (closed circles), or located receptive fields elsewhere on the mouse skin (open circles). (0, 0, 0)

is the intersection between spinal levels L3 and L4 at the midline (n = 19 neurons from 1 mouse). An example trace showing response of dorsal horn neurons to mechanical tapping applied to the plantar surface of the ipsilateral hindpaw. **(d)** Sensorimotor coordination of cannulated and wild type mice, as measured on the sticky tape removal test (n = 10 implanted, 10 wild type. P = 0.248). **(e)** Swing time of ipsilateral vs. contralateral paws of implanted mice (n = 5, P = 0.48). **(f)** Stance time on ipsilateral vs. contralateral hind paws of implanted mice (n = 5, P = 0.403). **(g)** Swing speed of ipsilateral vs. contralateral hind paws of implanted mice (n = 5, P = 0.1607). **(h)** Schematic of mouse with implanted cannula and attached fiber optic cable. **(i)** Representative image of ChR2 expression in the dorsal horn and c-Fos expression after stimulation in mice injected intraspinally with AAVDJ-CaMKIIa-ChR2-eYFP. c-Fos stimulation parameters were 10 minutes at 10 Hz, 20% duty cycle, 1–2 mW of total power from the fiber optic cannula. Scale bar: 250 µm. **(j)** Spontaneous response score of AAVDJ:CaMKIIa:ChR2-eYFP spinal cord injected mice vs. YFP controls. (n = 5 ChR2, 5 YFP, $P = 1.9\times10^{-5}$). **(k)** Computational modeling of light transmission through white-matter. **(l)** Fraction of light transmitted as a function of depth. **(m)** Individual SOM-ChR2 mouse results for light-intensity threshold for behavioral response.

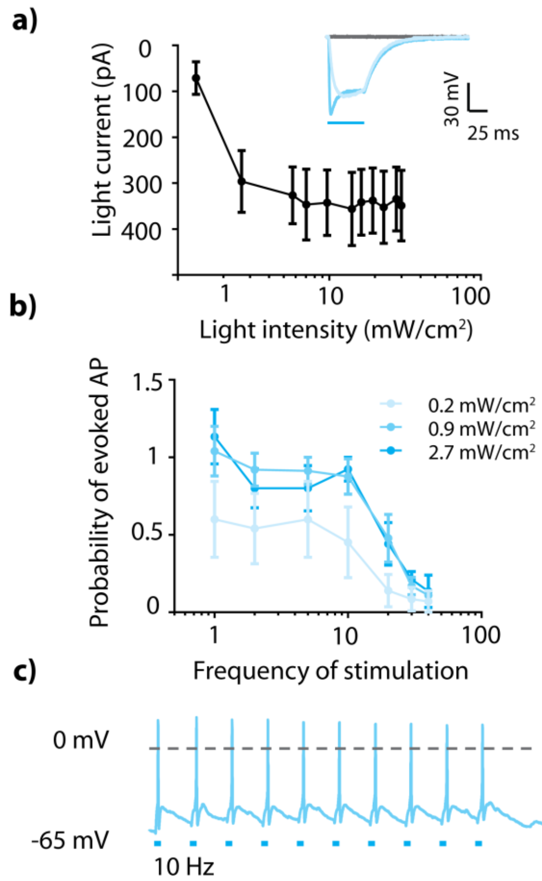

Figure S3: Electrophysiological recordings from somatostatin interneurons. Related to Figure 2.

(a) Light evoked current amplitude is dependent on light intensity (n = 10), inset: Representative traces of 50 ms light evoked current at different light intensity (grey: no light, light blue: 0.2 mW/cm^2, Dark blue: 0.9 mW/cm^2). **(b)** Light evoked action potential probability depends on the stimulation frequency (n = 10). **(c)** Representative trace of light evoked action potential at 10 Hz light stimulation.

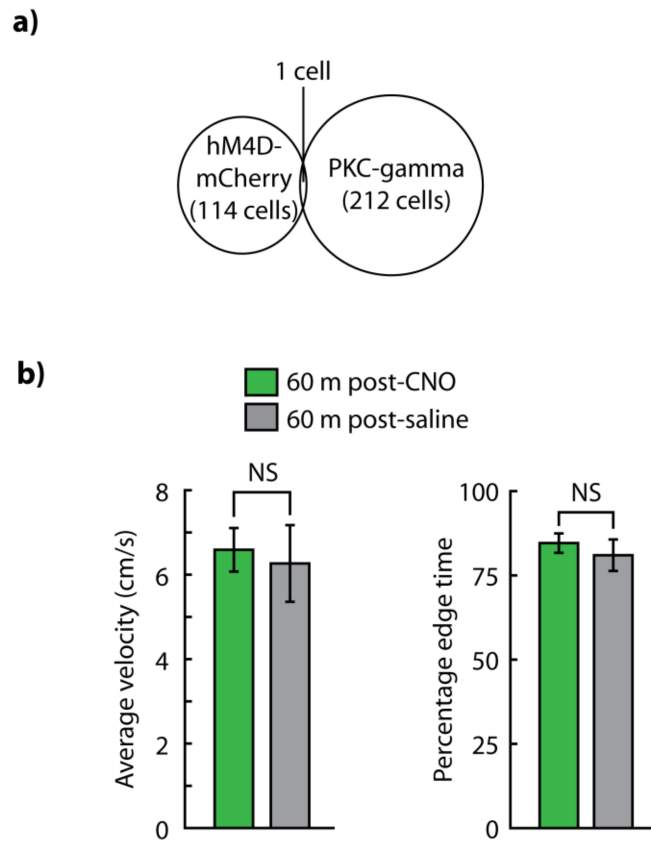

Figure S4: Chemogenetic inhibition of somatostatin interneurons. Related to Figure 2.

(a) Quantification of data from Figure 2h **(b)** Post-CNO measures of locomotion in the open-field test in hM4D+ mice. Left: average velocity (n = 5 post-CNO, n = 5 post-saline, P = 0.74). Right: time in center-zone (n = 5 post-CNO, n = 5 post-saline, P = 0.24).