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Supplemental information

Optogenetic activation of the inhibitory nigro-collicular circuit evokes contralateral orienting movements in mice

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SUPPLEMENTAL FIGURES AND LEGENDS

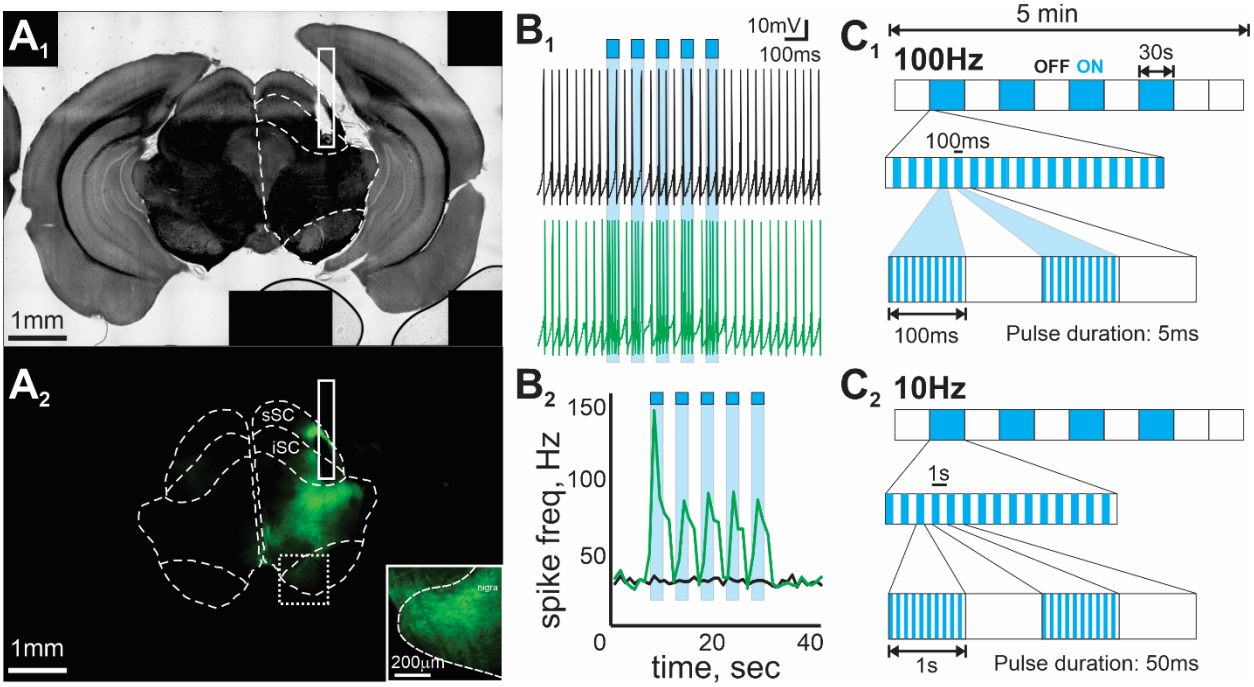

Figure S1. Optogenetic stimulation of nigral afferents in the SC. Related to Figure 1, Figure 3 and START Methods. (A$_1$) A bright-field photomicrograph of a coronal section of a brain slice obtained from a mouse after performing the behavioral recording and optogenetic stimulation. The solid white rectangle indicates the location of the optic fiber above the lateral iSC. Scale bar = 1mm. (**A$_2$**) Fluorescent micrograph (GFP) of the same slice shown in (**A$_1$**). The solid rectangle shows the location of the optic fiber. Note that this area is not green because the fiber optic probe is there. Scale bar = 1mm. Inset, Fluorescent micrograph (20X - GFP) of the section marked by the dashed square in (A$_2$). Scale bar = 200µm. (**B$_1$**) Current-clamp traces recorded from nigral neurons obtained from mice injected either with control virus or with Chronos-GFP (black and green traces, respectively). Cyan bars represent the light pulses (100ms/100Hz). (**B$_2$**) Plot depicting the absolute spike frequency obtained from the recordings shown in B$_1$. (**C**) Schematics representing the different patterns of light stimulation used during the behavioral sessions. A single 5 min session consists of a 30-sec baseline of no stimulation, followed by consecutive 30 sec periods of stimulation (light ON, cyan squares) and no stimulation (light OFF, white squares) at different frequencies. The different stimulation patterns aim to modify the number of light ON/OFF transitions, where the RD is evoked, without changing the overall length of stimulation. (**C$_1$**) For the 100Hz stimulation experiments, each of the 30s light ON period consisted of a series of 100ms stimulation followed by 100ms without light. Each 100ms stimulation consisted of a sequence of 5ms light pulses at 100Hz. During the 30s OFF periods, there was no stimulation. (**C$_2$**) For the experiments with 10Hz stimulation, during the 30s ON periods, the pattern consisted of a series of 50ms pulses at 10Hz. Each ON period was followed by an OFF period with no stimulation.

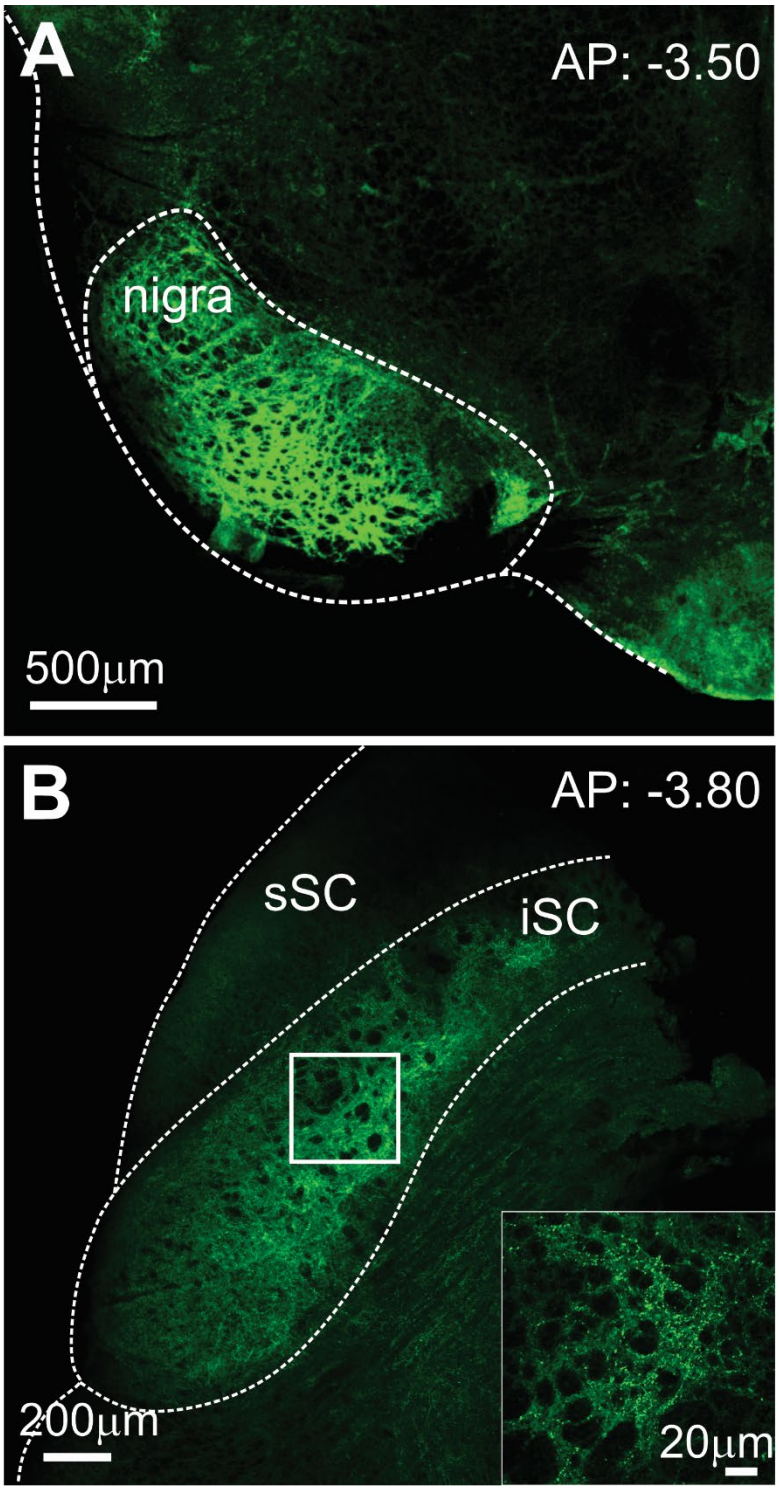

Figure S2. Distribution of the nigral terminals in the SC. Related to Figure 3. (A) Confocal fluorescent micrography of a coronal slice (AP: -3.50mm from bregma) showing the expression of the Chronos-GFP virus in the nigra. Scale bar = 500µm. (**B**) Confocal fluorescent micrograph of a coronal slice (AP: -3.80mm from bregma) showing the distribution of nigral terminals in the iSC. Scale bar = 200µm. The solid square shows the region within the SC where the 40x high magnification image in the inset was obtained. Scale bar is 20µm. iSC is intermediate/deep layers of SC and sSC indicated superficial layers of the SC.

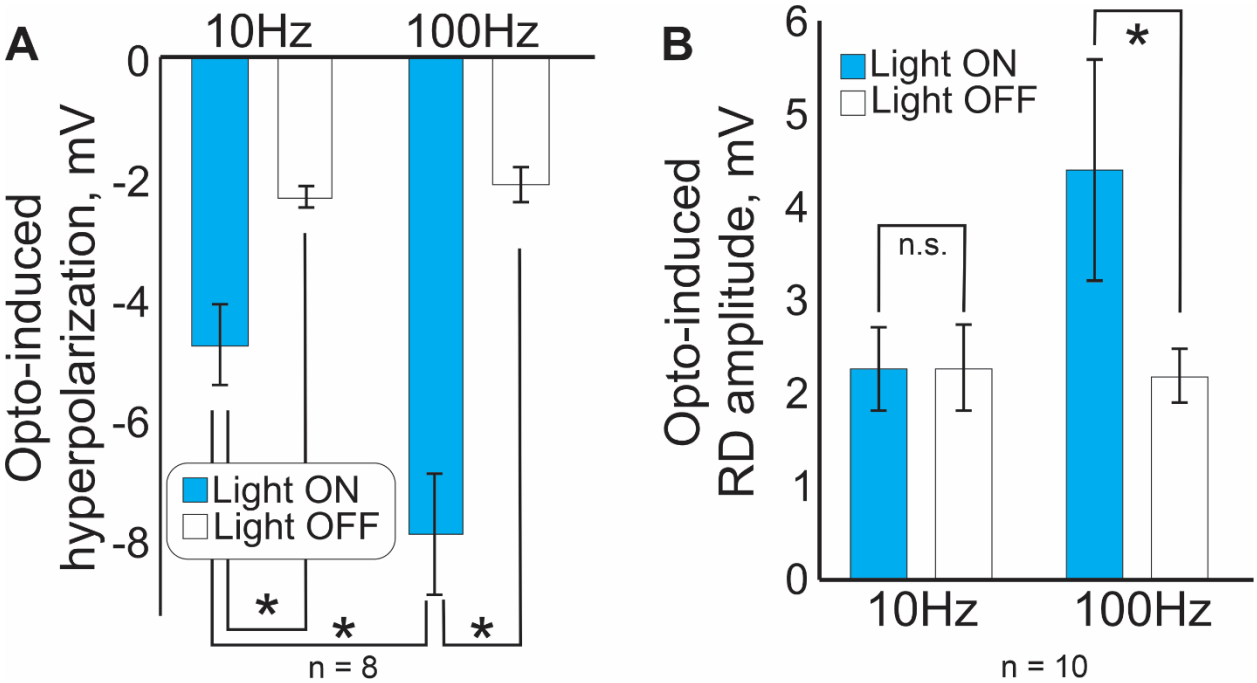

Figure S3. High-frequency (100Hz) but not low frequency (10Hz), stimulation of GABAergic nigral terminals in the SC evokes RD in PDB neurons. Related to Figure 3 and Figure 4. 100Hz frequency light stimulation evoked contralateral orienting movements whereas 10Hz stimulation did not. (**A**) Plot depicting the mean peak amplitude and SE of the hyperpolarization induced by 500ms optogenetic stimulation at either 10Hz (ON: -4.79±0.68; OFF: -2.34±0.17, n=8, paired t-test(7)=--3.45 p=0.0108) or 100Hz (ON: -7.94±1.01; OFF: -2.13±0.29, n=8, paired t-test(7)=-5.83, p=0.00025). (**B**) Plot depicting the mean amplitude and SE of the light-evoked post-inhibitory RD evoked after optogenetic stimulation of nigral terminals at 10Hz (ON:2.25±0.45; OFF:2.26±0.45, n=10, paired t-test(9)=-0015, p=0.49) and 100Hz (ON:4.36±1.00; OFF:2.17±0.29, n=10, paired t-test(9)=2.78, p=0.012). These figures show that 100Hz stimulation induced a larger hyperpolarization than 10Hz, which is capable to induce robust post-inhibitory RD in PDB neurons. The *ex vivo* results are consistent with the results shown in Figure 4 of the main text wherein we show that 100Hz but not 10Hz light stimulation of Chronos-expressing GABAergic nigral afferents in the SC results in reliable contralateral turning *in vivo*.

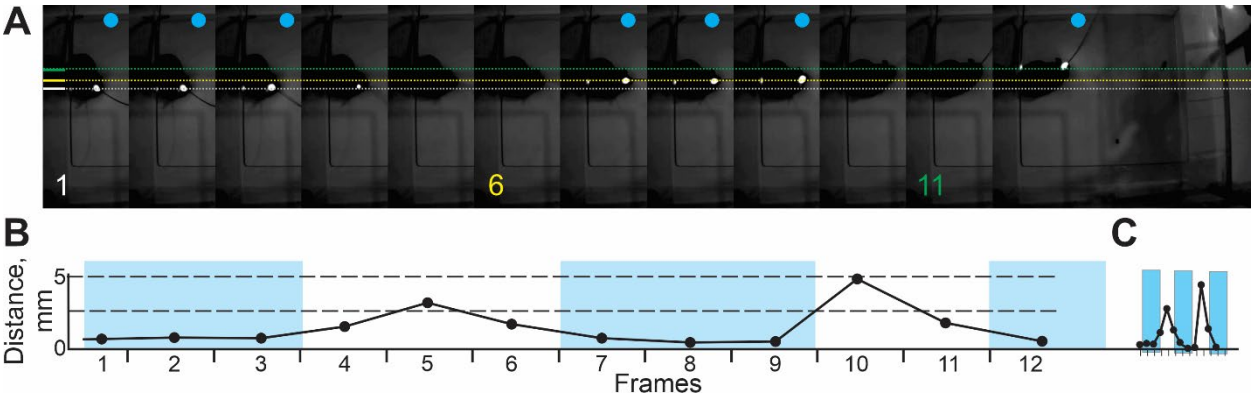

Figure S4. Orienting movements occur at the offset of the optogenetic activation of GABAergic nigral terminals in the SC. Related to Figure 4 and STAR Methods. Based on our finding that optogenetic activation of nigral afferents produces RD and RD-evoked spikes at the end of the hyperpolarization in PDB neurons *ex vivo* (Figure 3), and the unexpected finding that optogenetic activation of nigral afferents in the SC produces contralateral orienting behavior *in vivo* (Figure 1), we hypothesized that it is the post-inhibitory rebound activation and spiking of the PDB neurons that produced the orienting behavior. To test this hypothesis, we examined videos on a frame-by-frame basis to determine when the movement started relative to the onset and offset of the light stimulation. (**A**) A sequence of ~4-sec video recording during a behavioral session in the open field. Each image shows a single video fame with the frame number indicated at the bottom (1, 6, and 11). Each frame is ~33ms apart (video rate 30fps). The cyan circle in each frame shows whether the light stimulation was ON. The dotted lines mark the changes in the head position in the y-axis at the frames indicated by the color (white 1st, yellow 6th, and green 11th frame). Optogenetic stimulation occurred in the right SC. The mouse begins to turn its head contralateral to the stimulation site within one frame from the offset of the light stimulation. (**B**) Head position distance in mm (black circles) measured between consecutive frames during light pulses (cyan shadings) and no stimulation (white). (**C**) A time-compressed representation of the plot shown in (B).

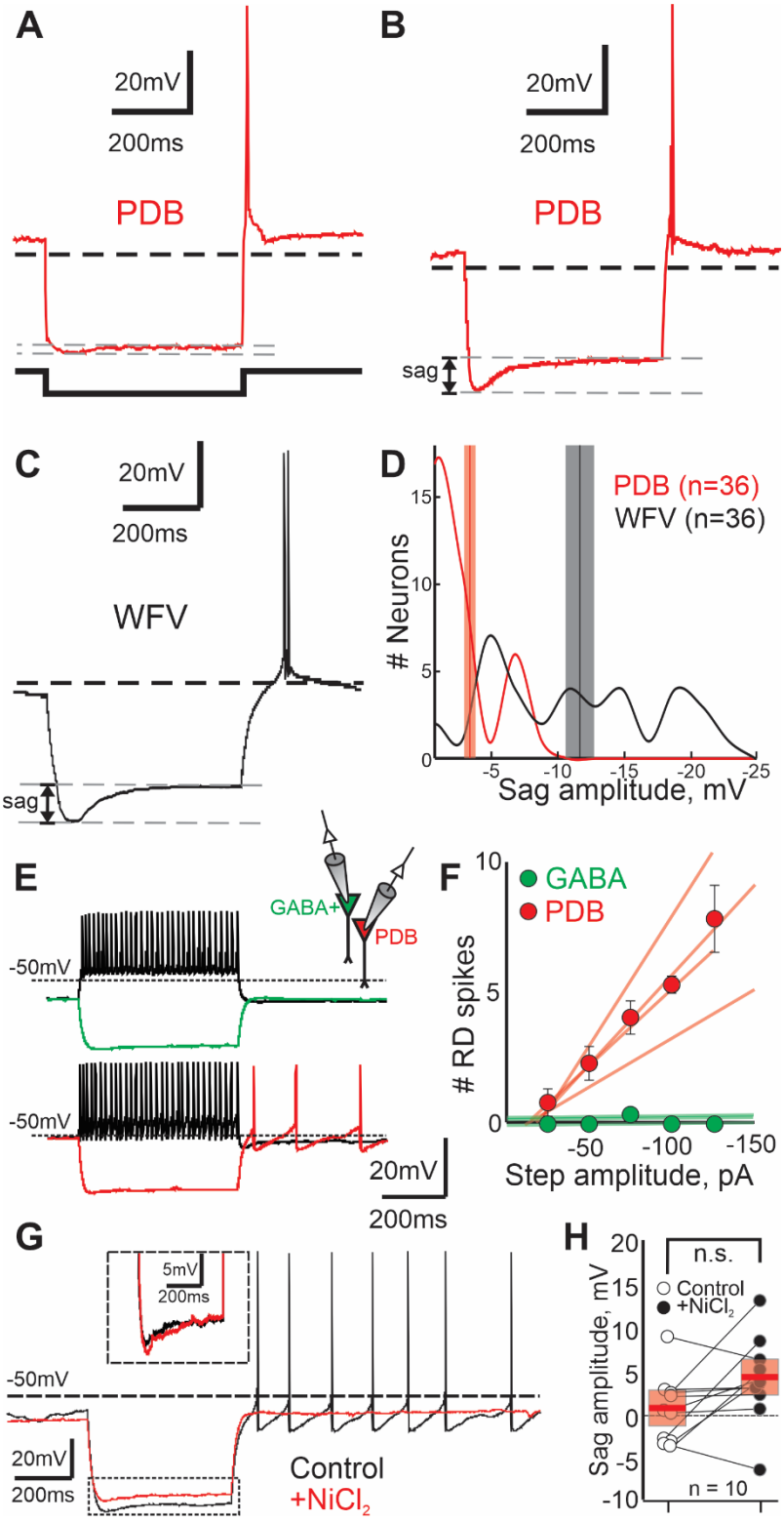

Figure S5. Related to Figure 5, Figure 6, and Figure 7. To determine the nature of the channels underlying the RD and RD-spikes in PDB neurons, we injected negative step currents into PBD neurons and measured the presence or absence of a voltage sag – a defining feature of I_h, thought to be instrumental in the development of the RD and RD-spikes. **(A)** We found that most PDB neurons showed very small to no sag voltage (red trace) upon injection of

negative step currents (black trace). **(B)** Only a small fraction of PDB neurons showed sag voltage with negative step current steps 8/37 (22%). **(C)** On the other hand, most wide-field vertical (WFV) neurons in the superficial layer of the SC present sag voltages with the same step current injections (black trace). The dashed gray lines (in A, B, and C) show the sag amplitude, measured as the difference between the voltage at the peak of the sag and the end of the negative step, after HCN channel inactivation. The black dashed lines indicate V_m = -60mV. Note that the kinetic of the RD in WFV is slower compared to PDB neurons. **(D)** The number of neurons plotted against the amplitude of the sag voltage recorded in WFV (black line) and PDB neurons (red line). The vertical lines show the mean sag amplitude, and the shaded rectangles show the means ± SEs. **(E)** Traces showing simultaneous double patch-clamp recordings from visually identified $GABA^+$ and PDB neurons (top and bottom panels) in the intermediate/deep layers of the SC. 500ms, -100pA current injections did not evoke RD or RD-spikes in GABA+ neurons (green traces) but did in PDB neurons (red trace). **(F)** Plot showing the number of post-inhibitory RD-spikes evoked by increasing negative step current injections in pairs of GABA+ and PDB+ recordings (n=4). Circles are the mean number of spikes evoked at each step current. Lines represent the linear regression calculated for the four pairs recorded. **(G)** Current clamp traces of a PDB neuron before and after application of $NiCl_2$ (black and red traces, respectively). The top inset shows time-compressed V_m traces from the dashed bottom rectangle, showing that there is no effect of $NiCl_2$ on the sag in PDB neurons. **(H)** Sag amplitude in mV from PDB neurons before and after bath application of $NiCl_2$ (n=10. Red bars, means ± SEs. n.s. = $p>0.05$, paired t-test).

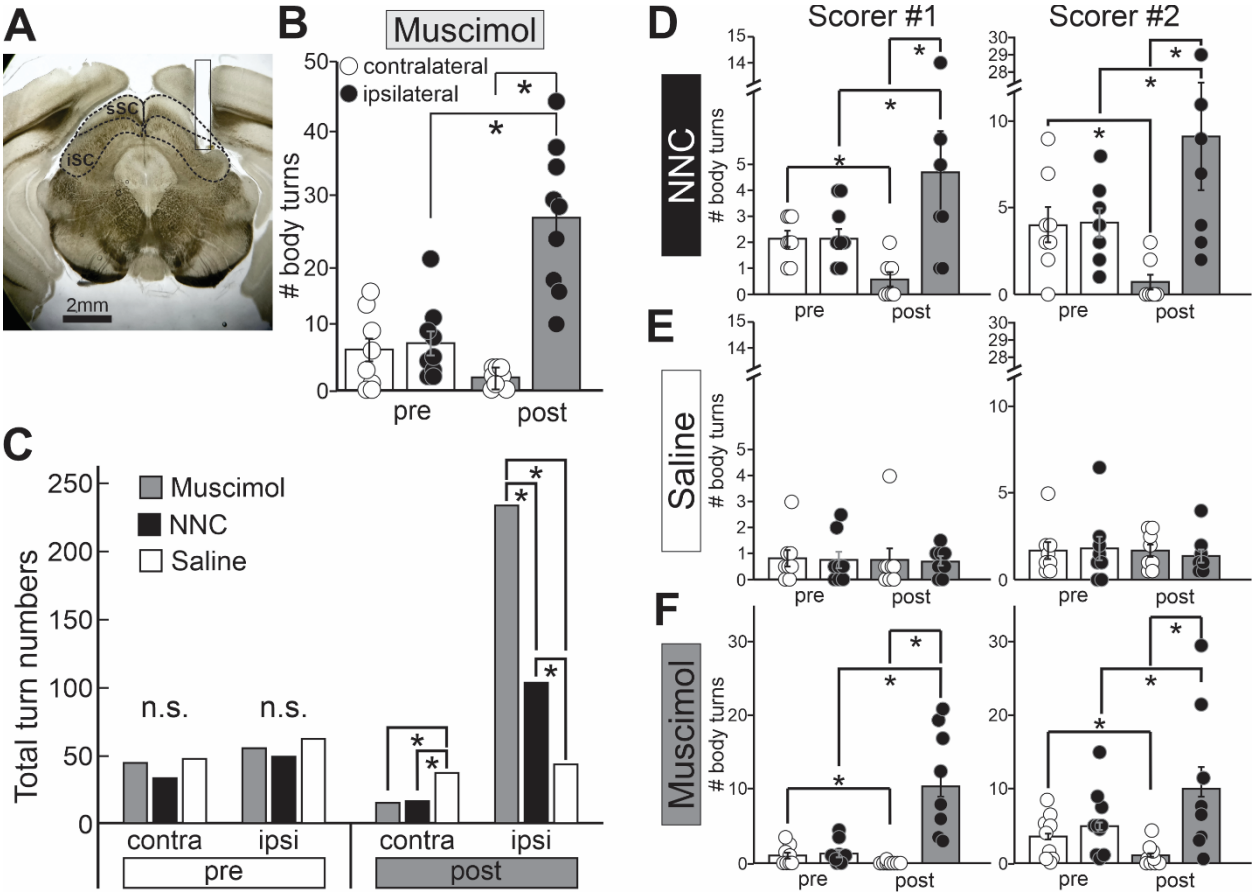

Figure S6. Related to Figure 7. (A) Micrograph of a mouse brain slice showing the SC and the location of the injection cannula in the right intermediate/deep SC. Scale bar = 2.0mm. **(B)** The number of rotations contralateral and ipsilateral to the injection site pre- and post-muscimol injection unilaterally into the SC. Rectangles represent the mean±SE number of rotations for all the mice. Circles show the number of rotations tracked for each mouse in four separate sessions using the automated tracking system pre- and post-injection of muscimol, a $GABA_A$ agonist. Muscimol injections induced a marked bias in the mouse rotations toward the ipsilateral side relative to the injection (contra: pre=6.89±1.50, post=1.89±0.51; t-test(7)=2.43, p=0.041; ipsi: pre=7.11±1.82, post=26.2±3.53 t-test(7)=-5.309, p=0.0007). **(C)** The total number of contralateral and ipsilateral rotations pre- and post-injection for all the mice (n = 8; 4 sessions) using the automated tracking system. There was a significant reduction in the number of contralateral rotations upon injection of both NNC and muscimol compared to the saline control. There was also a significant increase in the number of ipsilateral rotations upon injection of muscimol compared to injection of NNC, and both treatments induced more ipsilateral rotations compared to the saline controls (ipsi post-injection: mus=233, NNC=103, saline=43). One-way ANOVA(2,24)=33.95, p<0.0001. **(D-F)** Two independent raters, blinded to the experimental conditions, also quantified rotations by counting the number of full rotations in randomly labeled 10-minute videos pre- and post-injection. Bars depict the mean±SE number of ipsilateral and contralateral rotations for all the mice (white and black circles, the total number of rotations per mice measured from four sessions) pre- and post-injection of NNC into the intermediate/deep SC. Paired t-test.