Supplemental Information

Chemogenetic stimulation of striatal projection neurons modulates responses to Parkinson's therapy.

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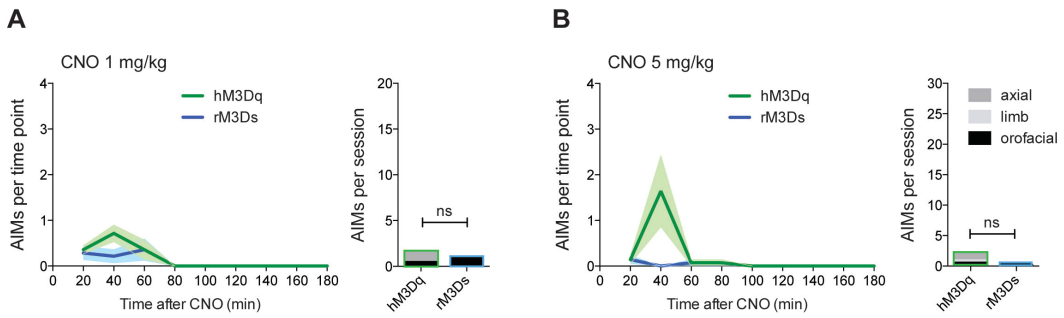
Supplemental Figure



Supplemental Figure 1. Activation of dSPNs by Gq- or Gs-DREADD does not induce dyskinesia in intact D1-Cre mice. Comparison of AIMs induced by CNO at the doses of 1 mg/kg (**A**) or 5 mg/kg (**B**) in mice transduced with hM3Dq and rM3Ds. Time course of AIMs after CNO injection, scored every 20 min until 180 min. Two-way ANOVA (*n = 7 per group*): (**A**) *CNO 1 mg/kg*: DREADD type, $F_{(1,12)} = 1.939$, $P = 0.189$; Time, $F_{(8, 96)} = 7.158$, $P < 0.001$; Interaction, $F_{(8, 96)} = 1.3$, $P = 0.253$; (**B**) *CNO 5 mg/kg*: DREADD type, $F_{(1,12)} = 2.755$, $P = 0.123$; Time, $F_{(8, 96)} = 4.183$, $P < 0.001$; Interaction, $F_{(8, 96)} = 4.408$, $P < 0.001$. Bar diagrams show the sum of AIM scores per session, with a representation of individual axial, limb, and orofacial scores by different shades of gray. Data are means ± SEM of $n = 7$ per group. Unpaired two-tailed Student's *t* test: showed no significant differences between *hM3Dq* and *rM3Ds* on either total AIMs or specific AIMs subtypes.

Supplemental Table S1: Basic electrophysiological properties of dSPNs transduced with Gs- or Gq-coupled DREADDs.

S1a: Data from intact mice

	rM3Ds-dSPN	hM3Dq-dSPN	t-test
Resting membrane potential (mV)	-89.7 +/- 1.33	-85.9 +/- 1.56	0.082
Action potential amplitude (mV)	89.1 +/- 2.37	91.0 +/- 1.71	0.520
Action potential threshold (mV)	-51.5 +/- 2.67	-51.4 +/- 1.22	0.976
Afterhyper-polarization (mV)	-10.0 +/- 1.05	-10.1 +/- 0.83	0.930
Input resistance (mOhm)	62.9 +/- 8.05	80.4 +/- 12.98	0.260
Rheobase (pA)	232.5 +/- 33.58	191.4 +/- 31.43	0.393

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S1b: Data from 6-OHDA-lesioned mice

	rM3Ds-dSPN)	hM3Dq-dSPN	t-test
Resting membrane potential (mV)	-90.7 +/- 1.73	-87.1 +/- 1.73	0.147
Action potential amplitude (mV)	80.7 +/- 2.88	82.3 +/- 2.27	0.678
Action potential threshold (mV)	-52.2 +/- 2.58	-48.9 +/- 1.12	0.273
Afterhyper-polarization (mV)	-11.6 +/- 1.48	-12.6 +/- 0.87	0.578
Input resistance (mOhm)	108.5 +/- 11.85	131.1 +/- 16.12	0.269
Rheobase (pA)	140.0 +/- 12.91	132.5 +/- 16.01	0.718

Basic electrophysiological properties of dSPNs transduced with Gs-coupled or Gq-coupled DREADD (rM3Ds and hM3Dq constructs, respectively). Data were recorded in the absence of CNO. Reported are mean +/- SEM and p-value of t-test (unpaired) from 7-9 neurons per group. No significant difference between type of DREADD was found on any parameter. Comparisons were performed separately for intact and 6-OHDA-lesioned mice, because the 6-OHDA lesion is known to change some of the basic properties of dSPNs (as shown in Fieblinger et al 2014, and confirmed in this study).

REFERENCE

Fieblinger T, Graves SM, Sebel LE, Alcacer C, Plotkin JL, Gertler TS, Chan CS, Heiman M, Greengard P, Cenci MA, et al. Cell type-specific plasticity of striatal projection neurons in parkinsonism and L-DOPA-induced dyskinesia. *Nat Commun.* 2014;5(5316.

Supplemental video legends

Video S1. Video recording representing a hemiparkinsonian D1-Cre mouse transduced with the hM3Dq DREADD, 80 min after an injection of vehicle. Note the lack of motor activation (the mouse sits still in the cage, as expected). The same behavior was seen after vehicle injection in 6-OHDA-lesioned D1-Cre mice transduced with the rM3Ds DREADD (video not shown).

Video S2. Video recording of the behavior elicited by CNO (1 mg/kg) in a hemiparkinsonian D1-Cre mouse transduced with the hM3Dq DREADD (80 min post injection). The video exemplifies the incremented horizontal activity (cf. Fig. 3E, distance travelled), vertical activity (cf. Fig. 3F, rearing events), and rotations contralateral to the lesion/transduction (cf. 3H) induced by CNO treatment. The mouse does not display any sign of axial nor limb AIMs (cf. Fig. 6A).

Video S3. Close-up of the orofacial region in a 6-OHDA-lesioned D1-Cre mouse transduced with the hM3Dq DREADD after treatment with CNO (1 mg/kg, the animal is the same as represented in S2, but here filmed 40 min after the drug injection). Despite the absence of axial and limb dyskinesia (cf. Fig. 6A and video S2), treatment with CNO clearly induces orofacial AIMs. Note the jaw movements and tongue protrusion.

Video S4. Video recording of CNO-induced dyskinesia (CNO 5 mg/kg) in a hemiparkinsonian D1-Cre mouse transduced with the rM3Ds DREADD, 80 min post injection (cf. Fig. 6B). Note the intermittent twisting movements of the upper trunk (axial dyskinesia), intermingled with contralateral rotation. The animal also presents bouts of limb dyskinesia: note the fluttering movements of the limb contralateral to the lesion (left) along one side of the body (these movements are rapid, and are best appreciated with slowmotion). The animal also presented mild orofacial dyskinesias, which are however not possible to discern from this particular video.

Video S5. An example of dyskinesias induced by L-DOPA (3 mg/kg) is here presented to allow comparisons with the preceding videos. In this example, L-DOPA induces continuous twisting movements of the trunk towards the side contralateral to the lesion (axial AIMs), which drive tight body turns. The forelimb is engaged in purposeless movements along the side of the body contralateral to the lesion.

Twitching of orofacial musculature and small jaw-openings are visible when the mouse turns towards the camera.

Video S6. Dyskinetic behaviors in a hemiparkinsonian D1-Cre mouse transduced with the rM3Ds DREADD, as elicited by combined treatment with CNO (1 mg/kg) and quinpirole (quin., 0.5 mg/kg; cf. Fig. 7B). Note the severe axial torsion towards the side contralateral to the lesion (concurring with a marked dystonic posture of the hindlimb). The forelimb contralateral to the lesion is constantly engaged in purposeless movements (some of which of conspicuous amplitude). The animal also displays orofacial dyskinesia (some up-and-down movements of the jaw are appreciable in slowmotion).

Supplemental Methods, Immunohistochemistry

The extent of DA denervation was verified in each animal by immunohistochemical staining for tyrosine hydroxylase (TH) according to the following protocol based on (24). *Day 1.* Sections were rinsed three times in 0.02 M potassium phosphate buffered saline (KPBS) pH 7.4 and pretreated with 3% hydrogen peroxide (H_2O_2) in 10% methanol/water to quench endogenous peroxidase activity. Sections were then preincubated for 1 h in blocking buffer, consisting of 5% normal serum in KPBS containing 0.25% Triton-X (KPBS/T). This was followed by overnight incubation at 4 °C with rabbit anti-TH (1:1000; Pel-Freez, Rogers, AR; #P40101-150). *Day 2.* After incubation with the primary antibody, sections were rinsed and incubated with the biotinylated goat anti-rabbit secondary antibody (1:200; Vector Laboratories, Burlingame, CA; BA1000). This was followed by incubation in an avidin–biotin–peroxidase solution (Vectastain Elite ABC; Vector Laboratories) for 1 h at room temperature. The immunocomplexes were visualized using 3,3-diaminobenzidine (DAB) and H_2O_2 (both from Sigma-Aldrich). Sections were then rinsed in KPBS/T, mounted onto chromalum-coated slides, and coverslipped using DPX mounting medium (Sigma-Aldrich). Densitometric analysis of TH immunostaining on regions of interest (ROI) were performed using the freesoftware *NIH Image J 1.43.* Images were digitized using a Nikon 80i microscope connected to a digital camera (Nikon DM1200F). Staining intensities were calibrated on optical density (O.D.) standards provided by the software and the average O.D. in the ROI was calculated after background subtraction. Measurements were carried out in 3 rostro-caudal sections per animal throughout the striatum. Values from the lesion side were expressed as a percentage of the values from the intact contralateral side.

The DREADD transduction was verified using an antibody that recognizes the fluorescent reporter protein mCherry (Anti-RFP antibody, 1:500; Abcam; # ab65856). *Day 1.* Free-floating sections were rinsed in Tris-buffered saline (TBS; 0.10 M Tris

and 0.14 M NaCl, pH 7.4), incubated for 5 minutes in TBS containing 3% H_2O_2 and 10% methanol, and then rinsed again. After 15 minutes incubation in TBS containing 0.2% Triton X-100, sections were blocked in serum solution, and then incubated overnight at 4°C with the primary antibody. *Day 2.* After rinsing, sections were incubated for 2 h in horse anti-mouse secondary antibodies (1:200; Vector Laboratories, Burlingame, CA; # BA2001). Sections were incubated for 1,5 hour in avidin-biotin-peroxidase solution. Immunocomplexes were visualized using DAB and H_2O_2 as described above.

To visualize the expression of phospho-ERK1/2 or phospho-PKA substrate in transduced neurons we performed double immunofluorescence with RFP. *Day 1* was performed as for RFP-DAB immunostaining protocol (see above). Free-floating sections were incubated overnight at 4°C with the following primary antibodies: rabbit polyclonal antibodies for phospho-Thr202/Tyr204–ERK1/2 (pERK, 1:400; Cell Signaling Technology; #9101L) or phospho-(Ser/Thr) PKA substrate (pPKA, 1:250; Cell Signaling Technology; #9621S) in combination with RFP antibody. *Day 2.* Sections were rinsed in TBS and incubated for 45 min with the following secondary antibodies: donkey anti-mouse Cy3-coupled (1:400; Jackson Immuno Research Laboratory; # 715-165-150), goat anti-rabbit Alexa Fluor 488-coupled (1:400; Invitrogen; # A-11001). After final rinsing steps, sections were mounted in polyvinyl alcohol mounting medium (PVA-DABCO, Sigma Aldrich). In some sections, the SPN phenotype of immunolabeled cells was verified using TO-PRO3 (1:8000, Invitrogen; # T3605), a double-stranded DNA-intercalating fluorescent molecule commonly used for laser confocal microscopy analysis, which revealed the pattern of heterochromatin clumps typical for SPNs (63). Alexa-488 was excited with 480 nm (filter 499–630), Cy3 with 514 nm (filter 538–681) and TO-PRO3 with 633 nm (filter 642-661). Overview confocal pictures of the DREADD-transduced striatum were taken using a 4x objective (0.10 NA).