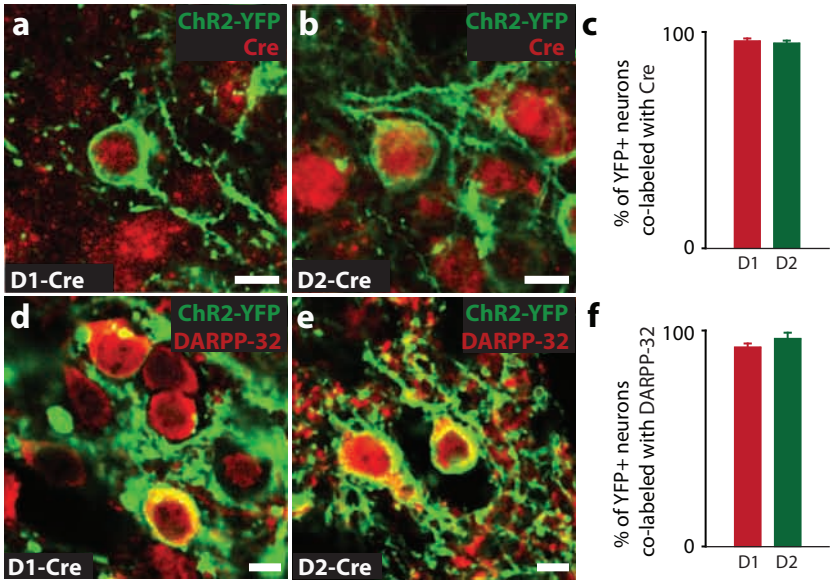
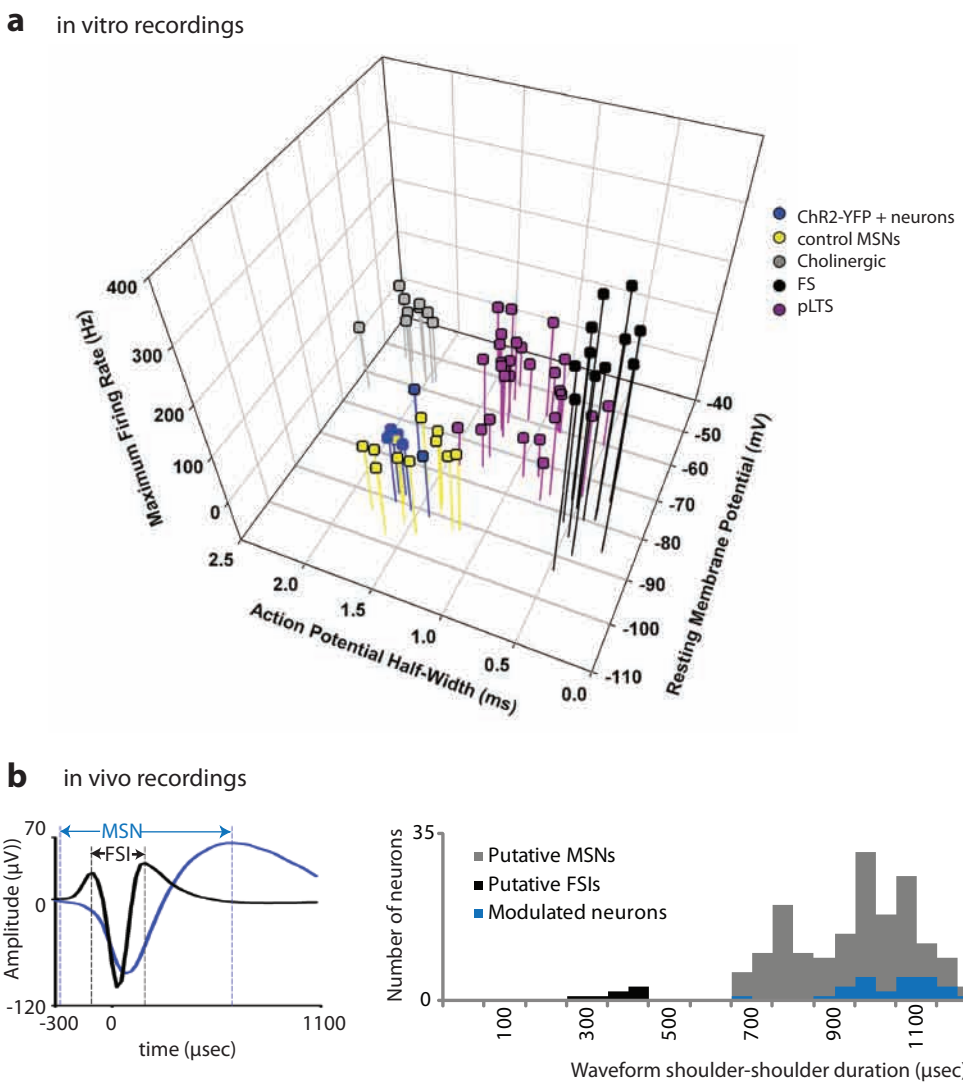
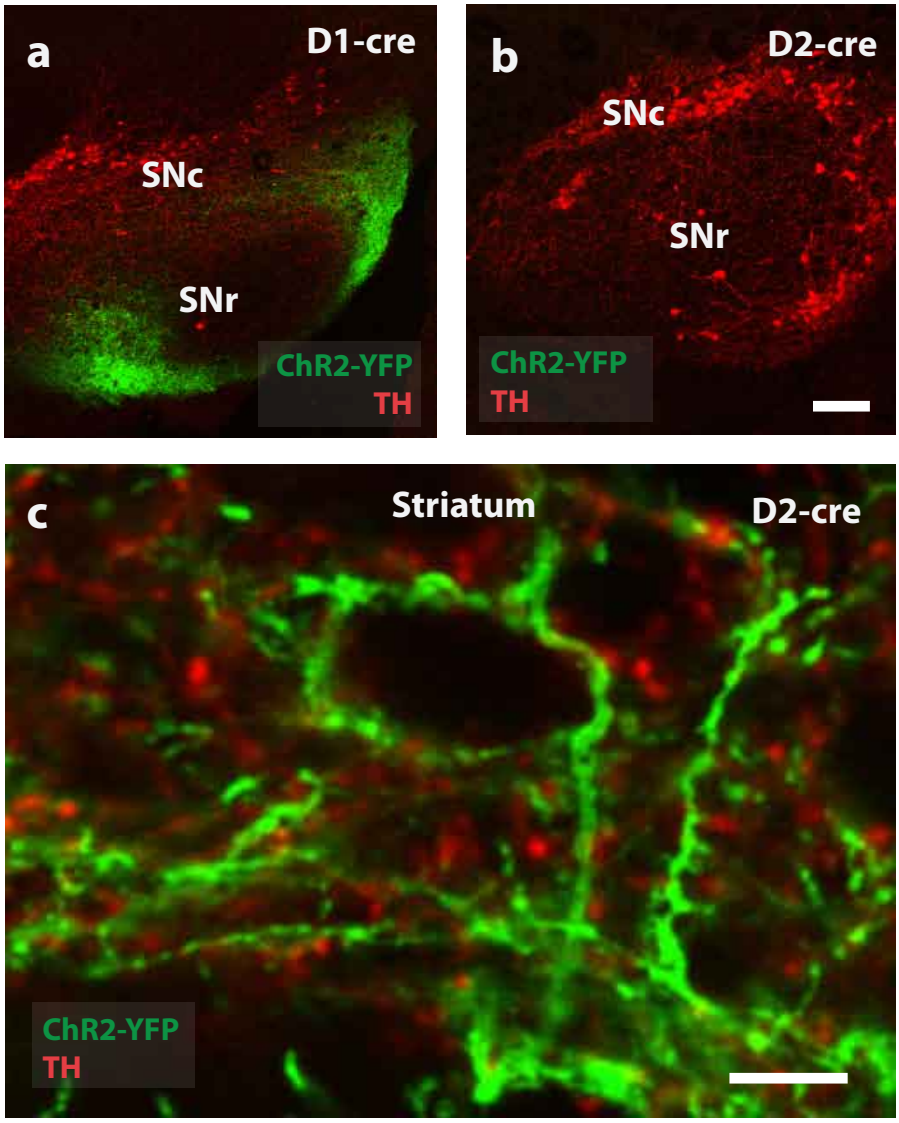


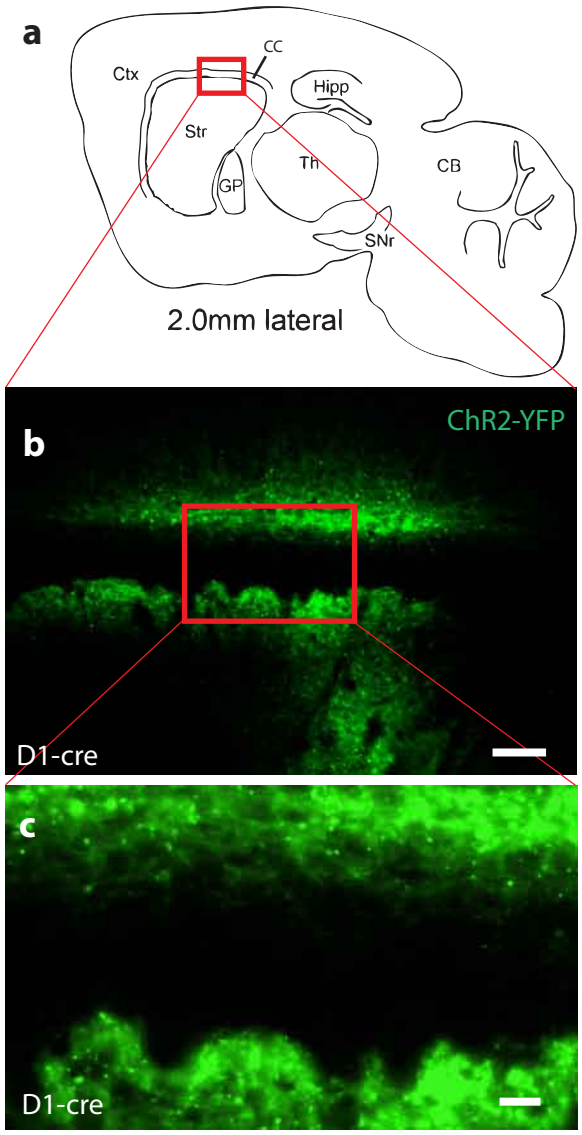
Supplementary Figure 1. ChR2-positive neurons in the striatum are Cre- and DARPP-32 positive. (a,b) Examples of ChR2-YFP and Cre expression D1-Cre and D2-Cre striatal sections. **(c)** Summary of percent ChR2-YFP-positive MSNs that are also co-labeled with Cre. **(d,e)** Examples of ChR2-YFP and DARPP-32 expression in D1-Cre and D2-Cre sections. Scale bars in a-b and d-e are 15 μM. **(f)** Summary of perecent ChR2-YFP positive MSNs that are also co-labeled with DARPP-32. Error bars are SEM.



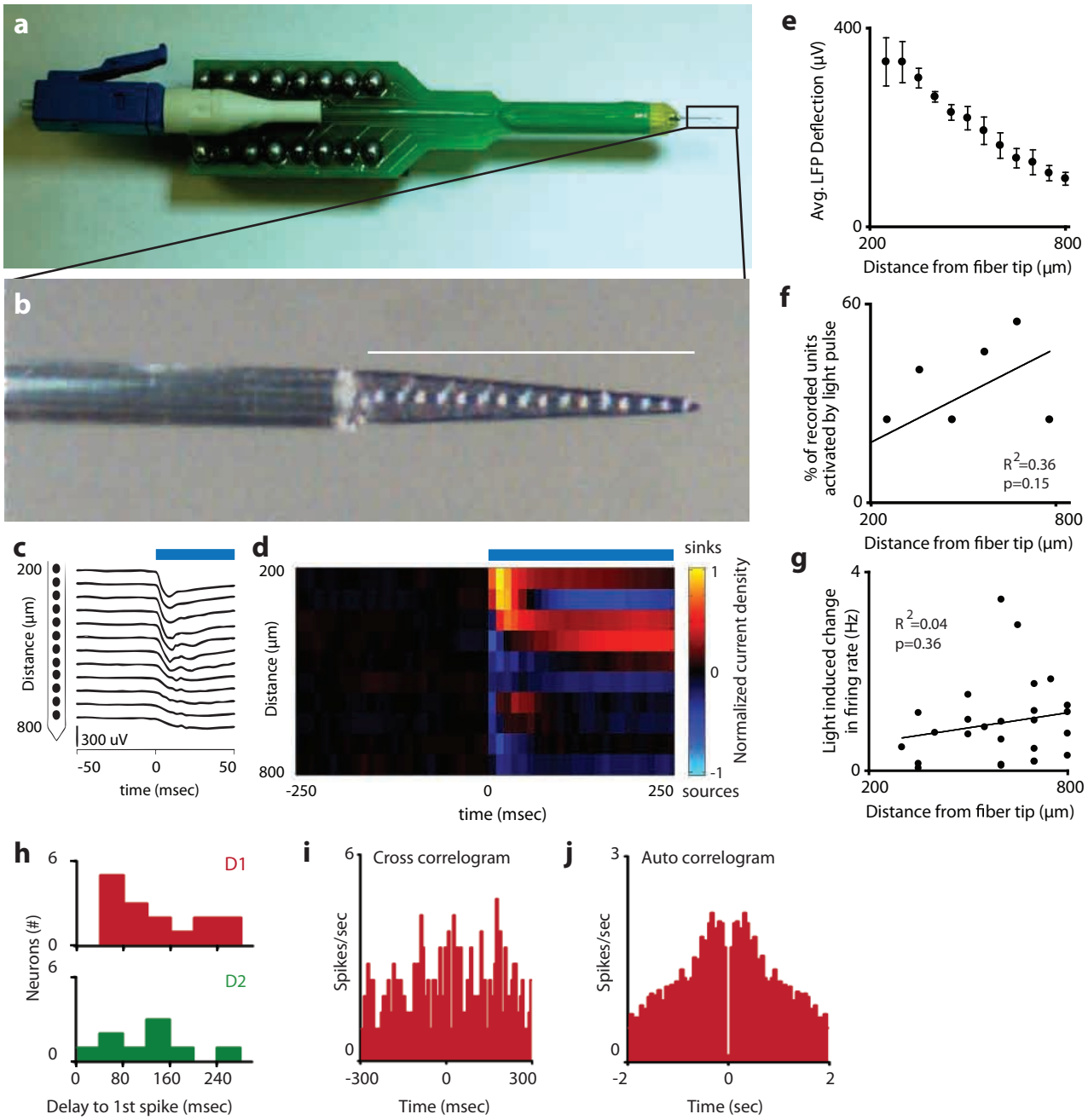
Supplementary Figure 2. ChR2-YFP+ neurons recorded in vitro and in vivo exhibit electrophysiological characteristics of striatal medium spiny neurons (MSNs). (a) Cluster analysis based on passive and active membrane properties recorded in whole-cell current clamp recordings from brain slices. **(b)** Spike width (shoulder-to-shoulder) distinguishes MSNs from fast-spiking interneurons (FSIs) in vivo. All light-modulated neurons (ChR2+) in vivo displayed slower spike waveforms consistent with MSNs.



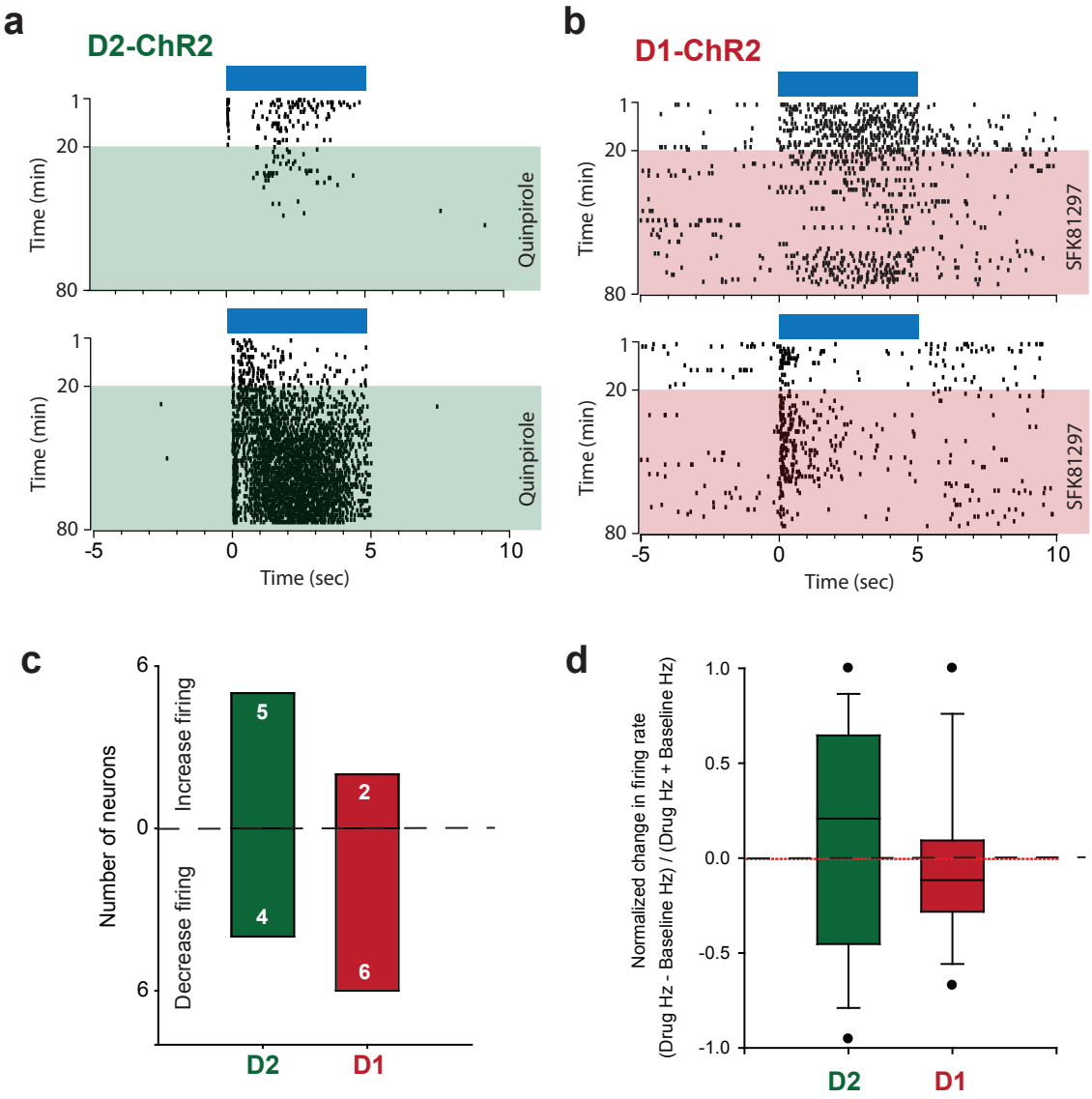
Supplementary Figure 3. ChR2-YFP is not expressed in TH+ midbrain dopamine neurons. Low-magnification sagittal image of the substantia nigra pars compacta (SNc) and pars reticulata (SNr) in D1-cre mice **(a)** or D2-cre mice **(b)** injected intrastriatally with AAV1-DIO-ChR2-YFP virus. Sections were stained with anti-tyrosine hydroxylase antibodies (TH, red). Endogenous ChR2-YFP fluorescence is shown in green. Axon terminals of ChR2-YFP+ striatonigral MSNs are evident in (a). No ChR2-YFP fluorescence was observed in TH+ SNc neurons in either D1- or D2-cre mice (0/71 cells in D1-cre, 0/88 cells in D2-cre). Scale bar in (a) and (b) is 100 um. High-magnification image of the striatum in D2-cre mice **(c)** injected intrastriatally with ChR2-YFP virus and stained for TH (red). ChR2-YFP+ neuropil is present, but no overlap with TH+ axon terminals is observed. Scale bar in (c) is 10 um.



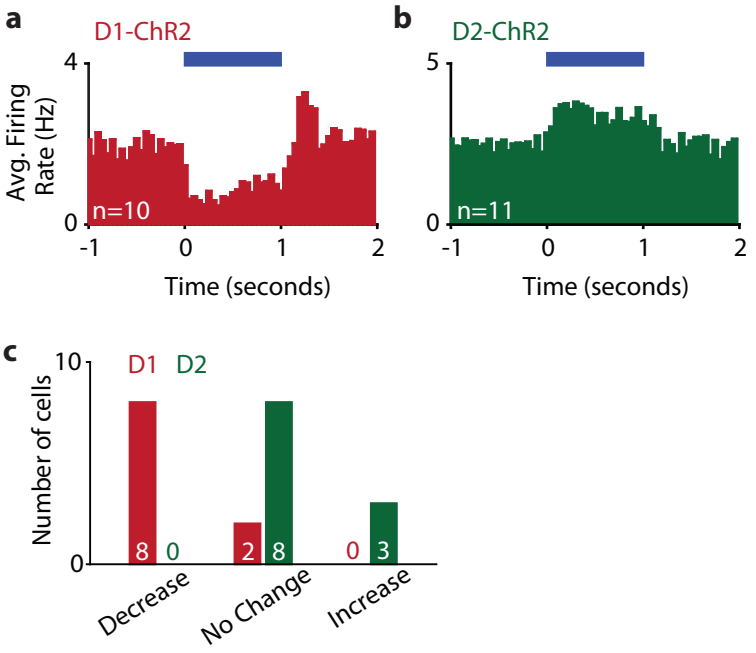
Supplementary Figure 4. Corticostriatal fibers are not labeled with ChR2-YFP in D1-cre mice. (a) Sagittal schematic of mouse brain, showing the cortex (Ctx), corpus callosum (CC), and striatum (Str). **(b)** ChR2-YFP fluorescence in a D1-cre mouse injected with AAV1-DIO-ChR2-YFP virus. In addition to striatal neurons, some cortical layer 6 neurons are labeled. Scale bar is 100 um. **(c)** Higher magnification image of corpus callosum shows no corticostriatal fibers labeled with ChR2-YFP. Scale bar is 20 um. Similar results were observed in 3 D1-cre mice examined.



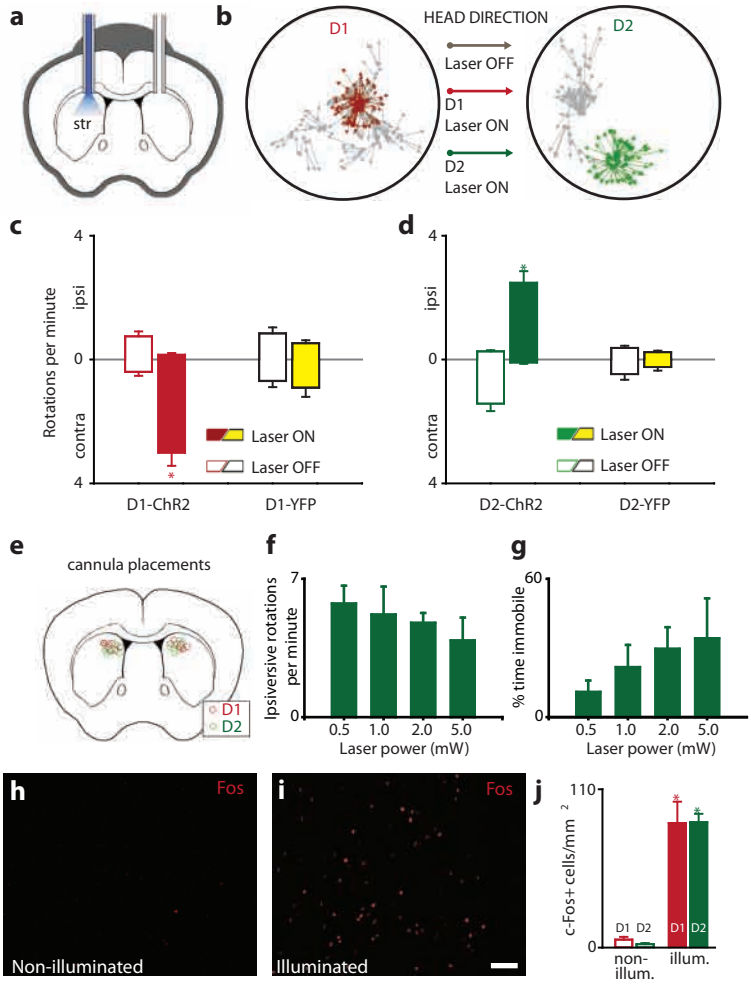
Supplementary Figure 5. Characterization of ChR2-mediated optical stimulation in the striatum. (a) An optrode recording unit (NeuroNexus) used for *in vivo* recordings. A multimode optical fiber (50 or 105um core) was attached to a linear silicon recording probe with 16 recording sites spaced at 50um intervals, shown enlarged in **(b)**. Because no units were ever recorded on the 4 recordings sites closest to the fiber tip, those sites were excluded from further analysis. **(c)** LFPs recorded in the striatum simultaneously recorded at various distances from the fiber tip before and after illumination with 473nm light (1 mW at tip), marked by the blue bar. **(d)** Current source density analysis of light-evoked LFPs. **(e)** Quantification of LFP deflection at different recordings sites (n=4 mice for d-e). No correlations were observed between, **(f)** number of recorded units modulated by light, or **(g)** the light-induced change in firing rate, as a function of distance from the fiber tip. **(h)** Average delay to first spike during illumination is plotted for all light-driven units recorded from D1-ChR2 (top) or D2-ChR2 (bottom) mice. **(i)** Cross correlogram between two representative light-driven units on the same recording probe in a D1-ChR2 mouse. **(j)** Auto correlogram of representative light-driven unit in a D1-ChR2 mouse. Error bars are SEM.



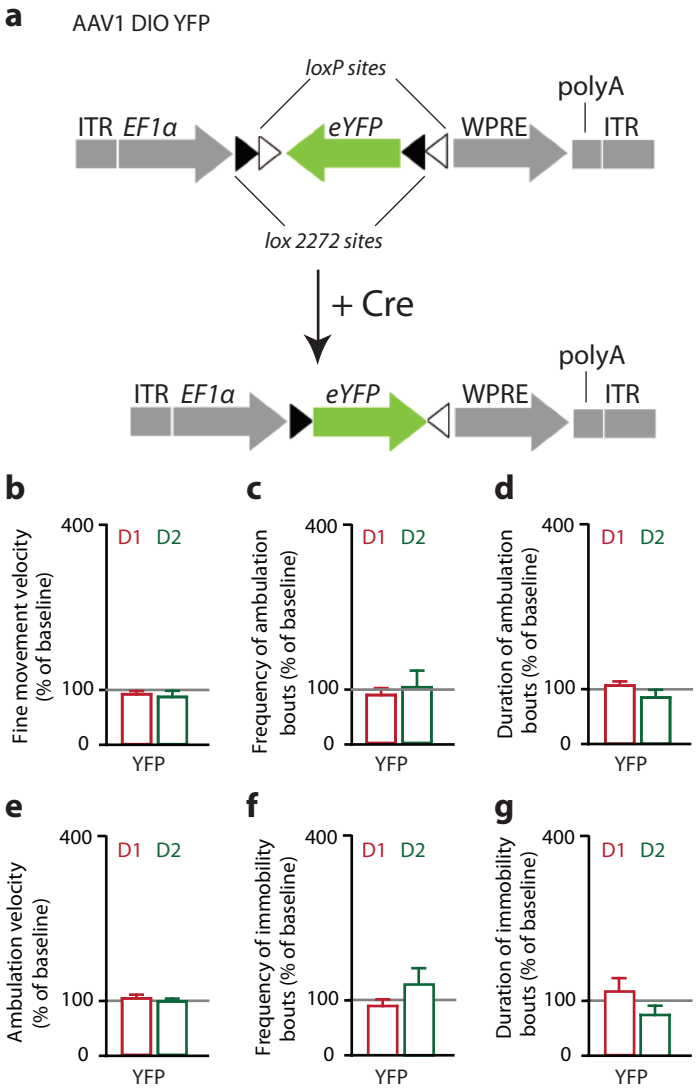
Supplementary Figure 6. Response of direct- and indirect-pathway MSNs *in vivo* to dopamine receptor agonists. (a,b) Single unit recording from direct- (D1, right) or indirect- (D2, left) pathway MSNs in anesthetized mice. The D2 agonist quinpirole (1 mg/kg) or the D1 agonist SKF81297 (1 mg/kg) was injected i.p. at 20 minutes. **(c)** Varied responses to quinpirole in D2-expressing MSNs (green bar) or SKF81297 (red bar) in D1-expressing MSNs. Number of neurons shown inside the bars. **(d)** Box plot showing no significant change in firing rate across the population of direct- (n=8) or indirect-pathway (n=9) MSNs was observed in response to D1- or D2-agonists, respectively.



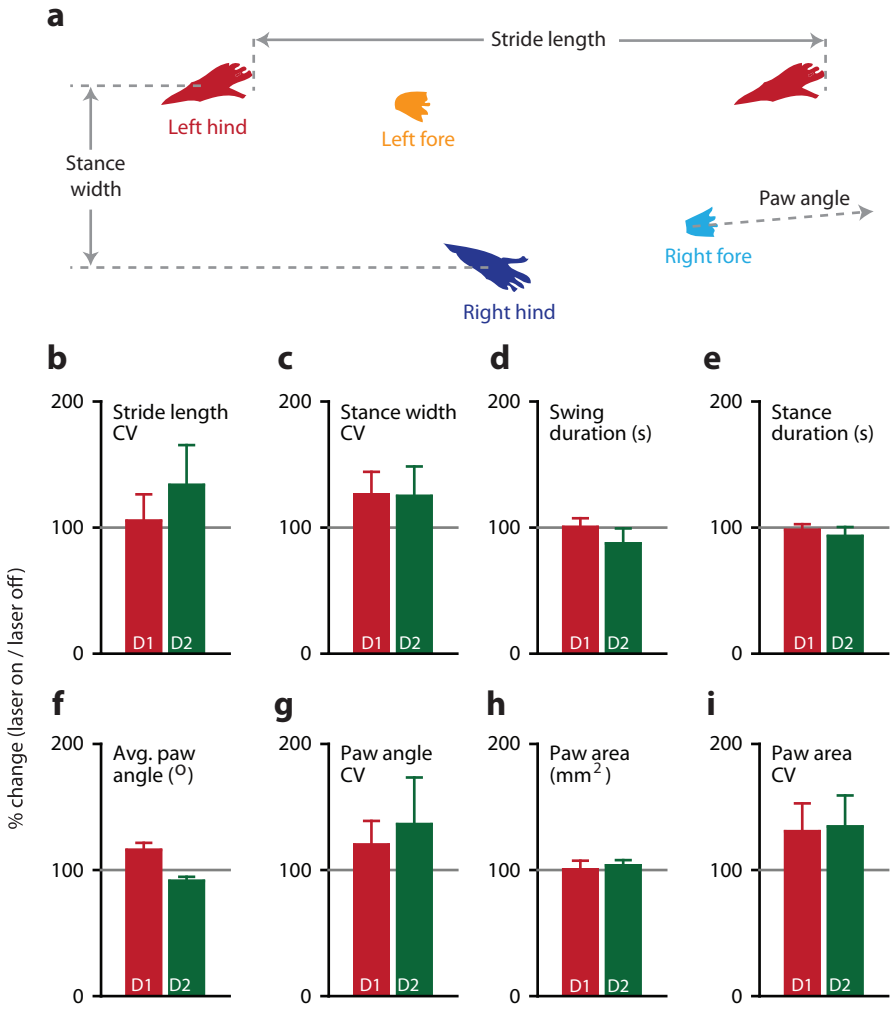
Supplementary Figure 7. Average firing rate of all LFP-modulated units recorded in SNr in response to striatal direct or indirect pathway activation. Single unit recordings from SNr. The blue bars mark the time of striatal illumination in D1-ChR2 **(a)** or D2-ChR2 **(b)** mice. **(c)** Total number of SNr cells whose firing rate decreased, remained constant, or increased in response to direct (D1) or indirect (D2) pathway activation.



Supplementary Figure 8. Unilateral in vivo activation of direct or indirect pathways elicits rotational behavior. (a) Schematic of cannula placement and unilateral fiber optic stimulation in dorsomedial striatum. **(b)** Examples of rotational behavior elicited by striatal illumination in D1-ChR2 (left) or D2-ChR2 (right) mice. Vectors represent head direction sampled every 300 ms. Grey vectors were sampled for 20 s prior to fiber optic illumination; colored vectors were sampled during 20 s of subsequent illumination. **(c)** Summary of ipsiversive and contraversive rotations with illumination (solid bars) and without illumination (open bars) for D1-ChR2 mice (red) or D1-Cre mice expressing a DIO-YFP control virus (D1-YFP, yellow). **(d)** Summary of rotational behavior with illumination (solid bars) and without illumination (open bars) in D2-ChR2 mice (green) or D2-YFP controls (yellow). **(e)** Cannula placements for all experiments in D1-ChR2 (red) and D2-ChR2 (green) mice. **(f)** Effect of laser power on rotational behavior in D2-ChR2 mice. **(g)** Effect of laser power on immobility in D2-ChR2 mice. Representative striatal Fos staining from non-illuminated **(h)** and illuminated **(i)** hemispheres in a D2-ChR2 mouse. Scale bar is 100 um. **(j)** Summary of Fos-positive cell density in non-illuminated and illuminated striata of D1-ChR2 and D2-ChR2 mice. Error bars are SEM.



Supplementary Figure 9. Expression of YFP alone does not alter alter motor behavior. (a) Schematic of the AAV DIO-YFP control virus, which enables cell-type specific expression of YFP only in Cre+ neurons. During striatal illumination of D1-YFP (red bars) or D2-YFP mice (green bars), no changes in **(b)** fine movement velocity, **(c)** frequency of ambulation bouts, **(d)** duration of ambulation bouts, **(e)** ambulation velocity, **(f)** frequency of immobility, or **(g)** duration of immobility were observed.



Supplementary Figure 10. Direct or indirect pathway activation in vivo does not change gait parameters. (a) Schematic of gait analysis parameters. No significant changes in **(b)** stride length variance, **(c)** stance width variance, **(d)** swing duration, **(e)** stance duration, **(f)** paw angle, **(g)** paw angle variance, **(h)** paw area contacting surface, or **(i)** paw area variance, were observed during bilateral stimulation of the direct (D1, red bars) or indirect pathway (D2, green bars). Error bars are SEM.

Supplementary Table 1

Mouse line	Marker	Cells counted/# of mice	Co-labeled with YFP	Co-labeled with DARPP-32	Co-labeled with Cre
D1-ChR2	Chat+	89/3	0 (0%)	-	-
	PV+	124/3	7 (6%)	-	-
	NPY+	102/3	2 (2%)	-	-
	Cre+	86/2	24 (28%)	-	-
	YFP+	83/3	-	76 (92%)	-
	YFP+	122/2	-	-	117 (96%)
D2-ChR2	Chat+	75/3	1 (2%)		
	PV+	101/3	0 (0%)		
	NPY+	82/3	0 (0%)		
	Cre+	84/2	14 (16%)		
	YFP+	73/3	-	71 (96%)	-
	YFP+	114/2	-	-	108 (95%)

Supplementary Table 2

	n	VRest (mV)	Capacitance (pF)	RInput (MΩ)
ChR2-YFP+	6	-88 ± 2	113 ± 10	162 ± 25
Control MSN	11	-92 ± 1 (p = .063)	96 ± 7 (p = .177)	142 ± 18 (p = .523)
Cholinergic	9	-57 ± 1	76 ± 4	229 ± 23
FS	22	-82 ± 2	74 ± 6	187 ± 25
pLTS	32	-63 ± 2	42 ± 1	828 ± 105

Note: p-values refer to comparisons between ChR2-YFP+ and Control MSN