Supporting Information

cAMP-producing chemogenetic activation of indirect pathway striatal projection neurons and the downstream effects on the globus pallidus and subthalamic nucleus in freely moving mice

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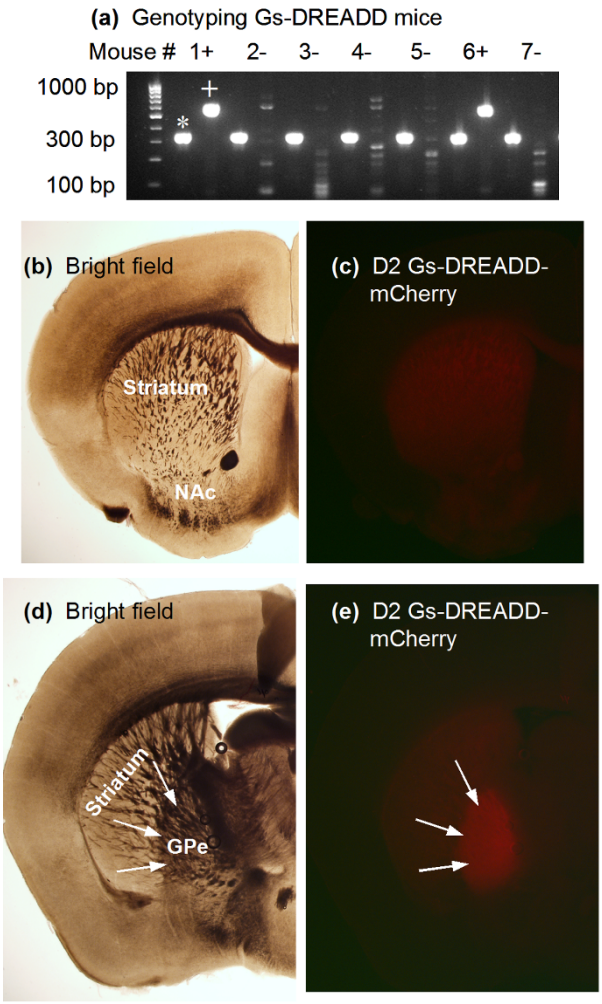

Fig. S1 Identification of Gs-DREADD positive mice and the expression pattern of Gs-DREADD in the brain. (a) PCR-based genotyping identifies Gs-DREADD positive mice. (b,c) Low power (1X objective) photomicrophs show a coronal brain section containing the anterior striatum. The bright field image (b) illustrates the gross anatomical structure of the anterior striatum, and the red fluorescent image (c) shows the red fluorescent mCherry-tagged Gs-DREADD expression in the dorsal and ventral striatum of the same brain section, both taken on a conventional fluorescence microscope. (d, e) Low power (1X objective) photomicrophs show a coronal brain section containing the posterior striatum and the GPe. The bright field image (d) illustrates the gross structure of the posterior striatum, and the red fluorescent image (e) shows the red fluorescent mCherry-tagged Gs-DREADD expression in the striatum of the same brain section; the strong signal in the GPe is due to the convergent D2-MSN projection to GPe (arrows), indicating selective Gs-DREADD expression in striatopallidal neurons or iMSNs.

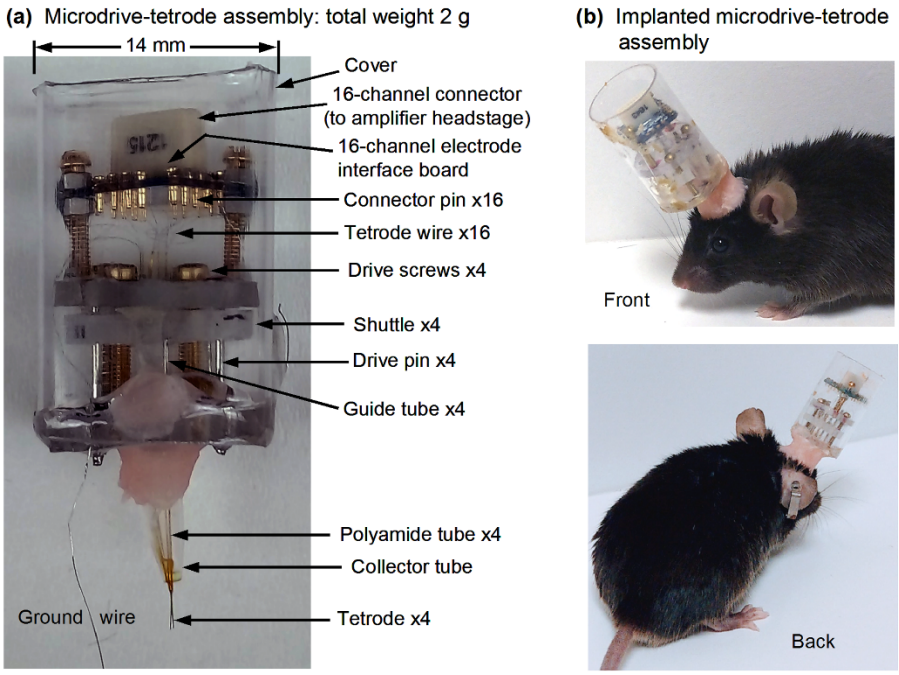

Fig. S2 Recording methods. (a) Our home-made lightweight (2 g) microdriveable tetrode recording assembly. (b) The recording assembly implanted in a D2 Gs-DREADD mouse.

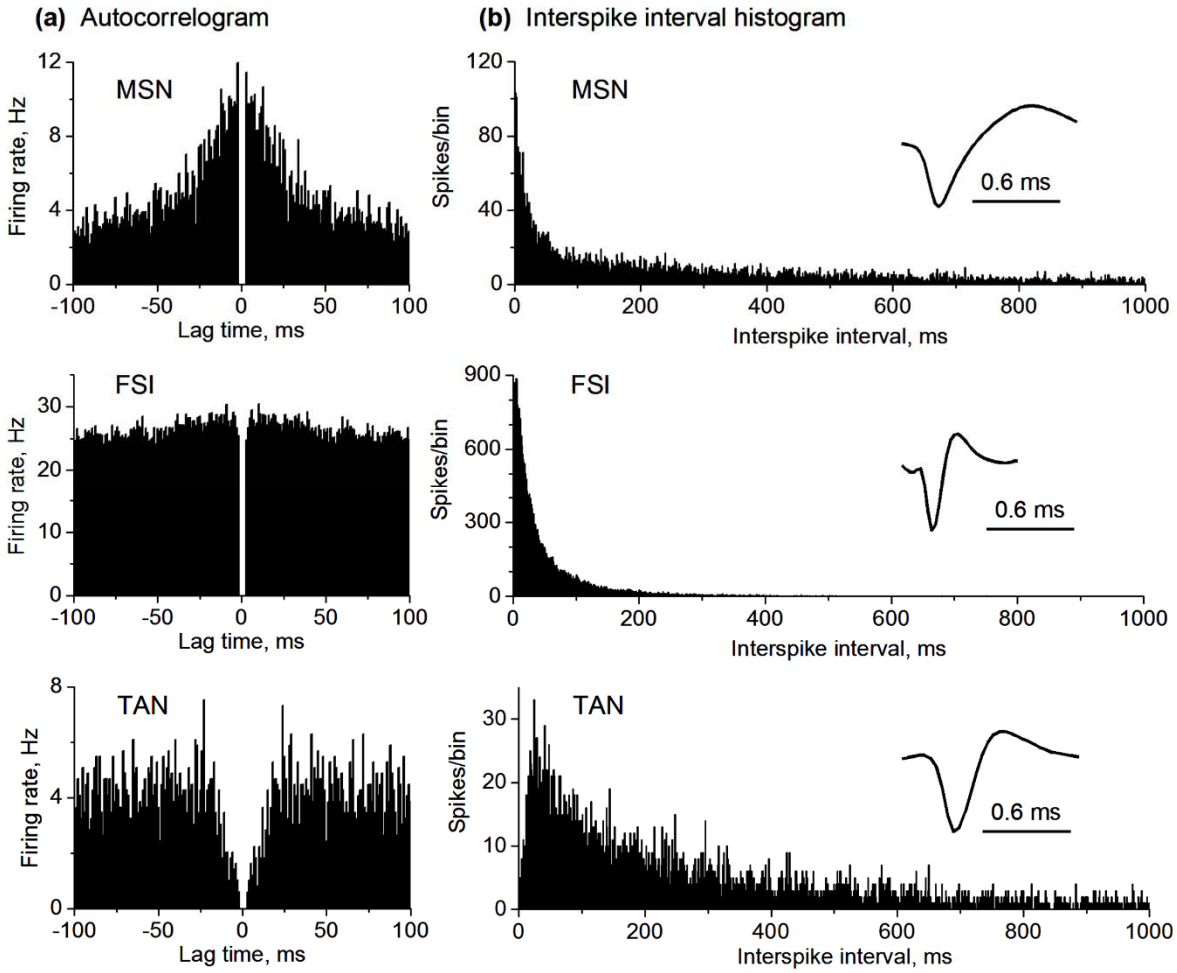

Fig. S3 Classification of single unit spikes of striatal neurons. Striatal neurons show distinctive characteristics allowing their classification into three categories: MSNs, fast-spiking interneurons (FSIs) and tonically active neurons (TANs). (a) Examples of auto-correlograms (1 ms bin width) for a MSN, a FSI and a TAN recorded in DREADD mice. (b) Examples of ISI histograms (1 ms bin width) and waveforms for a MSN, a FSI and a TAN recorded in DREADD mice.

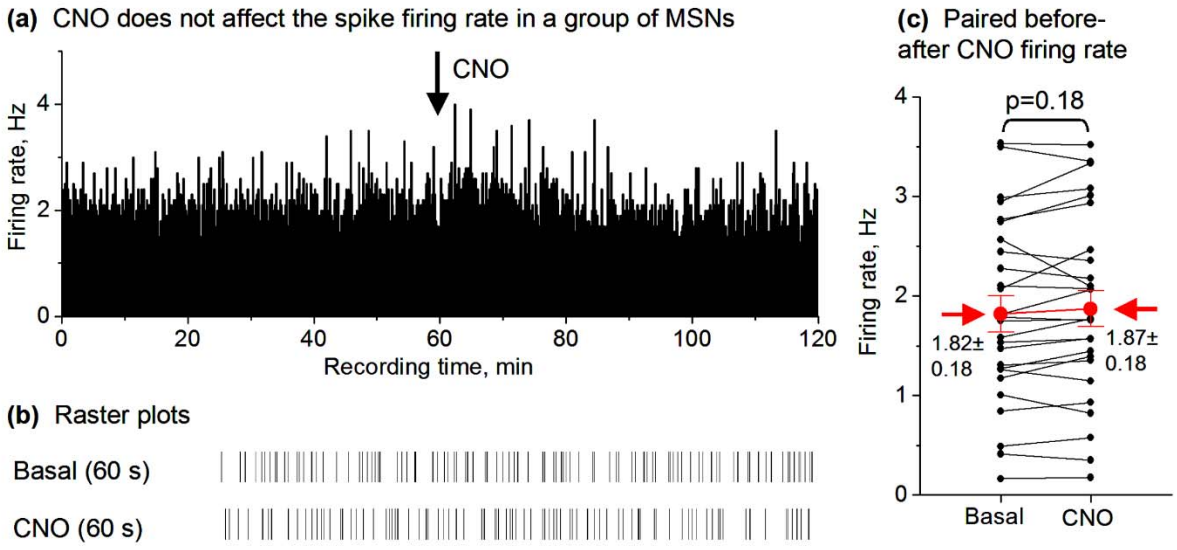

Fig. S4 CNO did not affect the spike firing of a subgroup of the recorded MSNs in D2 Gs-DREADD positive mice. (a) Spike firing rate histogram of a MSN recorded in DREADD positive mouse showing no significant change in the firing rate after 2.5 mg/kg CNO injection. Firing rates were calculated and plotted as the average frequency of discharge in 10-s bins. (b) Raster plots showing the spikes in a representative 60 s before and after CNO injection of an isolated MSN. (c) Paired before-after scatter plot showing that CNO did not increase the firing rate of the 27 MSNs in D2 Gs-DREADD positive mice (n=3), p= 0.18, paired t-test.

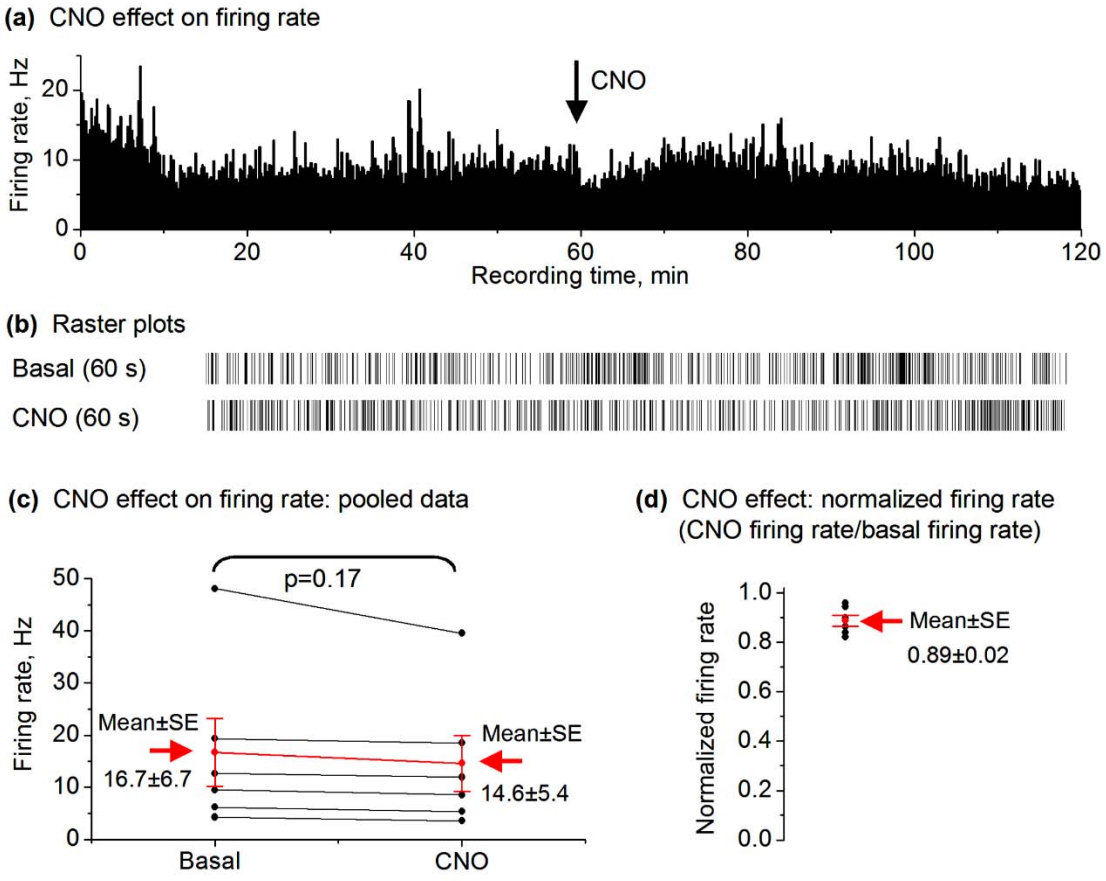

Fig. S5 CNO did not affect the spike firing of a small subgroup of GPe neurons (6 out of total 33) in D2-MSN Gs-DREADD positive mice. (a) Spike firing rate histogram of a GPe neuron recorded in DREADD positive mouse showing no significant change in the firing rate after 2.5 mg/kg CNO injection. Firing rates were calculated and plotted as the average frequency of discharge in 10-s bins. (b) Raster plots showing the spikes in a representative 60 s before and after CNO injection of an isolated GPe neuron. (c) Paired before-after scatter plot showing that CNO did not decrease the firing rate of the 6 GPe neurons in D2 Gs-DREADD positive mice (n=3), *p*=0.17, paired *t*-test. (d) CNO did not affect the normalized firing rate in GPe neurons. Each black dot represents the normalized firing rate of a single GPe neuron. The mean ± SE are shown in red and indicated by the arrow.

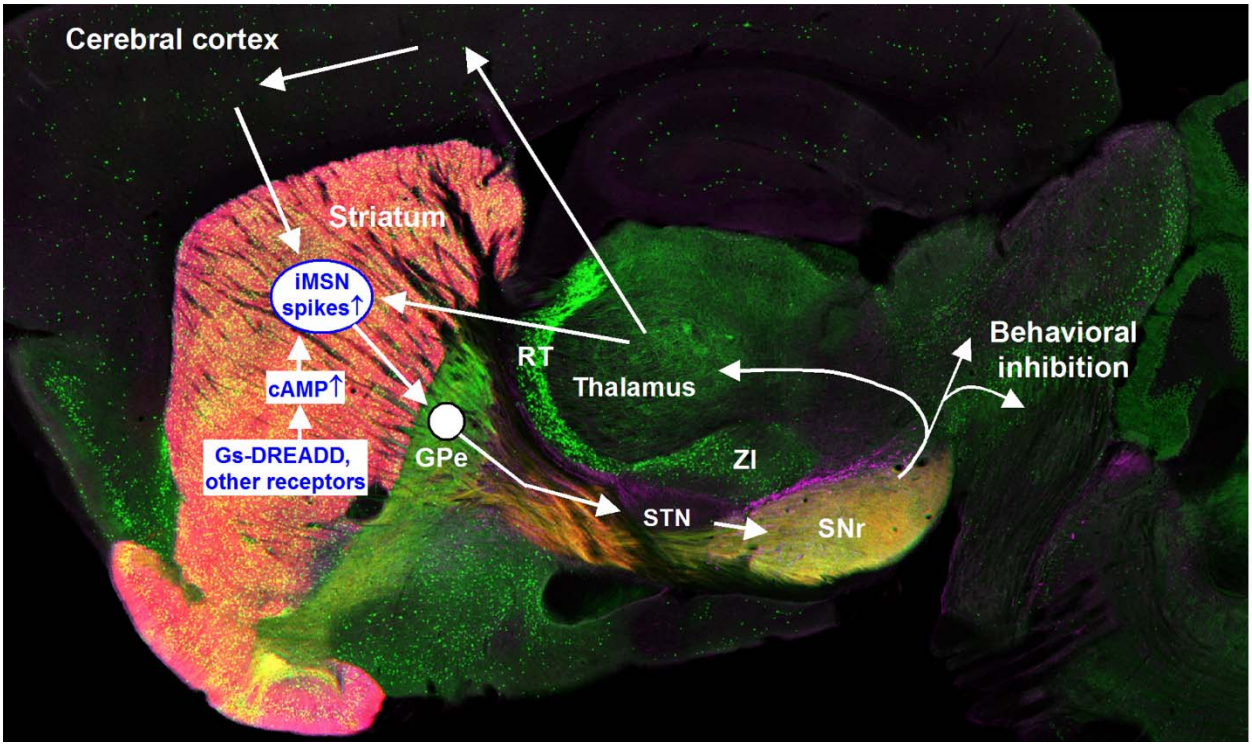

Fig. S6 Summary diagram. cAMP-producing chemogenetic activation increases the spike firing in iMSNs, leading to an inhibition of GPe neurons spike firing and an excitation of a subgroup of neurons in the STN. These effects eventually lead to an inhibition of mouse behavior. Our results reveal the neurophysiological function of intracellular cAMP on iMSNs. The background image is a laser scanning confocal picture of a mouse sagittal brain section (from our image database) that shows the main components of the basal ganglia. RT, reticular thalamus; SNr, substantia nigra pars reticulata; ZI, zona incerta.