

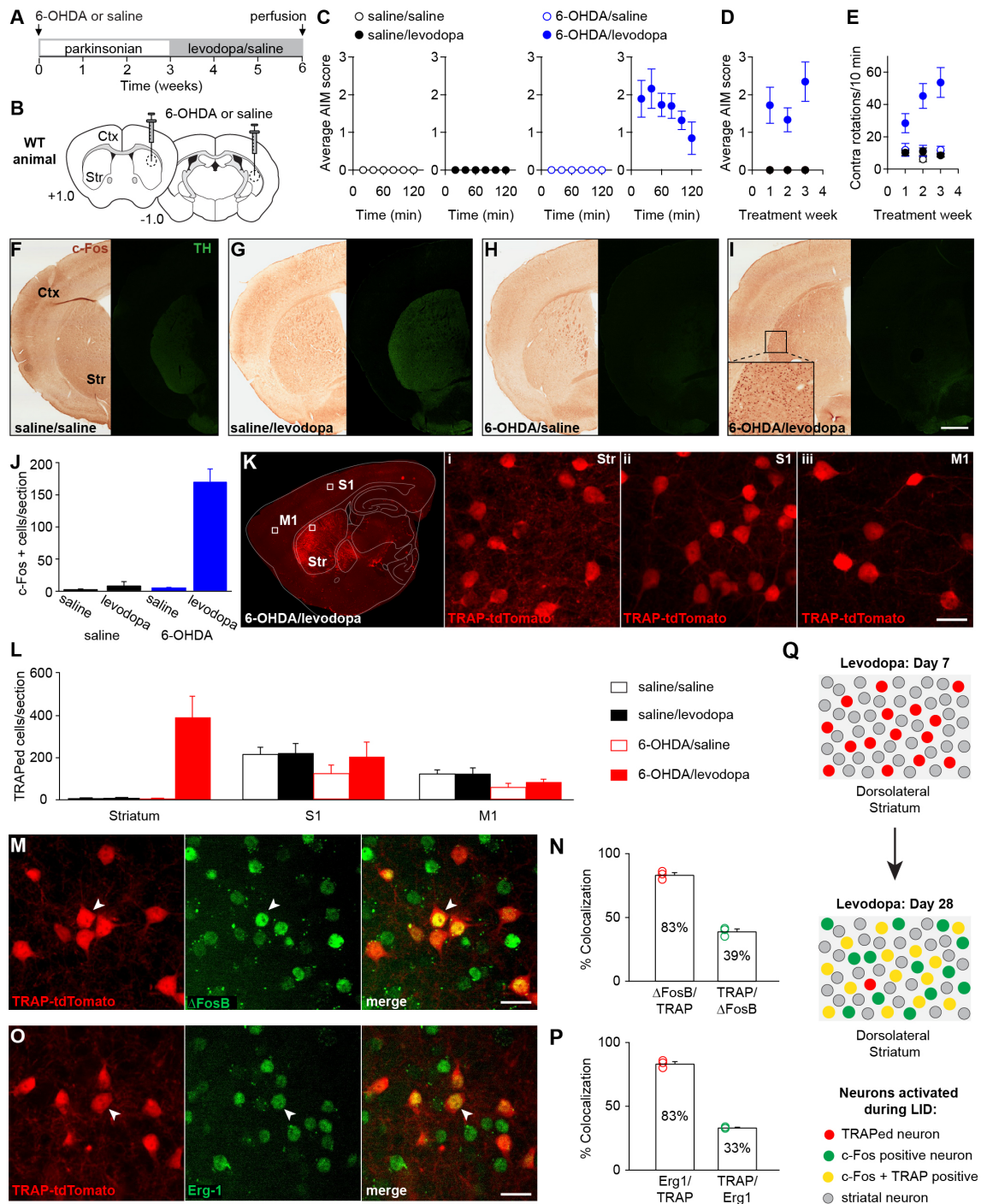
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**Supplemental Information**

**A Subpopulation of Striatal Neurons**

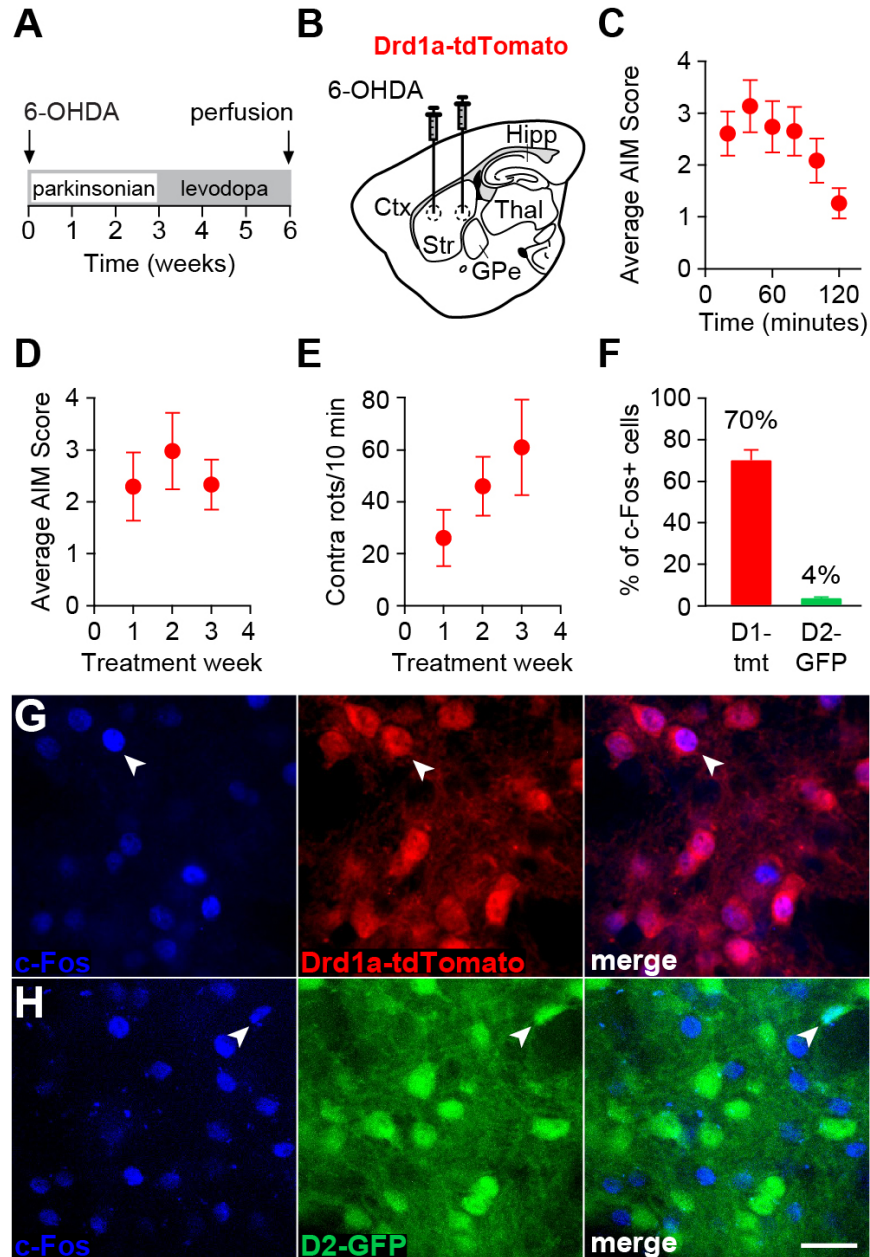
**Mediates Levodopa-Induced Dyskinesia**

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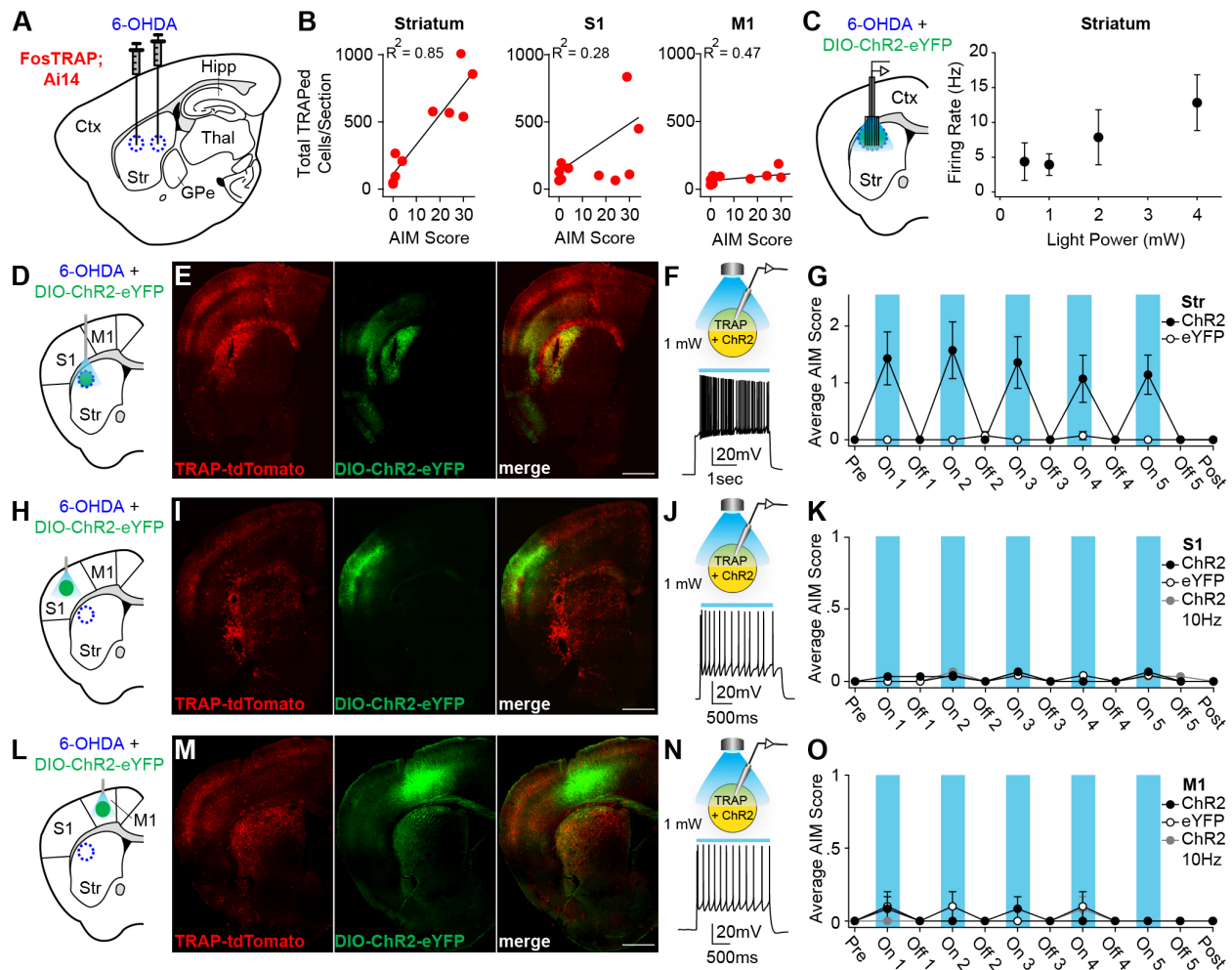
**Figure S1. Related to Figure 1. Levodopa-induced dyskinesia is associated with c-Fos expression.** A-J. Wild-type mice were separated into four experimental groups based on a combination of intracerebral injection (saline or 6-OHDA) and subsequent chronic drug treatment (saline or levodopa). A. Experimental timeline. B. Coronal schematic showing sites of 6-OHDA or saline injection in the dorsolateral striatum. C. Average abnormal involuntary movement (AIM) score measured after intraperitoneal (IP) injection of levodopa or saline in 6-OHDA-treated or control groups. D. Average composite AIM score over 3 weeks of treatment. E. Contralateral rotations after IP injection of levodopa or saline over 3 weeks of treatment. F-I. Representative

coronal histological images of striatal tissue stained with anti-c-Fos (left panels) and anti-TH (right panels) in mice from each of the four experimental groups. I. Inset: 20x image showing c-Fos positive nuclei in the dorsolateral striatum. J. Average number of c-Fos-positive nuclei per striatal section in all four experimental groups. K-L. FosTRAP mice were separated into four experimental groups, same as above. K. Representative sagittal section showing TRAPed cells in the striatum (Str), primary somatosensory cortex (S1), and primary motor cortex (M1) in a 6-OHDA/levodopa treated mouse. i, ii, iii, 40x confocal images of TRAP-tdTomato cells in the striatum, S1, and M1, respectively. L. Average number of TRAP-tdTomato positive cells per section in the striatum, S1, and M1 of animals from each group. M-P. FosTRAP mice were administered levodopa 2 hours prior to perfusion. TRAPed cells reflect those activated during a levodopa session approximately 2 weeks prior, while  $\Delta$ FosB or Erg-1 positive neurons reflect cells activated during the terminal levodopa session. M. Confocal images showing colocalization of TRAP-tdtomato (left),  $\Delta$ FosB (middle), and merged image (right). N. Percent colocalization of  $\Delta$ FosB with TRAP-tdTomato (left) and TRAP with  $\Delta$ FosB (right). O. Confocal images showing colocalization of TRAP-tdtomato (left), Erg-1 (middle), and merged image (right). P. Percent colocalization of Erg-1 with TRAP-tdTomato (left) and TRAP with Erg-1 (right). Q. Schematic representing the striatal neurons reproducibly activated during each levodopa administration. F-I, K (left) scale bar equals 1 mm. K (right), M, O, scale bar equals 20  $\mu$ m. Arrowhead shows colocalization. Data is displayed as average  $\pm$  SEM.

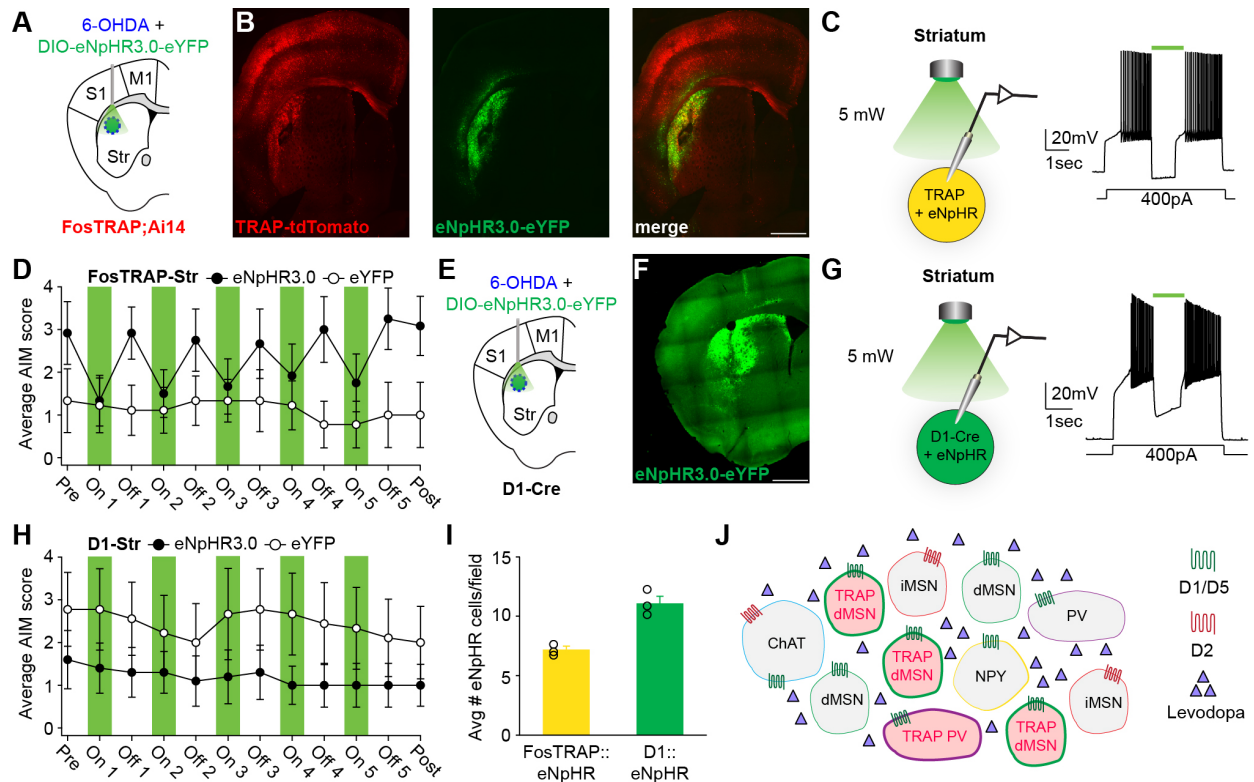


**Figure S2. Related to Figure 2. c-Fos activated neurons are primarily direct pathway cells.**

A-B. Drd1a-tdTomato mice were rendered parkinsonian with intrastriatal 6-OHDA, then treated with daily levodopa. After the last levodopa injection, mice were time-perfused for assessment of c-Fos expression. A. Experimental timeline. B. Sagittal schematic showing 6-OHDA injection sites. C. Average AIM scores after IP injection of levodopa. D. Average composite AIM score over 3 weeks of levodopa treatment. E. Contralateral rotations over 3 weeks of treatment. F. Percent of c-Fos positive cells that colocalize with Drd1a-tdTomato or D2-GFP. G. Representative 40X images from the striatum of a Drd1a-tdTomato mouse immunostained for c-Fos. Left: c-Fos, middle: Drd1-tdTomato, right: merged. H. Representative 40X images from the striatum of a D2-GFP mouse immunostained for c-Fos. Left: c-Fos, middle: D2-GFP, right: merged. White arrowheads denote colocalization. G-H, scale bar equals 20 μm. Data is displayed as average ± SEM.



**Figure S3. Related to Figure 3. Optogenetic reactivation of TRAPed striatal neurons, but not TRAPed S1 or M1 cortical neurons, causes dyskinesia in the absence of levodopa.** A. FosTRAP mice were injected with 6-OHDA in the striatum. B. Correlation between the average number of TRAPed cells per section vs total AIM score. C. Left: Coronal schematic of optrode recording configuration. Right: Average peak firing rate (in one second bins) achieved by optogenetically labeled TRAPed striatal neurons during 30 second blue light pulses at various light intensities. D, H, L. Schematic diagrams showing sites of AAV and 6-OHDA injection in the DLS, S1, and M1 of FosTRAP mice. E, I, M. Representative coronal sections showing colocalization of TRAP-tdTomato (left), ChR2-eYFP (middle), and merged (right) in DLS, S1, and M1. In E, note optical fiber track in DLS. F, J, N. Schematic of whole-cell recording configuration for slice validation of ChR2 (top). Light-evoked spiking of a TRAPed neuron in the striatum (F), S1 (J), or M1 (M) during illumination with 473 nm light (bottom). G. Average AIM scores during light on and off epochs for striatal reactivation experiments. K. Average AIM scores during continuous light on/off epochs and 10Hz light on/off epochs for S1 reactivation experiments. O. Average AIM scores during continuous light on/off epochs and 10Hz light on/off epochs for M1 reactivation experiments. E, I, M scale bar equals 1 mm. Data is displayed as average  $\pm$  SEM.



**Figure S4. Related to Figure 4. Optogenetic inhibition of TRAPed striatal neurons, but not a random subset of direct pathway striatal neurons, ameliorates dyskinesia.** FosTRAP (A-D) or *Drd1*-Cre mice (E-H) were treated with intrastriatal 6-OHDA and daily levodopa. The striatum was injected with DIO-eYFP or DIO-eNpHR3.0. A, E. Schematic diagram showing sites of AAV and 6-OHDA injection in the DLS of FosTRAP and D1-Cre mice, respectively. B. Representative coronal section showing colocalization of TRAP-tdTomato (left), eNpHR3.0-eYFP (middle), and merged (right) in the striatum. Optical fiber track can be seen in the area of eYFP signal. C, G. Schematic of whole-cell recording configurations for slice validation of eNpHR3.0 (left). Optical inhibition of a striatal neuron with 562 nm light (5mW, right) during a 400 pA current injection to evoke spiking. D, H. Average AIM scores during light on and off epochs for striatal inhibition experiments. F. Representative coronal section showing expression of eNpHR3.0-eYFP in D1-Cre mouse. I. Average number of eNpHR3.0-positive cells per field (adjacent to optical fiber track) in FosTRAP-eNpHR3.0 and D1-eNpHR3.0 animals. J. TRAPed cells are proposed as a specific subset of striatal neurons, composed primarily of dMSNs, that are necessary for the development of levodopa-induced dyskinesia. B, F, scale bar equals 1 mm. Data is displayed as average  $\pm$  SEM.