Supporting Information

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SI Methods

Buffers and Reagents for Dissociation. HABG: Hibernate-A (Brainbits) with 2% (vol/vol) B27 supplement (Invitrogen), 0.25% Glutamax (Invitrogen), and 1% penicillin/streptomycin. Papain buffer: Hibernate-A minus Ca^{2+} (Brainbits) with 0.25% Glutamax. Papain and DNase (Worthington product codes PAP2 and D2, respectively) were distributed as lyophilized aliquots.

Papain Dissociation of Medium Spiny Neurons for Flow Cytometry. The following protocol for dissociation of medium spiny neurons (MSNs) for flow cytometry represents the current, highest-yield version. Previous versions produced some of the data presented, but other than a reduced yield of intact neurons, they did not demonstrably affect GFP profiles. Mice were euthanized by CO_2 asphyxiation. Brains were removed and dissected immediately. One or both striata were removed and stored in room temperature HABG for no more than 20 min while remaining samples were dissected. Striata were minced in 1 mL HABG in a Petri dish using a razor, producing pieces no thicker than 0.5 mm. During dissection, the papain solution was prepared. Contents of one vial of papain were resuspended in each 5 mL of papain buffer required, and incubated at 37 °C for 20 min. Contents were then filtered (0.45 μm), and DNase was added (one vial DNase per 10 mL papain solution). Papain solution was aliquotted (1 mL per sample), and minced tissue bits were added using razor-trimmed pipette tips on a P1000 pipette, minimizing transfer of HABG [(*i*) below]. Tissue was incubated with gentle rotation at 31–32 °C for 30 min. After papain incubation, tissue pieces were transferred as above into 1–2 mL room temperature HABG and incubated for at least 5 min. Fire-polished, silanized Pasteur pipettes (end width narrowed to ∼0.8 mm) were used for trituration: Material was pipetted up and down for 10–20 rounds, ∼4 s per round, until most tissue was in suspension (*ii*). Afterward, using razor-blunted pipette tips, dissociated tissue was filtered (*iii*) (70 μm) and either used immediately for flow cytometry (after addition of propidium iodide or DAPI) or subjected to an additional BSA cushion purification step (described below). The latter was usually only done when sorting was performed, as it removes the majority of debris but sacrifices some yield, and is unnecessary for simple analysis and GFP quantitation. For further purification, dissociated tissue (∼2 mL in HABG) was gently placed on top of a 3-mL cushion of HABG plus 2% (wt/vol) BSA in a 15-mL conical tube (*iv*). The material was centrifuged at 20 × *g* (slow acceleration/deceleration) for 20 min to pellet neurons. After centrifugation, the top layer (devoid of neurons) and most of the BSA cushion layer were aspirated, leaving 0.5–1 mL of HABG + BSA. Pelleted cells were resuspended in the remaining HABG + BSA, refiltered (70 μm), and used for flow cytometry (propidium iodide or DAPI added).

We found the total time, when performed by someone experienced in the protocol, is ∼3 h for 10 samples. It may take as much as twice as long as this for a novice. Because many facilities require advanced scheduling of flow cytometers, to minimize the time neurons are kept on ice before use in the cytometer, this time should be factored in.

i) We found it worked well to bring tissue into the tip, holding the pipette vertically. The tissue pieces settle toward the bottom after a few seconds. When the tip/tissue contacts the papain solution, it will break the surface tension, and the tissue chunks will flow into the papain, minimizing HABG transfer that would happen by simply expelling the tissue into the papain solution. Gently wiggling the pipette sometimes helps. If chunks need to be knocked off the wall, a small air bubble (created by gently releasing the plunger while holding vertically out of solution) will knock the tissue off the inside of the tip, allowing it to settle for transfer as above.

ii) The details of the trituration are as follows. Using a pipette aid, tissue is drawn up; in 2 mL media, it should take ∼2 s. Tissue is expelled immediately afterward, taking ∼2 s. This cycle is repeated 10–20 times. Great care should be taken to prevent bubbles from forming (which limit yield), but the rate of drawing up and expulsion can be increased if pieces are getting small and still not breaking up. By the end, some pieces will likely remain, but this is often white matter, which is hard to break up and poor in neurons.

iii) The filtration was best accomplished using cut P1000 tips (opening ∼5 mm), holding the tip in the mesh and slowly forcing ∼800 μL through each time. A cut tip is preferable because a substantial amount of material will be caught in the filter, but a wider area of filtration prevents clogging. Care must be taken not to tear the filter membrane when holding the pipette tip in it, but it must be held in place firmly enough that material actually passes through the filter membrane rather than collecting above it.

iv) Layering the filtrate above the BSA cushion is difficult, but is made much more simple using cut P1000 tips. We recommend the following technique. The filtrate is retrieved in a cut tip, and the tip is then gently placed in contact with, but not below, the surface of the BSA cushion. Slow expulsion of the filtrate will allow it to remain above the cushion, whereas rapid expulsion forces it below the surface, preventing the formation of a clean interface. The above can be repeated until all of the filtrate is in a clean layer above the cushion. It should be noted that, should the samples be disturbed (e.g., dropped accidentally) before centrifugation, the samples can still be centrifuged as above to prevent loss of the sample. The neurons will still be visible by flow cytometry; however, debris events will be nearly as pervasive as if the BSA cushion filtration were skipped.

Small-Scale Adeno-Associated Virus Production and Purification. Viral capsids were assembled by cotransfection (TransIT LT1; Mirus) of 32 μg adeno-associated viral (AAV) vector with 96 μg AAV rep2/cap1 helper plasmid (pDP1rs; PlasmidFactory) onto a pair of 15-cm dishes of AAV-293 cells (Stratagene). Media were changed 24 h posttransfection, and virus was harvested 72 h posttransfection. First, cells were scraped, spun down, and resuspended in 5 mL PBS. Next, cells were lysed by three successive freeze-thaw cycles (freeze in dry ice/ethanol, thaw in 37 °C water bath). Finally, the lysate was clarified by spinning at 3,700 × *g* for 20 min at 4 °C. Clarified lysate was stored at −80 °C until the next purification step. AAV was purified from lysate using an iodixanol gradient. First, a discontinuous iodixanol [OptiPrep, 60% (wt/vol) iodixanol solution; Sigma] density gradient was prepared and underlayed into Beckman Ultra-Clear tubes (344322) as follows. First (top) layer, 3 mL: 15% (wt/vol) iodixanol in PBS, 1 mM $MgCl_2$ (PBSM) with 0.86 M NaCl. Second layer, 2 mL: 25% (wt/vol) iodixanol in PBSM. Third layer, 2 mL: 40% (wt/vol) iodixanol in PBSM. Fourth (bottom) layer, 2 mL: 54% (wt/vol) iodixanol in PBSM. Viral lysate was then added up to the top of the tubes, and the tubes were sealed and centrifuged at 60,000 rpm (240,000 × *g* average) for 3 h at 18 °C. Fractions (∼250 μL each) were collected

and analyzed either by silver stain or by genome copy titration quantitative (Q)PCR to identify fractions enriched with virus. Fractions were pooled, and concentration/LRS (lactated Ringer's solution) buffer exchange was carried out with two successive spins in Amicon Ultra-4 100K columns (Millipore), first at 1,500 × *g* for 25 min, then at 4,000 × *g* for 15 min, at 18 °C. Concentrated virus was titered by QPCR and used for delivery in vivo.

Stereotaxic Delivery of AAV to Mouse Striatum. Animals (4 or 5 wk of age) were given preemptive analgesia (0.1 mg/kg buprenorphine, 1.5 mg/kg meloxicam) and anesthetized (10 mg/kg xylazine, 120 mg/kg ketamine). The head was shaved, the animal was mounted into a stereotaxic frame, and the scalp was sterilized before a 1-cm midline incision to the scalp. Stereotaxic coordinates (left striatum) were 0.7 mm anterior, 1.8 mm left, and 3.5 mm ventral to bregma. A syringe (5 μL; Hamilton) carrying 3 μL virus was lowered and left in place for 5 min. Virus was injected at 0.2 μL/min, and the needle was left in place for 3 min postinjection, raised by 0.5 mm, and then left for a further 4 min before removal and wound closure. Animals were fed wet food for the first day postoperative, and were singly housed after surgery until euthanasia.

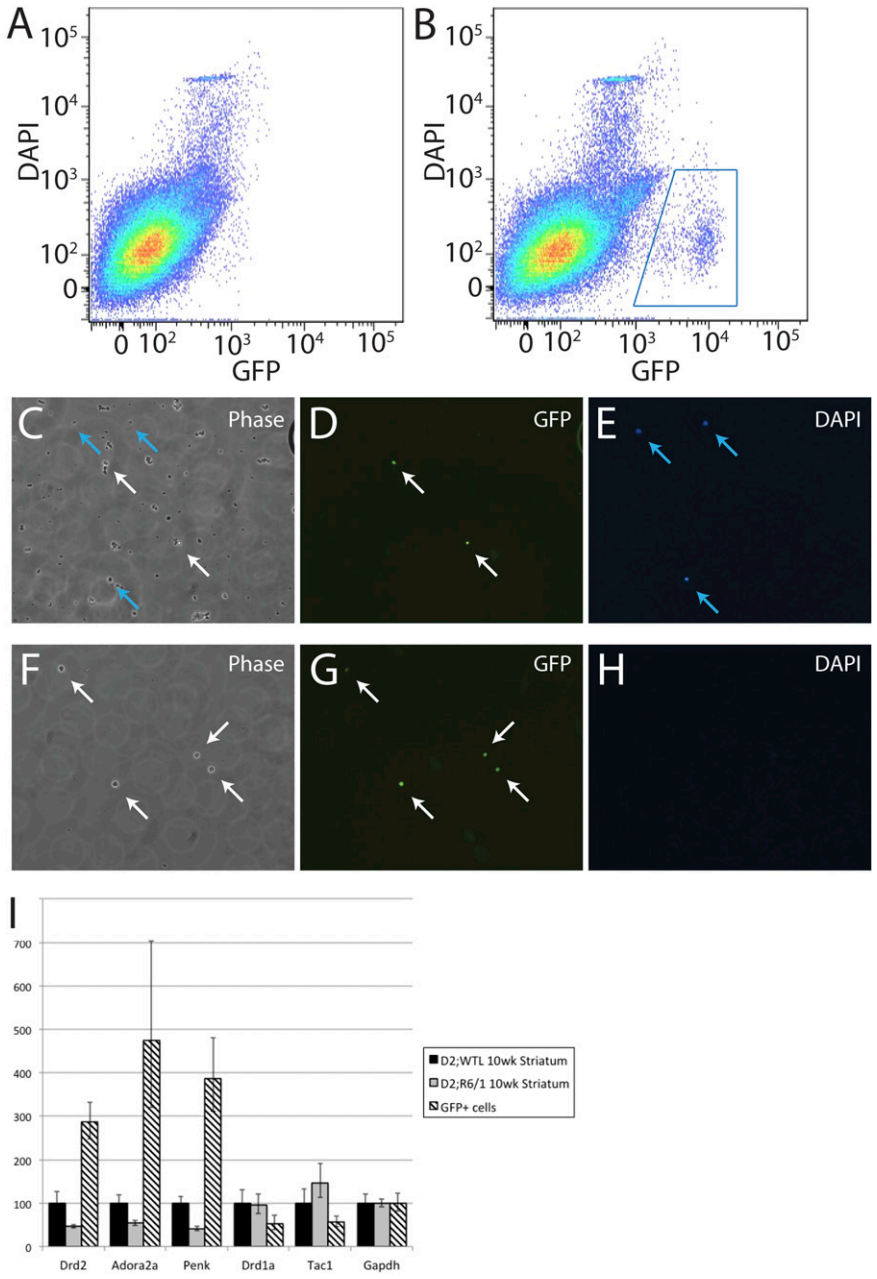

Fig. S1. Flow cytometry isolation of D2GFP medium spiny neurons. In adapting previous protocols (1), we demonstrated a population of GFP$^+$, DAPI$^-$ events in animals carrying the D2GFP (GFP driven by dopamine receptor D2 promoter elements) transgene (*B*, blue gate), absent when the transgene is not present (*A*). Before sorting, debris makes up the large majority of events (*C*), as only a few GFP$^+$ cells are visible (*D*, white arrows) along with some unintact, DAPI$^+$ cells (*E*, blue arrows). After sorting this GFP$^+$, DAPI$^-$ population, the only events visible (*F*) are GFP$^+$ (*G*, white arrows) and DAPI$^-$ (*H*; no DAPI$^+$ objects visible). (*I*) QPCR was used to compare transcripts known to be enriched in either indirect MSNs (*Drd2*, *Adora2a*, and *Penk*) or direct MSNs (*Drd1a* and *Tac1*). WTL, wild-type littermate of R6/1. D2, carrying the Drd2-GFP transgene. Error bars indicate SD of three technical replicates.

1. Lobo MK, Karsten SL, Gray M, Geschwind DH, Yang XW (2006) FACS-array profiling of striatal projection neuron subtypes in juvenile and adult mouse brains. *Nat Neurosci* 9:443–452.