SUPPLEMENTARY MATERIAL

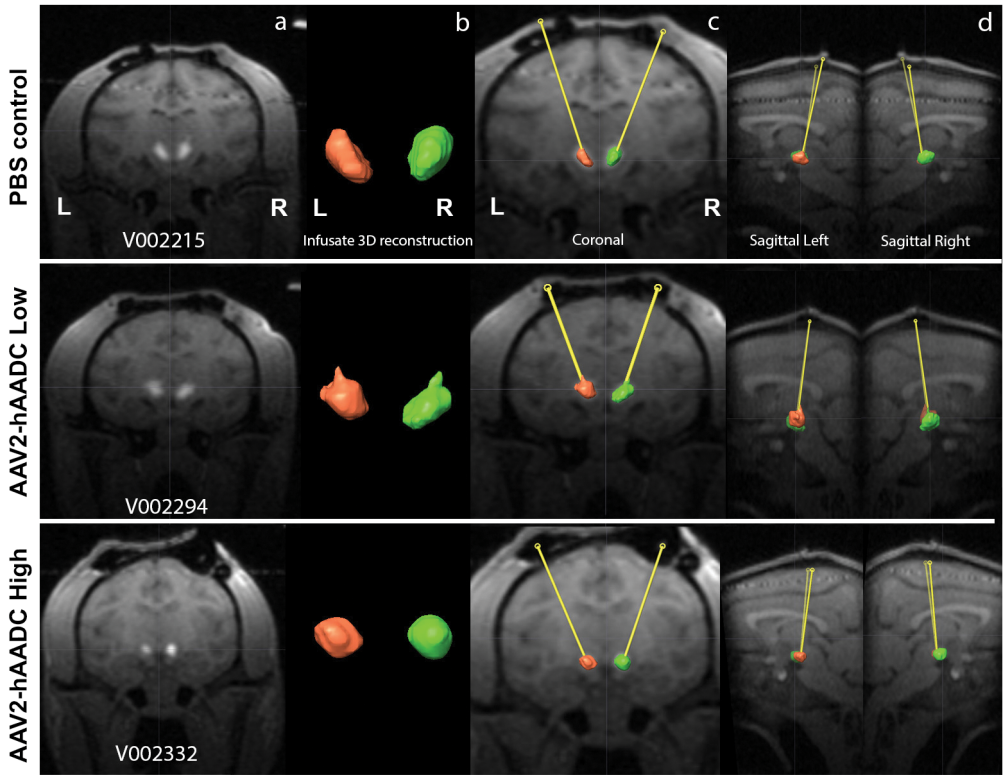


Figure S1. Three-dimensional reconstructions of representative infusions to the substantia nigra of nonhuman primates. Panel presents representative real-time T1-weighted magnetic resonance (MR) images (a) acquired at the conclusion of 30 µL of AAV2-hAADC co-infused with gadolinium for an animal of each experimental group. (b) 3D reconstructions of mid-brain infusions into the right (green) and left (orange) substantia nigra were generated. Superimposition of infusion reconstructions on MR images in the coronal (c) and sagittal (d) planes show location of infusate volume in the brain.

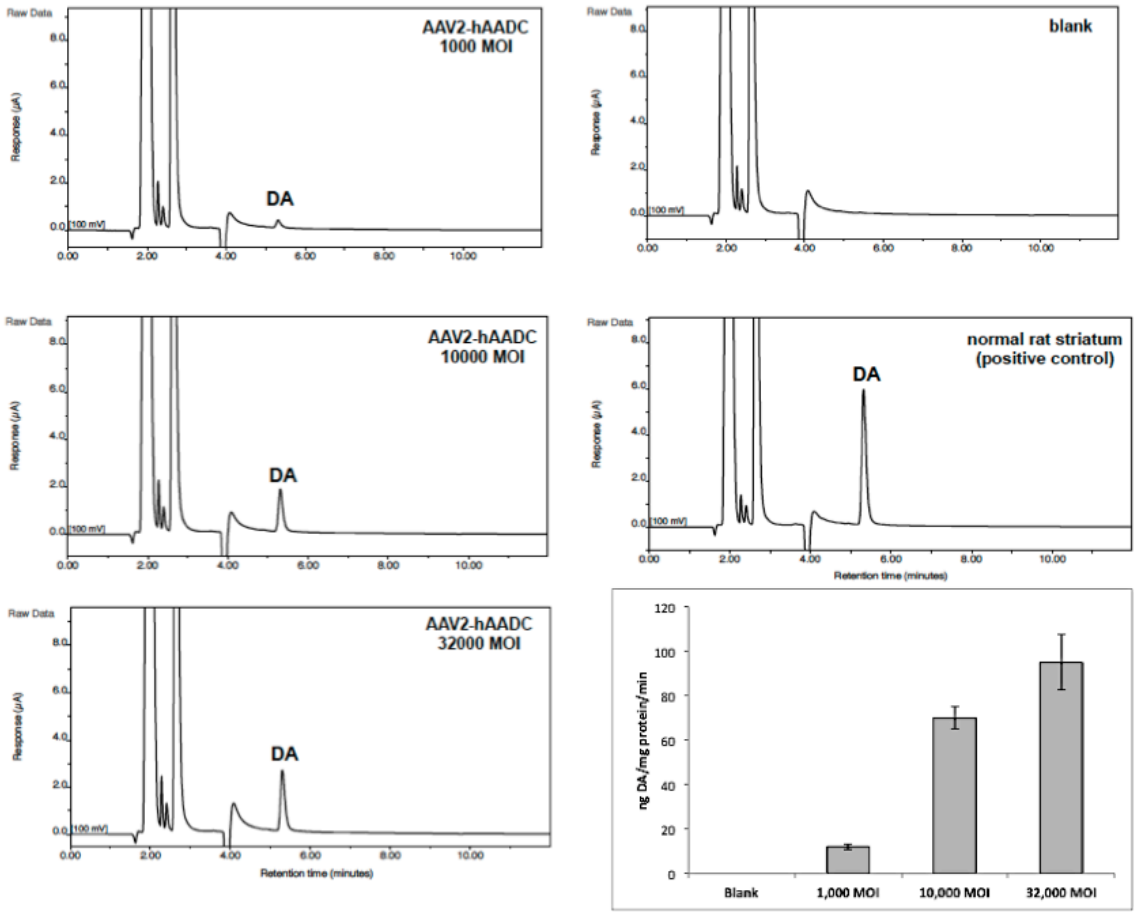


Figure S2. AAV2-driven transgenic AADC activity. Representative chromatograms show the rate of dopamine (DA) formation by AADC enzyme in the homogenates of cells transduced with 1000, 10000 and 32000 AAV2-hAADC multiplicity of infection (MOI), non-transduced cells (blank) and naïve rat striatum, which was used as a positive control. Histogram on the right shows the quantitative analysis of HPLC data (n=2 per group).

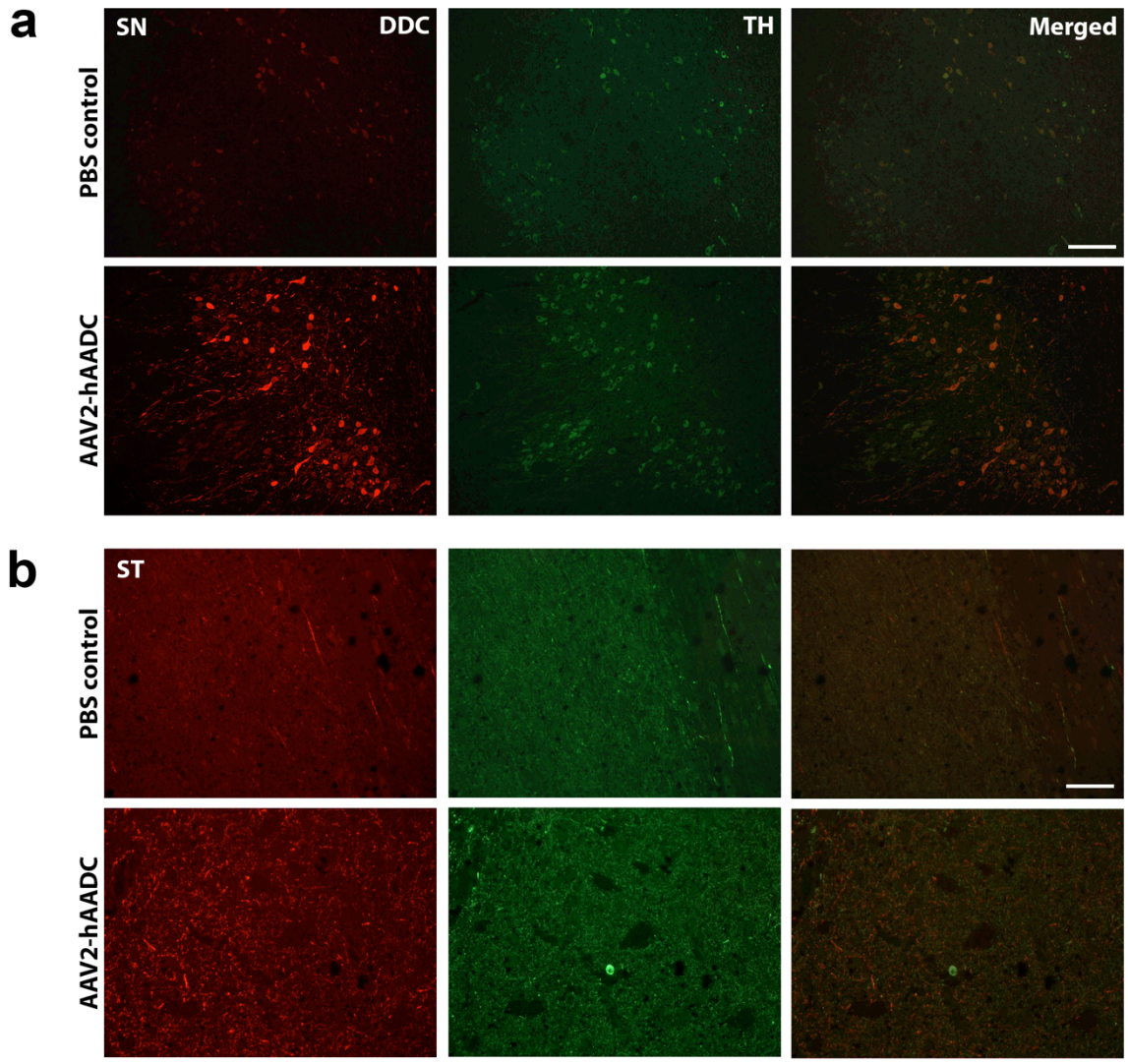


Figure S3. **AADC expression in striatum (a) and mid-brain (b) after AAV2-hAADC delivery to mid-brain.** Double immunofluorescent staining against TH and DDC in the mid-brain shows some more intensely stained nigral cell bodies in AAV2-hAADC receiving animals than in PBS-treated controls. Similarly, DDC immunofluorescence in striatum demonstrated the presence of the transgenic AADC in nigrostriatal terminals that widely covered both caudate and putamen nuclei. Images show AADC staining in transduced fibers in AAV2-hAADC-treated animals is more intense than endogenous signal in PBS control group. Scale bar: a, 200 μm; b, 400 μm.

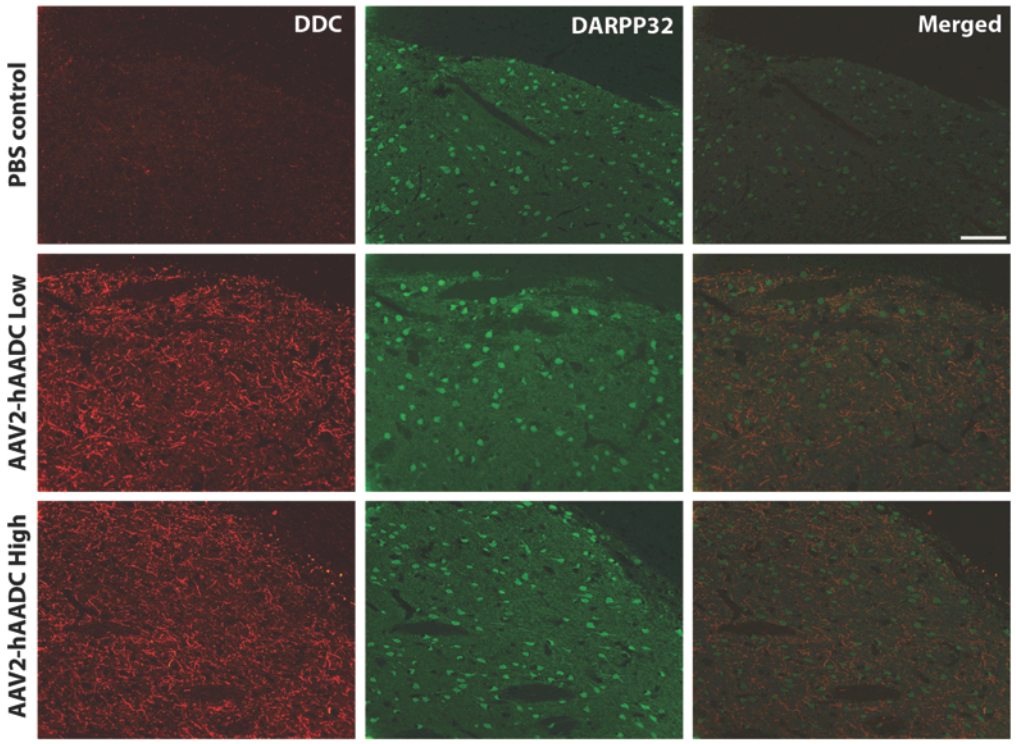
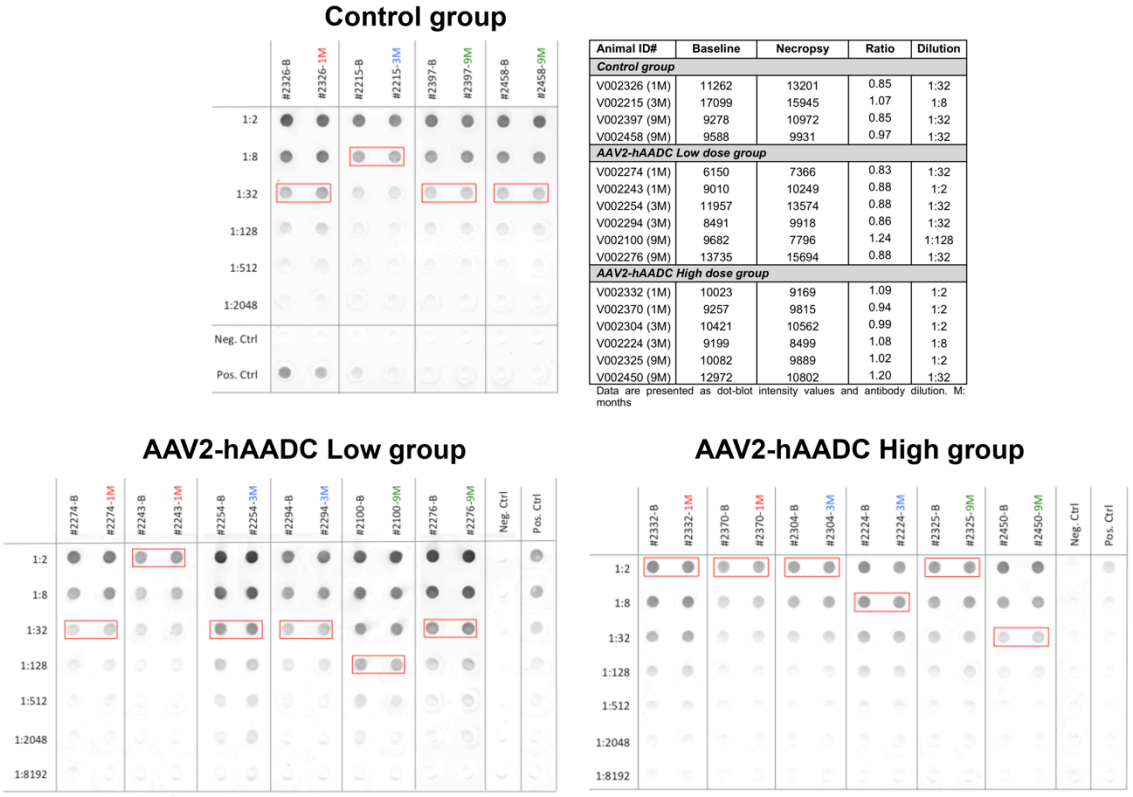


Figure S4. **Striatal medium spiny neurons do not express AADC.** Double immunofluorescent staining against DDC and DARPP32 (medium spiny neuron marker) in the striatum demonstrated the presence of the transgenic AADC in nigrostriatal terminals but not in striatal neurons. Scale bar: 200 μm.



Animal ID#	Baseline	Necropsy	Ratio	Dilution
Control group				
V002326 (1M)	11262	13201	0.85	1:32
V002215 (3M)	17099	15945	1.07	1:8
V002397 (9M)	9278	10972	0.85	1:32
V002458 (9M)	9588	9931	0.97	1:32
AAV2-hAADC Low dose group				
V002274 (1M)	6150	7366	0.83	1:32
V002243 (1M)	9010	10249	0.88	1:2
V002254 (3M)	11957	13574	0.88	1:32
V002294 (3M)	8491	9918	0.86	1:32
V002100 (9M)	9682	7796	1.24	1:128
V002276 (9M)	13735	15694	0.88	1:32
AAV2-hAADC High dose group				
V002332 (1M)	10023	9169	1.09	1:2
V002370 (1M)	9257	9815	0.94	1:2
V002304 (3M)	10421	10562	0.99	1:2
V002224 (3M)	9199	8499	1.08	1:8
V002325 (9M)	10082	9889	1.02	1:2
V002450 (9M)	12972	10802	1.20	1:32

Data are presented as dot-blot intensity values and antibody dilution. M: months

Figure S5. Lack of antibody production against AADC protein. Dot-blot assay showed that all animals presented anti-AADC antibodies at baseline. No significant increase of the anti-AADC antibody titer was detected in blood samples collected at necropsy for AAV2-hAADC-treated animals regardless of the dose. Data in table correspond to dot-blot intensity and antibody dilution. Red squares highlight dots comprehended in the intensity reading window (≤20,000 to ≥5,000). M: months.

Table S1. Vd/Vi ratios

Animal ID	Left			Right		
	Vd (mL)	**Vi (mL)**	**Vd/Vi**	**Vd (mL)**	**Vi (mL)**	**Vd/Vi**
Pilot group						
MCY32743	0.0580	0.030	1.93	0.0700	0.028	2.50
MCY34023	0.0600	0.026	2.31	0.1000	0.046	2.17
Vd/Vi (Mean±SD)		**Left**	**2.1±0.3**		**Right**	**2.3±0.2**
Control group						
V002326	N/A	0.030	N/A [a]	N/A	0.030	N/A [a]
V002215	0.0640	0.030	2.13	0.0628	0.030	2.09
V002397	0.0792	0.030	2.64	0.0744	0.030	2.48
V002458	N/A	0.030	N/A*	N/A	0.030	N/A*
AAV2-hAADC Low dose group						
V002243	0.0741	0.030	2.47	0.1009	0.030	3.36
V002274	0.0613	0.030	2.04	0.0475	0.030	1.58
V002254	0.0983	0.030	N/A [a]	0.1147	0.030	N/A [a]
V002294	0.0679	0.030	2.26	0.0728	0.030	2.43
V002100	0.0810	0.030	2.70	0.0780	0.030	2.60
V002276	0.0620	0.030	2.07	0.0687	0.030	2.29
AAV2-hAADC High dose group						
V002332	0.0603	0.030	2.01	0.0585	0.030	1.95
V002370	0.0288	0.030	0.96	0.0271	0.030	0.90
V002224	0.0664	0.030	2.21	0.0761	0.030	2.54
V002304	0.0587	0.030	1.96	0.0603	0.030	2.01
V002325 [b]	N/A	0.030	N/A	N/A	0.030	N/A
V002450	0.0660	0.030	2.20	0.0744	0.030	2.48
Vd/Vi (Mean±SD)		**Left**	**2.1±0.4**		**Right**	**2.2±0.6**

[a]: excluded due to reflux or perivascular leakage; [b]: no post-infusion scans; N/A: not available; SD: standard deviation; Vd: volume of diffusion; Vi: volume of infusate.

Table S2. Transgenic AADC expression in nigrostriatal fibers

NHP ID	Section ID	Striatum			
		Lt Cd	Lt Put	Rt Cd	Rt Put
Control group					
V002326 (1M)	F	-	-	-	-
	G	-	-	-	-
	H	-	-	-	-
	I	-	-	-	-
V002215 (1M)	F	-	-	-	-
	G	-	-	-	-
	H	-	-	-	-
	I	-	-	-	-
V002397 (3M)	F	-	-	-	-
	G	-	-	-	-
	H	-	-	-	-
	I	-	-	-	-
V002458 (9M)	F	-	-	-	-
	G	-	-	-	-
	H	-	-	-	-
	I	-	-	-	-
AAV2-hAADC Low dose					
V002243 (1M)	F	++	++	++	++
	G	+++	+++	++	++
	H	+++	+++	+++	+++
	I	+++	+++	+++	++
V002274 (1M)	F	+	+	++	++
	G	++	++	+++	+++
	H	++	++	+++	+++
	I	-	-	++	+++
V002254 (3M)	F	-	N/A	-	N/A
	G	+	+	+	+
	H	+	+	+	+
	I	++	++	++	++
V002294 (3M)	F	++	N/A	+	+
	G	+++	+++	++	++
	H	+++	+++	+++	++
	I	+++	+++	+++	+++
V002100 (9M)	F	+++	+++	+++	+++
	G	+++	++	+++	++
	H	+++	++	+++	++
	I	+++	++	+++	++
V002276 (9M)	F	++	++	++	-/+
	G	+++	++	++	++
	H	+++	+++	+++	++
	I	+++	++	+++	++

AAV2-hAADC High dose					
V002332 (1M)	F	+++	++	++	++
	G	+++	++	+++	+++
	H	+++	++	+++	+++
	I	+++	++	+++	N/A
V002370 (1M)	F	++	+	+++	+++
	G	++	++	+++	+++
	H	++	++	+++	+++
	I	+++	++	+++	+++
V002224 (3M)	F	+	+	++	++
	G	+++	++	+++	+++
	H	+++	+++	+++	+++
	I	+++	+++	+++	+++
V002304 (3M)	F	+++	++	+	+
	G	+++	++	++	+
	H	+++	+++	++	++
	I	+++	++	+++	++
V002325 (9M)	F	-	-	+	+
	G	++	++	+	-
	H	++	++	++	+++
	I	++	+	++	+
V002450 (9M)	F	++	++	+++	+++
	G	+++	+++	+++	+++
	H	+++	+++	+++	+++
	I	+++	+++	+++	+++

AADC-ir fiber staining rating: -: absent (endogenous AADC staining); +: low intensity / sparse fiber staining; ++: intermediate intensity / many positive fibers; +++: high intensity / dense fiber staining.. Abbreviations: Cd: caudate nucleus; M: months; N/A: not available; Put: putamen.

Table S3. Off-target of AADC expression in AAV2-hAADC Low dose group

NHP ID		Cx[a]	GP	Th	MFB	STN	SNpr	RN	SC	Others
V002243 (1M)	L	-	-	-	-	C (++)	-	-	-	F (+) dorsal to PPT and central tegmental field
	R	-	F (+)	F (++)/C (+)	-	C (++)	F (+)[b]	-	-	-
V002274 (1M)	L	-	-	-	-	-	-	F (+)	-	F (+) parabrachial pigmented nucleus
	R	-	-	-	-	-	F (+)[b]	F (+)	-	F (+) parabrachial pigmented nucleus
V002254 (3M)	L	-	F (+)	-	-	F/C (+)	-	-	-	F (+) corona radiata/IC
	R	-	F (+)	F/C (+)	-	F/C (+)	F (+)[b]	-	-	F (+) corona radiata/IC, reticular formation (dorsal to SN)
V002294 (3M)	L	-	-	-	-	F/C (+)	-	C (+)	F (+) Cv,T,L	F (+) corona radiata/IC C (+) central tegmental field
	R	-	F (+)	F (+)	-	F/C (+)	-	C (+)	-	C (+) central tegmental field
V002100 (9M)	L	-	-	-	-	-	-	F (+)	-	Cortical fibers
	R	-	-	-	-	-	-	F (+)	-	Occular nucleus
V002276 (9M)	L	-	-	F (++)/C (+)	-	F/C (+)	-	C (+) mag.	-	F (+) corona radiata/IC
	R	-	-	F (++)/C (+)	-	F/C (+)	-	-	-	-

AADC-ir fiber staining rating: -: absent (endogenous AADC staining); +: low intensity / sparse fiber staining; ++: intermediate intensity / many positive fibers; +++: high intensity / dense fiber staining. [a]Cortical region surrounding cannula entry point. [b]Corticospinal (thick) fibers. Abbreviations: C: cells; Cv: cervical; F: fibers; GP: globi pallidi; HTh: hypothalamus; IC: internal capsule; L: lumbar; mag.: magnocellularis; MFB: medial forebrain bundle; M: months; PPT: pedunculopontine tegmental nucleus; RN: red nucleus; SC: spinal cord; SNpc: substantia nigra pars compacta; SNpr: substantia nigra pars reticulata; STN: subthalamic nucleus; T: thoracic; Th: thalamus; VTA: ventral tegmental area.

Table S4. Off-target of AADC expression in AAV2-hAADC High dose group

NHP ID		Cx [a]	GP	Th	MFB	STN	SNpr	RN	SC	Others
V002332 (1M)	L	-	-	-	-	F/C (+)	C (++)	F (+)	-	F (+) central tegmental field
	R	-	-	-	-	F/C (+)	C (++)	F (+)	-	F (+) central tegmental field
V002370 (1M)	L	-	F (+)	F/C (+)	-	F/C (+)	F (++)		F (+) Cv,T,L	F (+) corona radiata/IC F/C (+) dorsal to SN and MFB
	R	-	F (+)	F/C (+)	-	F/C (+)	F (++)	-	-	F/C (+) dorsal to SN and MFB
V002224 (3M)	L	-	-	F (++)/C (+) along tract	F (+)	-	-	F (+)	-	Cortical fibers F (+) dorsal to SN and MFB – anterior pulvinar nucleus
	R	-	-	F (++)	-	C (+)	F (+)	F (+)	-	F (+) occular nucleus and dorsal to SN
V002304 (3M)	L	-	F/C (+)	F(++)/C (+)	-	C (++)	-	-	F (+) Cv	F (+) dorsal to PPT, central tegmental field
	R	-	F/C (+)	F (++)/C (+)	-	C (++)	F (+)[a]	-	F (+) Cv	-
V002325 (9M)	L	F/C(+)	-	C (+) along tract	C (+)	F/C (+)	-	F (+) parv./ mag.	F (++) Cv,T,L	F (+) corona radiata/IC
	R	F/C(+)	F (+)	C (+) along tract	C (+)	F/C (+)	F (+)[a]	F (+) parv./ mag.	F (++) Cv,T,L	F (+) corona radiata/IC, reticular formation (dorsal to SN)
V002450 (9M)	L	F/C(+)	F (+)	F (++)	F (++)	F/C (+)	F (+++)	C (+) mag.	-	F (+) corona radiata/IC F (++) comb system, brain stem and central tegmental field
	R	F/C(+)	F (+)	F (++)	F (++)	F/C (+)	F (+++)	F (++) C (+)	-	F (++) comb system, brain stem and central tegmental field

AADC-ir fiber staining rating: -: absent (endogenous AADC staining); +: low intensity / sparse fiber staining; ++: intermediate intensity / many positive fibers; +++: high intensity / dense fiber staining. [a]Cortical region surrounding cannula entry point. [b]Corticospinal (thick) fibers. Abbreviations: C: cells; Cv: cervical; F: fibers; GP: globi pallidi; IC: internal capsule; L: lumbar; mag.: magnocellularis; MFB: medial forebrain bundle; M: months; parv.: parvocellularis; PPT: pedunculopontine tegmental nucleus; RN: red nucleus; SC: spinal cord; SNpr: substantia nigra pars reticulata; STN: subthalamic nucleus; T: thoracic; Th: thalamus.

Table S5. Monoamine levels in the CSF

Animal ID	Dopamine		DOPAC		HVA		Serotonin		5-HIAA	
	Bsln	Ncrp	Bsln	Ncrp	Bsln	Ncrp	Bsln	Ncrp	Bsln	Ncrp
Pilot group										
MCY32743	BLQ	BLQ	10	BLQ	340	220	ND	ND	139	102
MCY34023	BLQ	BLQ	BLQ	BLQ	273	206	ND	ND	104	85
Control group										
V002326(1M)[a]	ND	BLQ	ND	BLQ	ND	149	ND	BLQ	ND	43
V002215(3M)	BLQ	BLQ	BLQ	BLQ	163	182	BLQ	BLQ	41	48
V002397(9M)	BLQ	BLQ	BLQ	BLQ	259	179	BLQ	BLQ	62	33
V002458(9M)	BLQ	BLQ	BLQ	BLQ	232	163	BLQ	BLQ	62	59
AAV2-hAADC Low dose group										
V002243(1M)	BLQ	BLQ	BLQ	BLQ	178	133	BLQ	BLQ	67	64
V002274(1M)	BLQ	11	BLQ	BLQ	191	159	BLQ	BLQ	61	56
V002254(3M)	BLQ	27	BLQ	18	169	142	BLQ	17	65	58
V002294(3M)	BLQ	BLQ	BLQ	BLQ	203	210	BLQ	BLQ	56	63
V002100(9M)	BLQ	BLQ	BLQ	BLQ	202	124	BLQ	BLQ	51	39
V002276(9M)[a]	BLQ	ND	BLQ	ND	235	ND	BLQ	ND	51	ND
AAV2-hAADC High dose group										
V002332(1M)	BLQ	BLQ	BLQ	BLQ	204	149	BLQ	BLQ	62	56
V002370(1M)	BLQ	BLQ	BLQ	BLQ	130	107	BLQ	BLQ	46	42
V002224(3M)	BLQ	BLQ	BLQ	BLQ	177	180	BLQ	BLQ	29	46
V002304(3M)	BLQ	BLQ	BLQ	BLQ	308	275	BLQ	BLQ	75	75
V002325(9M)	BLQ	BLQ	BLQ	BLQ	185	171	BLQ	BLQ	32	66
V002450(9M)	BLQ	BLQ	BLQ	BLQ	155	169	BLQ	BLQ	62	92

All data presented as ng/mL. [a]CSF sample was contaminated with blood and not analyzed. BLQ: below limit of quantification; Bsln: baseline; Ncrp: necropsy, ND: not determined.

Table S6. Serum and CSF vector DNA levels

Animal ID #	Blood serum 4 h	Blood serum necropsy	CSF necropsy
Control group			
V002326 - 1M	< 10	< 10	< 10
V002215 - 3M	< 10	< 10	< 10
V002397 - 9M	< 10	< 10	< 10
V002458 - 9M	< 10	< 10	< 10
AAV2-hAADC Low dose group			
V002243 - 1M	< 10	< 10	< 10
V002274 - 1M	< 10	< 10	< 10
V002254 - 3M	< 10	< 10	< 10
V002294 - 3M	< 10	< 10	< 10
V002100 - 9M	< 10	< 10	< 10
V002276 - 9M	< 10	< 10	< 10
AAV2-hAADC High dose group			
V002332 - 1M	1,917 ± 43	< 10	< 10
V002370 - 1M	< 10	< 10	< 10
V002224 - 3M	76 ± 25	< 10	< 10
V002304 - 3M	462 ± 70	64 ± 9	< 10
V002325 - 9M	4,794 ± 49	< 10	< 10
V002450 - 9M	< 10	59 ± 24	< 10

All data reported in DNA copies/mL; M: months

Table S7. Serum and CSF AADC protein levels

Animal ID #	Blood serum baseline	Blood serum necropsy	CSF necropsy
Control group			
V002326 - 1M	204	191	BDL
V002215 - 3M	BDL	BDL	BDL
V002397 - 9M	BDL	BDL	BDL
V002458 - 9M	575	311	278
AAV2-hAADC Low dose group			
V002243 - 1M	BDL	BDL	BDL
V002274 - 1M	BDL	BDL	BDL
V002254 - 3M	2,773	3,298	BDL
V002294 - 3M	BDL	210	278
V002100 - 9M	272	309	141
V002276 - 9M	808	1,997	BDL
AAV2-hAADC High dose group			
V002332 - 1M	BDL	BDL	BDL
V002370 - 1M	BDL	BDL	BDL
V002224 - 3M	BDL	BDL	BDL
V002304 - 3M	BDL	BDL	BDL
V002325 - 9M	2,584	17,863	512
V002450 - 9M	153	367	BDL

All data reported in pg/mL; BDL: below detection limit of 62.5 pg/mL; M: months

Table S8. Anti-AAV2 neutralizing antibody titers

Animal ID #	Baseline	Necropsy
Control group		
V002326 - 1M	<1:25	<1:25
V002215 - 3M	1:25	1:25
V002397 - 9M	1:50	not tested*
V002458 - 9M	<1:25	<1:25
AAV2-hAADC Low dose group		
V002243 - 1M	<1:25	<1:25
V002274 - 1M	<1:25	<1:25
V002254 - 3M	<1:25	<1:25
V002294 - 3M	<1:25	<1:25
V002100 - 9M	<1:25	<1:25
V002276 - 9M	<1:25	<1:25
AAV2-hAADC High dose group		
V002332 - 1M	<1:25	1:50
V002370 - 1M	<1:25	1:100
V002224 - 3M	1:25	1:1600
V002304 - 3M	<1:25	1:200
V002325 - 9M	<1:25	1:800
V002450 - 9M	<1:25	1:800

M: months, *sample contaminated

Supplementary Materials and Methods

In vitro AADC activity assay

AADC activity from AAV2-hAADC vector was determined by a cell-based in vitro assay adapted from a previously published method [1, 2]. Briefly, transfected and non-transfected cells (blank) were rinsed in ice-cold PBS and lysed in 100 µL of 0.25M sucrose solution by mechanical disruption using glass beads (0.1 mm) in a Bullet Blender (Next Advance). Positive control striatum tissue was homogenized in 10 volumes of 0.25M sucrose solution and lysed by mechanical disruption as well. After centrifugation for 20 min at 10,000 x g and 4°C, the supernatant was collected and kept on ice. To measure enzymatic conversion of L-DOPA into dopamine, 40 µL of the supernatant was added to 360 µL of an incubation mixture containing 50mM sodium phosphate buffer, pH 7.2; 0.1mM ascorbic acid, 0.1mM pyridoxal-5'-phosphate, 1mM DL-dithiothreitol, 0.1mM EDTA, and 0.1mM pargyline. Samples were pre-incubated at 37°C for 5 min and the reaction was initiated by addition of 20 µL of L-DOPA (Sigma, St. Louis, MO; 0.3mM final concentration) and allowed to proceed for 30 min at 37°C, whereupon it was stopped by addition of 80 µL ice-cold 0.4M perchloric acid. The mixture was then centrifuged at 3000 x g for 10 min. The supernatant was stored at -80°C until further HPLC analysis. Non-enzymatic conversion was determined by substituting water for the L-DOPA substrate. Some homogenates were spiked with L-DOPA solution and benserazide (0.01mM) to serve as a control.

HPLC analysis

Dopamine was determined by HPLC coupled to an electrochemical detector (ECD). Supernatant (30 µL) was injected onto the HPLC-ECD system (CoulArray 5600A; ESA, Chelmsford, MA) and was separated on a reverse-phase, analytical column MD-150X3.2 (ESA, Chelmsford, MA) with a mobile MD-TM phase flow rate of 0.5 mL/min. Samples were quantified by comparison with a DA standard solution(Sigma-Aldrich, St. Louis, MO) of known concentration. HPLC-ECD software was used to quantify the area under the peaks. The DC protein assay kit (Bio-Rad) was used to determine the protein concentration in the primary supernatant (homogenate in 0.25M sucrose). AADC activity was expressed as ng DA/min/mg protein.

1. Nagatsu, T, Yamamoto, T, Kato, T (1979). A new and highly sensitive voltammetric assay for aromatic L-amino acid decarboxylase activity by high-performance liquid chromatography. Anal Biochem 100:160-165.
2. Jonkers, N, Sarre, S, Ebinger, G, Michotte, Y (2001). Benserazide decreases central AADC activity, extracellular dopamine levels and levodopa decarboxylation in striatum of the rat. J Neural Transm 108:559-570.