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Parkin is protective for substantia nigra dopamine neurons in a tau gene transfer neurodegeneration model

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Abstract

Parkin is a ubiquitin ligase involved in the ubiquitin-proteasome system. Elevating parkin expression in cells reduces markers of oxidative stress while blocking parkin expression increases oxidative stress. In parkin gene knock down mouse and fly models, mitochondria function is deficient. Parkin is neuroprotective against a variety of toxic insults, while it remains unclear which of the above properties of parkin may mediate the protective actions. One of the models for which parkin is protective is overexpression of alpha-synuclein, a protein that self-aggregates in Parkinson disease. The microtubule-associated protein tau is another protein that self-aggregates in specific neurodegenerative diseases that also involve loss of dopamine neurons such as frontotemporal dementia with parkinsonism linked to chromosome 17, progressive supranuclear palsy and corticobasal degeneration. We recently developed a tau-induced dopaminergic degeneration model in rats using adeno-associated virus vectors. In this study, we successfully targeted either a mixed tau/parkin vector or mixed tau/control vector to the rat substantia nigra. While there was significant loss of dopamine neurons in the tau/control group relative to uninjected substantia nigra, there was no cell loss in the tau/parkin group. We found no difference in total tau levels between tau/control and tau/parkin groups. Parkin therefore protects dopamine neurons against tau as it does against alpha-synuclein, which further supports parkin as a therapeutic target for diseases involving loss of dopamine neurons.

Keywords

Adeno-associated virus; Gene therapy; Neurodegenerative disease; Parkin; Substantia nigra; Tau

Parkin is an E3 ubiquitin ligase involved in the ubiquitin-proteasome system (UPS) [3]. Defective parkin function is associated with neurodegeneration because familial mutations that result in loss of ubiquitin ligase function result in autosomal recessive juneville Parkinson disease (PD) [3]. Because the post-mortem neuropathology of this form of PD generally lacks Lewy bodies, the intraneuronal aggregates of alpha-synuclein among other proteins, parkin appears to be important in the aggregation of alpha-synuclein and other proteins [3]. Parkin also appears to be a pan-neuroprotective agent against a number of different toxic insults including elevated expression of substrates for parkin ubiquitination [15,22,29,30] as well as

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other toxins that are not thought to be related to the UPS [2,9,17,26]. Increasing parkin expression reduces oxidative damage [10] while blocking parkin expression increases oxidative damage [7,19], which may explain the general protection from parkin against a variety of insults. The effects of parkin on markers of oxidative stress may be a result of parkin's role in mitochondria function as parkin knockout transgenic mice [19] and flies [8] have deficient mitochondria. Parkin loss-of-function mutants resulted in degeneration of dopaminergic neurons which could be rescued by increased glutathione s-transferase expression in transgenic flies [28]. The oxidative damage that occurs with loss of parkin function is therefore amenable to therapeutic intervention.

The microtubule-associated protein tau is damaging to neurons when it gets hyperphosphorylated and aggregates into filaments [1,5]. The filaments form neurofibrillary tangles (NFT) in the cytoplasm in many neurodegenerative diseases such as Alzheimer disease (AD), frontotemporal dementia with parkinsonism associated to chromosome 17 (FTDP-17), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) [1,5]. Tau is thought to be a causal factor for neurodegeneration in these tangle-related diseases. Overexpressing a mutant form of tau (P301L) that is associated with FTDP-17, in transgenic mice leads to pronounced degeneration and NFT formation in the CNS [14]. Tau expression and NFT formation can be targeted to either the cholinergic basal forebrain [13] or the dopaminergic substantia nigra (SN) [12] of the rat with a viral vector approach, the latter causing significant loss of dopamine neurons [12]. Because there is tau pathology in the SN in AD [24], FTDP-17 [18], PSP [23] and CBD [4,27], and SN dopaminergic degeneration occurs in FTDP-17 [18], PSP [23] CBD [27], the tau-induced degeneration targeted to the SN in rats mimics some features of, and provides a model for, diseases with neurofibrillary degeneration.

One of the treatments for which parkin is protective is mutant alpha-synuclein, which has been demonstrated in several systems including neuronal cultures, transgenic flies, and rats injected with viral vectors for alpha-synuclein [15,22,30]. Like tau, alpha-synuclein self-aggregates in specific neurode-generative diseases [3,5]. The respective pathologies for tau or alpha-synuclein, NFTs or Lewy bodies, are frequently found co-expressed in several neurodegenerative diseases [5,6], and the two proteins may interact in a pathological manner [6]. Although there is evidence that alpha-synuclein [25] but not tau [21] is ubiquitinated by parkin, we hypothesized whether the general neuroprotective actions of parkin apply to tau-induced dopaminergic degeneration in the SN because of the similarities of alpha-synuclein and tau in neurodegeneration and because parkin and tau interact in vitro [21].

Plasmids for the reporter control green fluorescent protein (GFP) or the P301L form of human tau including exons 2, 3 and 10 (four repeat microtubule-binding domains, 4R2N) were described [13]. The expression cassette used to drive expression has the hybrid cytomegalovirus/chicken β-actin promoter and the 3′ enhancer woodchuck hepatitis virus post-transcriptional regulatory element all flanked by AAV2 terminal repeats. A cDNA for human parkin was provided by M. Farrer (Mayo Clinic Jacksonville, FL) and inserted into the AAV cassette. The plasmids were packaged in recombinant AAV2 by described methods [12]. Human embryonic kidney 293 cells were co-transfected with one of the AAV2 terminal repeat-containing plasmids and the AAV2 packaging plasmid. The cell lysate was applied to a discontinuous gradient of iodixinol (OptiPrep, Greiner Bio-One, Longwood, FL) and centrifuged. The AAV was then removed and concentrated and washed using Millipore (Billerica, MA) Biomax 100 Ultrafree-15 units. AAV vector stocks were titered for physical particles, or copies of vector genomes, by dot-blotting against standard curves of known amounts of DNA using non-radioactive Psoralen-Biotin and BrightStar kits from Ambion (Austin, TX). Male Sprague–Dawley rats (3 months old, from Harlan, Indianapolis, IN) were anesthetized with a cocktail of 3 ml xylazine (20 mg/ml, from Butler, Columbus, OH), 3 ml ketamine (100 mg/ml, from Fort Dodge Animal Health, Fort Dodge, IA), and 1 ml

acepromazine (10 mg/ml, from Boerhinger Ingelheim, St. Joseph, MO) administered intramuscularly at a dose of 1 ml/kg. Viral stocks were injected using a microinjection pump as described [12]. The stereotaxic injection coordinates for the SN were 5.3 mm posterior, 2.1 mm lateral, 7.6 mm ventral [20] with a dose of 2×10^{10} AAV particles in 4 μl per injection. All animal care and procedures were in accordance with institutional IACUC and NIH guidelines.

Animals received parkin vector alone or a mixture of tau/GFP or tau/parkin vectors with a total of 34 rats in the study. Vector-derived parkin expression was confirmed by infecting 293 cells (1×10^6 cells treated with 4×10^{10} particles; cells harvested 4 days later) and injecting the SN with the parkin vector (2×10^{10} particles; survival interval 12 weeks). The soluble fraction from cells or the brain was prepared for westerns as described [12,13]. Samples were normalized for protein content by Bradford assay and subjected to 4–20% SDS/PAGE. Antibodies for immunoblots were polyclonal E1 for human tau (1:1000), parkin from Cell Signaling (1:1000), ubiquitin from DAKO (1:1000), and GAPDH from Sigma (1:1000). The soluble fraction was prepared from rat SN by previously described methods [21] and tau levels were compared with and without parkin co-expression. For peroxidase immunostaining, anesthetized animals were perfusion fixated and processed as described [12]. Primary antibodies for immunostaining included: Tau (human-specific from Zymed, 1:2000); parkin (Cell Signaling, 1:1000, GFP (Molecular Probes, 1:25000), TH (Pel-Freeze, 1:2000). The number of SN pars compacta neurons expressing TH immunoreactivity was estimated by unbiased stereology using the Micro-Brightfield Inc. (Williston, VT) system as described [12]. Eight sections evenly spaced throughout the SN pars compacta structure were analyzed for each probe. Optical dissectors were 50 μm × 50 μm × 16 μm cubes spaced in a systematic random manner 150 μm × 150 μm apart and offset 2 μm from the section surface. The fractionator sampling was optimized to yield about 150 counted cells per animal, for Gundersen error coefficients <0.10. To test for a modulating effect of parkin, a 1:1 equal mixture of tau/GFP vector was injected on one side and a 1:1 equal mixture of tau/parkin was injected on the other in nine rats. The tissues were processed 5 weeks after the injections, a sufficient interval for tau-induced cell loss in this model [12]. The two sides were compared pairwise (*t*-test) and also with a group of five untreated treats by ANOVA/Dunnett's multiple comparison test.

Infecting the 293 cells with the AAV2 parkin resulted in robust expression of ~50 kDa band on parkin immunoblots not seen in controls (Fig. 1A). In dissected SN, the AAV2 parkin also produced consistent transgene product expression on westerns, although all rat brain samples contained much less of a band at ~44 kDa, consistent with endogenous rat parkin (Fig. 1B). Targeting of vector-derived parkin expression to the SN was confirmed by immunostaining (Fig. 1C). We tested whether the parkin expression led to changes in overall protein ubiquitination with ubiquitin immunoblots with the samples from Fig. 1B. Changes in the amount of ubiquitinated species were not detected in the parkin group relative to control (3/group, not shown).

The human parkin expression in the SN was combined with expression of human P301L tau by mixing the 2 vectors 1:1. This clearly led to co-expression of both parkin and tau as evidenced with immunostaining (Fig. 2). While co-localization with double labeling was not attempted, the same regions of the SN expressed both parkin and tau as adjacent sections had many positive cells for either marker with overlapping expression pattern (Fig. 2A–D).

We tested if parkin could protect dopaminergic neurons in our tau-induced degeneration model in the rat SN. There was a visible trend of more TH staining on the side of the parkin co-expression as in Fig. 2E. Stereological analysis of TH neurons in the SN showed more neurons remaining on the side of the tau/parkin injections relative to the side with tau/GFP (Table 1). We also compared a group of uninjected controls, which had the expected stereological

estimates of ~10,000 dopamine neurons/SN [12]. Consistent with a lesioning effect of the tau, there were significantly less cells in the tau/GFP group than in the uninjected group. However, consistent with a protective effect of parkin, the tau/parkin group did not differ from uninjected control (Table 1).

A possible mechanism for parkin to protect neurons from tau damage would be targeting tau for protein degradation, because of its ubiquitin ligase activity in the UPS. We attempted to prepare both soluble and aggregated insoluble tau, but failed to detect human tau in the insoluble fractions in this study, probably because insufficient amount of starting material for fractionation. We evaluated the mixed vector samples for soluble tau levels with 5/group (Fig. 3). We observed human-specific tau expression in both the tau/GFP and tau/parkin vector groups, and vector-derived parkin expression only in the ones injected with the tau/parkin mixture, as expected. The tau blots were stripped and reprobed for GAPDH and the bands were analyzed using the Scion (Frederick, MD) imaging program. Levels of tau were expressed as ratio to GAPDH to normalize protein loading. There was no significant difference in tau levels between the tau/GFP and tau/parkin groups (*t*-test); however a trend in lower tau levels was observed in the tau/parkin group.

It was clear that the tau/GFP group underwent significant loss of SN dopamine neurons while the tau/parkin group did not. Parkin therefore appears to also protect against tau-related neurotoxicity as it does against alpha-synuclein [15,22,29,30] and other toxic proteins and chemicals [2,9,17,26]. The tau-induced lesion in this study was partial compared to what is possible with higher doses of AAV2 tau [12] and especially the more efficient AAV8 tau vectors [11] which produce motor deficits. For instance, we ran the bilaterally injected rats for amphetamine-stimulated rotational behavior one month after by described methods [12] and found no side-to-side differences. The side-to-side difference in the number of cells in the SN was not sufficient to be detected behaviorally and we would like to test if parkin is also protective against larger, behaviourally significant tau vector lesions in the future. We investigated two potential mechanisms in this study, ubiquitination and tau degradation. The ubiquitin western blots suggested that widespread ubiquitination was not involved, although the method may not have been sufficiently specific for analyzing just the transduced cells. We did not observe an effect of parkin on tau degradation although the data suggest that this issue may be worth studying further in the future. The relationship of parkin levels and oxidative stress [7,10,19] leads to the hypothesis of an oxidative damage mechanism in this animal model of neurofibrillary disease. While it remains unclear how parkin can protect against tau or the other toxic treatments for which it is protective including proteotoxic stress and alpha-synuclein induced degeneration [2,9,15,17,22,26,29,30], the protective property is important because there are no existing methods to inhibit tau-induced neurodegeneration with drugs. The tau-related diseases such as AD, FTDP-17, CBD and PSP are pernicious and basically untreatable, so targeting enzymes such as parkin or other enzymes that may process tau and block its toxicity could potentially be worthwhile for developing a new treatment. Clinical trials for specific neurodegenerative diseases are underway and/or being pursued with AAV vectors [16]. The preclinical data showing efficacy of parkin gene transfer against alpha-synuclein induced [15,29], six-hydroxydopamine induced [17] and tau-induced dopaminergic neurodegeneration support further exploration of parkin as a therapeutic target for diseases involving loss of dopamine neurons such as PD, FTDP-17, CBD and PSP.

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References

1. Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res. Brain Res. Rev 2000;33:95–130. [PubMed: 10967355]
2. Darios F, Corti O, Lucking CB, Hampe C, Muriel MP, Abbas N, Gu WJ, Hirsch EC, Rooney T, Ruberg M, Brice A. Parkin prevents mitochondrial swelling and cytochrome c release in mitochondria-dependent cell death. Hum. Mol. Genet 2003;12:517–526. [PubMed: 12588799]
3. Dawson TM, Dawson VL. Molecular pathways of neurodegeneration in Parkinson's disease. Science 2003;302:819–822. [PubMed: 14593166]
4. Di Maria E, Tabaton M, Vigo T, Abbruzzese G, Bellone E, Donati C, Frasson E, Marchese R, Montagna P, Munoz DG, Pramstaller PP, Zanusso G, Ajmar F, Mandich P. Corticobasal degeneration shares a common genetic background with progressive supranuclear palsy. Ann. Neurol 2000;47:374–377. [PubMed: 10716259]
5. Dickson DW. Tau and synuclein and their role in neuropathology. Brain Pathol 1999;9:657–661. [PubMed: 10517505]
6. Giasson BI, Forman MS, Higuchi M, Golbe LI, Graves CL, Kotzbauer PT, Trojanowski JQ, Lee VM. Initiation and synergistic fibrillization of tau and α-synuclein. Science 2003;300:636–640. [PubMed: 12714745]
7. Greene JC, Whitworth AJ, Andrews LA, Parker TJ, Pallanck LJ. Genetic and genomic studies of Drosophila parkin mutants implicate oxidative stress and innate immune responses in pathogenesis. Hum. Mol. Genet 2005;14:799–811. [PubMed: 15689351]
8. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. Mitochondrial pathology and apoptotic muscle degeneration in Drosophilia parkin mutants. Proc. Natl. Acad. Sci. U.S.A 2003;100:4078–4083. [PubMed: 12642658]
9. Hyun DH, Lee M, Halliwell B, Jenner P. Effect of overexpression of wild-type or mutant parkin on the cellular response induced by toxic insults. J. Neurosci. Res 2005;82:232–244. [PubMed: 16130151]
10. Hyun DH, Lee M, Hattori N, Kubo S, Mizuno Y, Halliwell B, Jenner P. Effect of wt or mutant Parkin on oxidative damage, nitric oxide, antioxidant defenses, and the proteasome. J. Biol. Chem 2002;277:28572–28577. [PubMed: 12034719]
11. Klein RL, Dayton RD, Leidenheimer NJ, Jansen K, Golde TE, Zweig RM. Efficient neuronal gene transfer with AAV8 leads to neurotoxic levels of tau or green fluorescent proteins. Mol. Therapy 2006;13:517–527.
12. Klein RL, Dayton RD, Lin WL, Dickson DW. Tau gene transfer, but not alpha-synuclein, induces both progressive dopamine neuron degeneration and rotational behavior in the rat. Neurobiol. Dis 2005;20:64–73. [PubMed: 16137567]
13. Klein RL, Lin WL, Dickson DW, Lewis J, Hutton M, Duff K, Meyer EM, King MA. Rapid neurofibrillary tangle formation after localized gene transfer of mutated tau. Am. J. Pathol 2004;164:347–353. [PubMed: 14695347]
14. Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, Gwinn-Hardy K, Murphy MP, Baker M, Yu X, Duff K, Hardy J, Corral A, Lin WL, Yen SH, Dickson DW, Davies P, Hutton M. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expression mutant (P301L) tau protein. Nat. Genet 2000;25:402–405. [PubMed: 10932182]
15. Lo Bianco C, Schneider BL, Bauer M, Sajadi A, Brice A, Iwatsubo T, Aebischer P. Lentiviral vector delivery of parkin prevents dopaminergic degeneration in an alpha-synuclein rat model of Parkinson's disease. Proc. Natl. Acad. Sci. U.S.A 2004;101:17510–17515. [PubMed: 15576511]
16. Mandel RJ, Burger C. Clinical trials in neurological disorders using AAV vectors: promises and challenges. Curr. Opin. Mol. Ther 2004;6:482–490. [PubMed: 15537049]
17. Manfredsson FP, Lewin AS, Muzyczka N, Burger C, Mandel RJ. rAAV-mediated nigral parkin over-expression is neuroprotective in the 6-OHDA rat model of Parkinson's disease. Mol. Therapy 2005;11 (Suppl. 1):24–25.
18. Mirra SS, Murrell JR, Gearing M, Spillantini MG, Goedert M, Crowther RA, Levey AI, Jones R, Green J, Shoffner JM, Wainer BH, Schmidt ML, Trojanowski JQ, Ghetti B. Tau pathology in a family

with dementia and a P301L mutation in tau. J. Neuropathol. Exp. Neurol 1999;58:335–345. [PubMed: 10218629]

19. Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M, Klose J, Shen J. Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. J. Biol. Chem 2004;279:18614–18622. [PubMed: 14985362]
20. Paxinos, G.; Watson, C. The Rat Brain in Stereotaxic Coordinates. Academic Press; Sydney: 1986.
21. Petrucelli L, Dickson D, Kehoe K, Taylor J, Snyder H, Grover A, De Lucia M, McGowan E, Lewis J, Prihar G, Kim J, Dillmann WH, Browne SE, Hall A, Voellmy R, Tsuboi Y, Dawson TM, Wolozin B, Hardy J, Hutton M. CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. Hum. Mol. Genet 2004;13:703–714. [PubMed: 14962978]
22. Petrucelli L, O'Farrell C, Lockhart RJ, Baptista M, Kehoe K, Vink L, Choi P, Wolozin B, Farrer M, Hardy J, Cookson MR. Parkin protects against the toxicity associated with mutant a-synuclein: protea-some dysfunction selectively affects catecholaminergic neurons. Neuron 2002;36:1007–1019. [PubMed: 12495618]
23. Poorkaj P, Muma NA, Zhukareva V, Cochran EJ, Shannon KM, Hurtig H, Koller WC, Bird TD, Trojanowski JQ, Lee VM, Schellenberg GD. An R5L tau mutation in a subject with a progressive supranuclear palsy phenotype. Ann. Neurol 2002;52:511–516. [PubMed: 12325083]
24. Schneider JA, Bienias JL, Gilley DW, Kvarnberg DE, Mufson EJ, Bennett DA. Improved detection of SN pathology in Alzheimer's disease. J. Histochem. Cytochem 2002;50:99–106. [PubMed: 11748299]
25. Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trocken-backer A, Schneider R, Mizuno Y, Kosik KS, Selkoe DJ. Ubiquitination of a new form of a-synuclein by parkin from human brain: implications for Parkinson's disease. Science 2001;293:263–269. [PubMed: 11431533]
26. Staropoli JF, McDermott C, Martinat C, Schulman B, Demireva E, Abeliovich A. Parkin is a component of an SCF-like ubiquitin ligase complex and protects postmitotic neurons from kainate excitotoxicity. Neuron 2003;37:735–749. [PubMed: 12628165]
27. Wakabayashi K, Oyanagi K, Makifuchi T, Ikuta F, Homma A, Homma Y, Horikawa Y, Tokiguchi S. Corticobasal degeneration: etiopathological significance of the cytoskeletal alterations. Acta Neuropathol 1994;87:545–553. [PubMed: 8091948]
28. Whitworth AJ, Theodore DA, Greene JC, Benes H, Wes PD, Pallanck LJ. Increased glutathione S-transferase activity rescues dopaminergic neuron loss in a Drosophilia model of Parkinson's disease. Proc. Natl. Acad. Sci. U.S.A 2005;102:8024–8029. [PubMed: 15911761]
29. Yamada M, Mizuno Y, Mochizuki H. Parkin gene therapy for alpha-synucleinopathy: a rat model of Parkinson's disease. Hum. Gene Ther 2005;16:262–270. [PubMed: 15761265]
30. Yang Y, Nishimura I, Imai Y, Takahashi R, Lu B. Parkin suppresses dopaminergic neuron-selective neurotoxicity induced by Pael-R in Drosophila. Neuron 2003;37:911–924. [PubMed: 12670421]

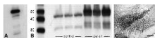

Fig. 1.

Parkin gene delivery with AAV vector. (A) Parkin immunoblot of soluble fraction from human embryonic kidney 293 cells treated with the human parkin AAV 4 days earlier. The ~50 kDa band was not observed in untreated or control GFP AAV treated cells under these blotting conditions. (B) Parkin immunoblot of dissected rat substantia nigra that were injected with control GFP or human parkin AAV vectors 12 weeks earlier at a dose of 2×10^{10} particles (3 rats/group, 50 μg protein loaded in each lane) showing parkin transgene product expression in the parkin vector group. Size markers in kDa in first lane. (C) Substantia nigra section from a parkin AAV-injected rat as in B processed for parkin immunostaining. Parkin staining in uninjected or GFP AAV injected tissues was blank under these conditions. Bar = 125 μm.

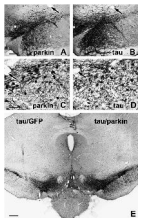

Fig. 2.

Tau/parkin co-expression and effect on dopamine neurons. (A) Parkin immunostaining in substantia nigra 5 weeks after injecting a mixture of tau/parkin AAV. (B) Adjacent section processed for human tau immunostaining. (C) Higher magnification of parkin immunostaining. (D) Higher magnification of tau immunostaining. Arrows point to the same blood vessel in A, B or C, D demonstrating co-expression on adjacent sections. (E) Tyrosine hydroxylase staining in midbrain. The tau/parkin AAV mixture was injected on one side (right side of panel) and the tau/GFP AAV mixture on the other at a total dose of 2×10^{10} particles in either case 5 weeks earlier. There were more tyrosine hydroxylase-positive neurons on the side of parkin co-expression (see Table 1). Bars, A = 100 μm; C=25 μm; E = 190 μm; A, B same magnification; C, D same magnification.

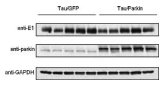

Fig. 3.

Levels of soluble tau with or without parkin co-expression. Human tau (E1) was detected at ~66 kDa in rats receiving a tau/GFP mixed vector or a tau/parkin mixture, and human parkin was detected at ~50 kDa only in the tau/parkin group as expected, with much lower levels of a ~44 kDa band consistent with rat parkin in both groups. The tau bands were normalized to the protein band for GAPDH in the same lane and compared for the tau/GFP and tau/parkin groups. No difference was found (*t*-test; $N = 5$/group).

Table 1

Parkin protects from tau-induced loss of dopamine neurons

Group	Dose (particles)	*N*	TH neurons	Comparison
Uninjected	–	5	9682 ± 143	
Tau/GFP AAV	2×10^{10}	9	7495 ± 558	$P < 0.01$ Tau/Parkin or uninjected
Tau/Parkin AAV	2×10^{10}	9	9264 ± 335	N.S. uninjected

Number of SN pars compacta neurons (±S.E.M.) immunoreactive for tyrosine hydroxylase (TH) estimated by stereology 5 weeks after injections in the case of the AAV vector groups. The tau/GFP was injected on the side contralateral to the tau/parkin in nine animals. There were fewer cells on the tau/GFP side relative to the tau/parkin side (paired *t*-test, $P < 0.01$). When an uninjected group was compared, there were fewer cells in the tau/GFP group relative to control but not in the tau/parkin group (ANOVA and Dunnett's multiple comparison test, $P < 0.01$).