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DOPA Decarboxylase Modulates Tau Toxicity

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ABSTRACT

BACKGROUND: The microtubule-associated protein tau accumulates into toxic aggregates in multiple neurodegenerative diseases. We found previously that loss of D_2-family dopamine receptors ameliorated tauopathy in multiple models including a *Caenorhabditis elegans* model of tauopathy.

METHODS: To better understand how loss of D_2-family dopamine receptors can ameliorate tau toxicity, we screened a collection of *C. elegans* mutations in dopamine-related genes (n = 45) for changes in tau transgene–induced behavioral defects. These included many genes responsible for dopamine synthesis, metabolism, and signaling downstream of the D_2 receptors.

RESULTS: We identified one dopamine synthesis gene, DOPA decarboxylase (DDC), as a suppressor of tau toxicity in tau transgenic worms. Loss of the *C. elegans* DDC gene, *bas-1*, ameliorated the behavioral deficits of tau transgenic worms, reduced phosphorylated and detergent-insoluble tau accumulation, and reduced tau-mediated neuron loss. Loss of function in other genes in the dopamine and serotonin synthesis pathways did not alter tau-induced toxicity; however, their function is required for the suppression of tau toxicity by *bas-1*. Additional loss of D_2-family dopamine receptors did not synergize with *bas-1* suppression of tauopathy phenotypes.

CONCLUSIONS: Loss of the DDC *bas-1* reduced tau-induced toxicity in a *C. elegans* model of tauopathy, while loss of no other dopamine or serotonin synthesis genes tested had this effect. Because loss of activity upstream of DDC could reduce suppression of tau by DDC, this suggests the possibility that loss of DDC suppresses tau via the combined accumulation of dopamine precursor levodopa and serotonin precursor 5-hydroxytryptophan.

Keywords: Aromatic amino acid decarboxylase, DOPA decarboxylase, Dopamine, Neurodegeneration, Serotonin, Tau

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Tauopathies are neurodegenerative diseases characterized by the toxic accumulation of abnormal conformers of the microtubule-associated protein tau (1). Alzheimer's disease and frontotemporal lobar degeneration are the most commonly occurring tauopathies (2). Various clinical strategies are being pursued to reduce tau toxicity, including reducing tau phosphorylation, tau cleavage, tau fibrillization, and tau expression levels (2). However, many candidate therapies have issues of nonspecificity and safety. There are currently no treatments that halt or reverse the accumulation of pathological tau in human patients (2).

We previously identified D_2-like dopamine receptor antagonist drugs ameliorating tauopathy phenotypes using a whole animal *Caenorhabditis elegans* model of tau toxicity (3). Pan-neuronal expression of human tau in *C. elegans* causes neurotoxicity, including a significant accumulation of tau aggregates, behavioral dysfunction, progressive neuron loss, and shortened lifespan (4). We demonstrated that loss of *dop-2* and *dop-3*, the two D_2-like dopamine receptors in *C. elegans*, significantly ameliorated tau-induced toxicity in tau transgenic *C. elegans* (3). Multiple studies have shown that overexpression of tau alters dopamine signaling by causing loss of dopaminergic neurons (5,6). In addition, activation of D_1 dopamine receptors has been shown to increase tau phosphorylation (7). One study addressed how blocking D_2-family dopamine receptors may suppress tauopathy using the D_2 receptor antagonist haloperidol (8); however, this drug is not specific to dopamine receptors (9). Therefore, to better understand how loss of D_2-like dopamine receptors suppresses tauopathy phenotypes, we performed a genetic screen of dopamine-related genes in tau transgenic *C. elegans.* We identified *bas-1*, the *C. elegans* homolog of DOPA decarboxylase (DDC), as a suppressor of tau-induced toxicity.

METHODS AND MATERIALS

C. elegans Strains and Transgenics

C. elegans strains used are listed in Supplemental Table S1. All strains were maintained at 20°C on standard nematode growth media plates containing OP50 *Escherichia coli* as previously described (10). Worms were grown on nematode growth media plates containing five times more peptone (5XPEP) prior to collection for protein studies. The $P_{bas\text{-}1}$*::bas-1::GFP* transgenic plasmid was a gift from Dr. Shi-Qing Cai (Institute of Neuroscience, Chinese Academy of Sciences, Shanghai, China). $P_{bas\text{-}1}$*::bas-1::GFP* and $P_{elt\text{-}2}$*::*mCherry were injected into N2

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worms at 100 and 20 ng/μL, respectively, and chromosomally integrated using ~3500R of gamma rays from a cesium source.

Behavioral Analysis

Worms were synchronized by timed egg lays and grown at 20°C for 4 days (approximately day 1 of adulthood for tau transgenic *C. elegans*). Swimming behavior was quantified as described previously (3) with a few modifications. A single worm was transferred to a shallow well in a Teflon-coated glass slide filled with M9 buffer. After allowing the worm to adjust to liquid for 10 seconds, thrashes (body bends) were counted for 1 minute. A thrash was considered a large movement of the head or tail of the worm that causes a significant displacement of the middle third. For initial behavior screening (see Table 1), at least 15 animals were assayed per group. For all other comparisons, 100 animals were assayed per group.

Lifespan Analysis

Lifespan assays were performed as described previously (11) with a few modifications. A total of 100 to 150 L4 stage worms per strain were transferred to 35-mm nematode growth media plates (25 worms per plate) seeded with 75 μL of 10 times OP50 *Escherichia coli* and 0.05-mg/mL fluorodeoxyuridine. Worms were maintained at 25°C. Dead worms were counted every day. A worm was considered dead when it did not respond to repeated poking by a platinum wire. Worms that crawled off the plate or died from unusual causes were censored from analysis.

Neurodegeneration Assays

tgT337;unc-47::GFP and tgT337;unc-47::GFP;*bas-1(ad446)* worms were synchronized by timed egg lays. Worms were mounted on 2% agarose pads containing 0.1% sodium azide as a paralytic at 4 or 7 days of age. The number of gamma-aminobutyric acidergic (GABAergic) neurons in the ventral nerve cord was counted with a DeltaVision microscope (Applied Precision, Isaquah, WA) using 60× magnification. Twenty animals were counted per strain per time point.

Immunoblotting

Protein samples were diluted with five times sample buffer (0.046 mol/L Tris, 0.005 mol/L ethylenediamine tetraacetate, 0.2 mol/L dithiothreitol, 50% sucrose, 5% sodium dodecyl sulfate, 0.05% bromophenol blue), boiled for 5 minutes, and centrifuged at 13,000*g* for 5 minutes prior to being loaded onto 4% to 15% precast sodium dodecyl sulfate polyacrylamide gel electrophoresis gradient gels (Bio-Rad Laboratories, Hercules, CA) and transferred to polyvinylidene difluoride membranes, as recommended by the manufacturer. A total of 5 to 10 μL of diluted sample was loaded for each analysis. Primary antibodies used were rabbit monoclonal anti-tau antibody (Rockland Immunochemicals, Limerick, PA) at 1:5000, mouse anti-phospho-tau antibody CP13 (Peter Davies, Litwin-Zucker Research Center for the Study of Alzheimer's Disease, The Feinstein Institute of Medical Research, Northwell Health, Manhasset, NY) at 1:1000, mouse anti-phospho-tau antibody (pSer396/pSer404) [PHF-1] (Peter Davies) at 1:1000, rabbit anti-phospho-tau antibody pS422 (Abcam, Cambridge, United Kingdom) at 1:500, goat anti–green fluorescent protein (GFP) antibody (Rockland Immunochemicals) at 1:5000, and mouse anti-tubulin antibody E7 (Developmental Studies Hybridoma Bank, Iowa City, IA) at 1:5000. Secondary antibodies used were anti-rabbit horseradish peroxidase (Jackson Immunoresearch, West Grove, PA), anti-mouse horseradish peroxidase (Jackson Immunoresearch, West Grove, PA), and anti-goat horseradish peroxidase (Rockland Immunochemicals), all at 1:5000. Enhanced chemiluminescence substrate (Bio-Rad Laboratories) was added to the membrane and chemiluminescence signals were detected with ChemiDoc-It Imager (UVP LLC, Upland, CA) and measured with UVP Software (Doc-ItLS Image Analysis Software 4.1).

Tau Protein Extraction

Staged young adult tau transgenic *C. elegans* were grown from eggs at 20°C for 3 days on 5XPEP plates (10), washed off plates in M9 buffer, and collected by centrifugation. Worms were snap frozen in liquid nitrogen and stored at –70°C. Tau fractions were obtained as described previously (4). A total of 2 μL of high-salt reassembly (RAB) buffer (0.1 mol/L 2-(*N*-morpholino)ethanesulfonic acid, 1 mmol/L ethylene glycol bis-2-aminoethyl ether-N,N′,N″,n′-tetraacetic acid, 0.5 mmol/L $MgSO_4$, 0.75 mol/L NaCl, 0.02 mol/L NaF, pH 7.0) containing phenylmethylsulfonyl fluoride and protease inhibitors was added per milligram of worm pellet and homogenized by sonication. A portion of the sample was saved for immunoblotting while the rest was centrifuged at 40,000*g*

Table 1. Genes That Modified Behavior Deficits in Tau Transgenic *Caenorhabditis elegans*

Gene [Human Homolog]	Allele (Mutation)	Motor Function Mutation Alone	Motor Function Tau Background
bas-1 [*DDC*]	*ad446* (deletion)	105% (p = .11)	254% (p = 9.1E-21)
	tm351 (deletion)	98% (p = .61)	202% (p = 7.6E-11)
egl-8 [*PLCB1*]	*n488* (deletion)	42% (p = 1.5E-10)	32% (p = 3.2E-5)
	sa47 (Q85Stop)	67% (p = 6.2E-16)	51% (p = 3.0E-5)
egl-10 [*RGS7*]	*n692* (W418Stop)	9% (p = 5.9E-10)	8% (p = 1.5E-4)
grk-2 [*GRK2*]	*gk268* (deletion)	30% (p = 1.4E-51)	50% (p = 5.6E-5)
unc-43 [*CAMK2D*]	*n1186* (Q67Stop)	29% (p = 6.0E-10)	23% (p = 5.0E-10)
	n498^{x} (E108K)[a]	5% (p = 1.2E-6)	28% (p = 6.5E-4)
tax-6 [*PPP3CC*]	*p675* (D259N)	34% (p = 2.5E-4)	38% (p = 4.7E-3)

Motor function was assessed via liquid thrashing assays. *C. elegans* strains carrying the mutation alone were compared with N2 (wild type) *C. elegans* strains, while *C. elegans* strains carrying the mutation and the tau transgene were compared with tau transgenic *C. elegans.* At least 15 animals were assessed per strain and Student's *t* tests were used to determine significance.

[a]Superscript x indicates a gain-of-function allele.

for 40 minutes. The supernatant was the RAB/soluble fraction. The pellet was extracted by adding 1 μL of radioimmunoprecipitation assay (RIPA) buffer (50 mmol/L Tris, 150 mmol/L NaCl, 1% Nonidet P-40 [Sigma-Aldrich, St. Louis, MO], 5 mmol/L ethylenediamine tetraacetate, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate, pH 8.0) containing phenylmethylsulfonyl fluoride and protease inhibitors per milligram of original worm pellet weight and centrifuged at 40,000*g* for 20 minutes. The supernatant was the RIPA/detergent-soluble fraction. The pellet was extracted by adding 1 μL 70% formic acid (FA) per milligram of original worm pellet weight and centrifuged at 13,000*g* for 15 minutes. The supernatant was the FA/detergent-insoluble fraction.

Catecholamine Measurements

Staged young adult *C. elegans* were grown from eggs at 20°C for 3 days on 5XPEP plates, washed off plates in M9 buffer, and collected by centrifugation. Approximately 150 μL of packed worms were snap frozen in liquid nitrogen and stored at –70°C. Pellets were extracted with 0.1 mol/L perchloric acid. A total of 50 μL was used for Pierce bicinchoninic acid protein determination. The remainder was centrifuged and the supernatant frozen at –70°C until assayed. The acid extract was put through an alumina extraction procedure before injection onto high-pressure liquid chromatography. Catecholamine levels were measured using electrochemical detection by a Dionex Choulochem III high-pressure liquid chromatography system (Thermo Fisher Scientific, Waltham, MA). The data was analyzed using the Chromeleon 7 software package (Dionex, Thermo Fisher Scientific). The catecholamine levels were normalized to the total protein.

RESULTS

Identification of Dopamine-Related Genes Involved in Tauopathy Phenotypes

Previous work in our lab demonstrated that the combined loss of *dop-2* and *dop-3*, D_2-like dopamine receptors, suppresses tauopathy phenotypes in our *C. elegans* tauopathy model (3). D_2-like dopamine receptors are G-protein coupled receptors that activate $G_{i/o}$-mediated signaling pathways and also act presynaptically to regulate dopamine release (12). Therefore, we crossed tau transgenic *C. elegans* with strains containing loss of function alleles in dopamine-related genes including genes involved in dopamine synthesis, metabolism, and downstream signaling (Supplemental Table S1). We assayed the resulting strains for behavioral deficits using liquid thrashing assays, which measure *C. elegans* responses to immersion in liquid (Supplemental Table S2). A few genes significantly modulated the swimming response of tau transgenic worms (Table 1). Most of these genes enhanced the tau-induced behavioral deficit. However, these enhancers of tau toxicity also caused significant impairment of locomotion in wild-type animals that do not carry the tau transgene, demonstrating that they influence behavior independent of tau.

Loss of DDC Suppresses Tau-Induced Behavioral Dysfunction

bas-1, the *C. elegans* gene for DDC, was the only suppressor of tau-related phenotypes identified in our screen. We tested two independent deletion alleles of *bas-1*, *ad446* and *tm351*, both of which rescued the behavioral dysfunction of tau transgenic *C. elegans* without altering behavior in wild-type *C. elegans* (Figure 1A, B; Table 1; Supplemental Figure S1). The allele *ad446* contains a deletion that removes almost the entire coding sequence of *bas-1*, including the catalytic site encoded by exon 4, while the allele *tm351* deletes only exon 2. Both alleles caused increases in levodopa (L-DOPA) levels and decreases in dopamine levels in *C. elegans*, which suggests that they are both functionally null (Supplemental Table S3). The allele *ad446* additionally deletes *basl-1*, a gene predicted to encode a nonfunctional paralog of DDC (13). We assayed the effect of deleting *basl-1* alone by examining *basl-1(ok703)* and found it did not alter the behavior of tau transgenic *C. elegans* (Supplemental Table S2).

Loss of DDC Decreases Tau Phosphorylation

Phosphorylation of tau is a normal mechanism for regulation of tau activity, but hyperphosphorylation of tau, commonly seen in tauopathies, is thought to be toxic and precede the formation of insoluble aggregates (14). We examined whether loss of *bas-1* modulated total tau levels or tau phosphorylation in tau transgenic *C. elegans*. We assessed tau phosphorylation with three different antibodies against phosphorylated tau: CP13 (pSer202), PHF-1, and pSer422 (15). Phosphorylation of tau at these three sites is elevated in brains of patients with Alzheimer's disease (16). Loss of *bas-1* did not alter total tau levels significantly but generally reduced the phosphorylation at all three sites probed (Figure 1C; Table 2).

Loss of DDC Decreases Tau Aggregation

The deposition of detergent insoluble aggregates of tau protein is a hallmark of human tauopathies (2). Detergent-insoluble tau increases in tau transgenic *C. elegans* with age and with disease-causing mutations in tau (4). To determine whether loss of *bas-1* affected the pathological aggregation of tau, we subjected tau transgenic *C. elegans* with or without *bas-1* null alleles to extraction with buffers of increasing solubilizing strength. We found that loss of *bas-1* did not change tau levels in soluble (RAB) fractions but reduced tau in detergent-soluble (RIPA) and detergent-insoluble (FA) fractions (Figure 1D; Table 2).

Loss of DDC Rescues Neurodegeneration, but Not Longevity of Tau Transgenic *C. elegans*

Neuronal loss occurs during the brain organ failure that causes premature death in tauopathy patients. Progressive neuronal loss and shortened lifespan are phenotypes seen in this tau transgenic *C. elegans* model (4). To determine whether loss of *bas-1* affected the neurodegenerative phenotype seen in *C. elegans*, we crossed tau transgenic *C. elegans* carrying the *bas-1* allele *ad446* with the transgenic strain EG1285. EG1285 carries a GFP reporter transgene (*Punc-47::GFP)*, which marks the cell bodies and processes of motor neurons within the ventral nerve cord of *C. elegans* (17). The resulting strain carries the *bas-1* null allele, the tau transgene, and the GFP reporter. We counted the number of GABAergic motor neurons and observed a significant rescue of degenerating GABAergic neurons in *bas-1* mutants at both 4 and 7 days of age (Figure 2).

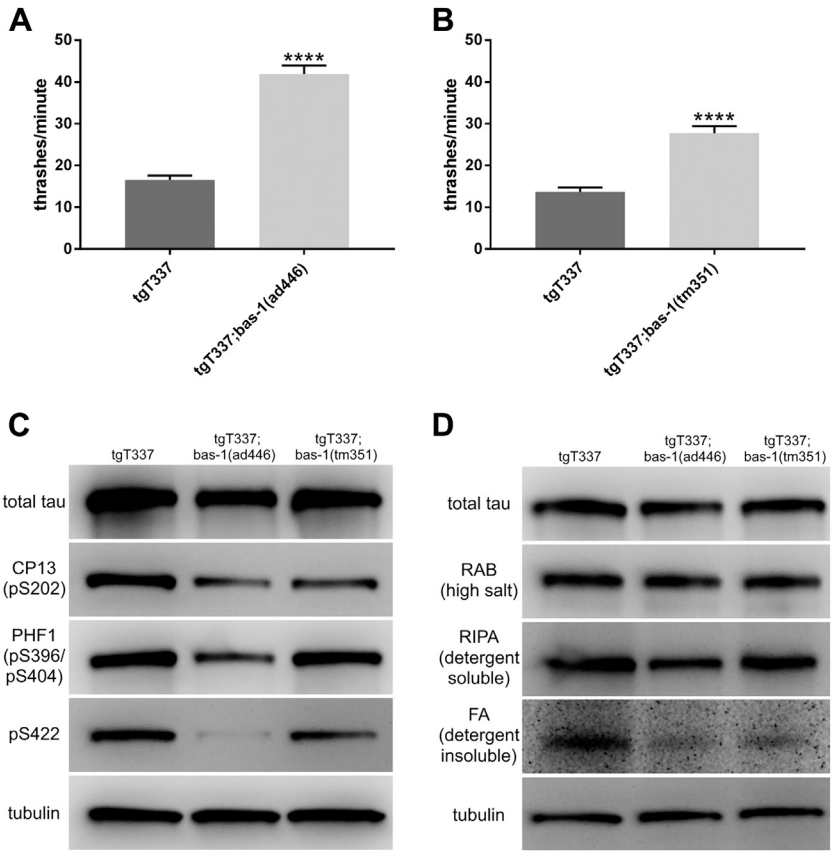

Figure 1. Loss of *bas-1* ameliorates behavioral deficits and reduces phosphorylated and insoluble tau in tau transgenic *Caenorhabditis elegans*. **(A)** Effect of *bas-1(ad446)* on liquid thrashing rate of tau transgenic (tgT337) worms. **(B)** Effect of *bas-1(tm351)* on liquid thrashing rate of tgT337 worms. Four-day-old *C. elegans* were placed in M9 buffer and thrashes were counted for 1 minute. Experiments were performed five times for a total of 100 worms per group. Data are displayed as mean ± SEM. Student's *t* test was used to compare tgT337 to tgT337;*bas-1* worms. ****p = 9.1E-21 for *ad446* and p = 7.6E-11 for *tm351*. **(C)** Effects of *bas-1* alleles on phosphorylation of tau in tau transgenic *C. elegans*. Representative immunoblots for tau phosphorylation at different sites. CP13 detects phosphorylation at Ser202. PHF-1 detects phosphorylation at Ser396/Ser404. pS422 detects phosphorylation at Ser422. Tubulin was used as a load control. **(D)** Effects of *bas-1* alleles on tau solubility in tau transgenic *C. elegans*. Representative immunoblots for tau after sequential extraction of tgT337 worms and *bas-1* mutants (tgT337;*bas-1(ad446)* and tgT337;*bas-1(tm351)*). Total tau is tau in lysates prior to sequential extraction. The reassembly (RAB) fraction is soluble tau, the radioimmunoprecipitation assay (RIPA) fraction is detergent-soluble tau, and the formic acid (FA) fraction is detergent-insoluble tau. Tubulin was used as a load control. Chemiluminescence signals were quantified for 4–6 replicates by Kruskal-Wallis test with post hoc Dunn's multiple comparisons test to determine significance. See Table 2 for values and statistics.

Tau transgenic *C. elegans* have shortened lifespans when compared with wild-type worms (4). However, we previously found that azaperone, a chemical suppressor of tau toxicity in tau transgenic *C. elegans*, was unable to extend lifespan (3). Similarly, loss of *bas-1* did not extend lifespan significantly in tau transgenic *C. elegans* (Supplemental Figure S2; Supplemental Table S4).

Overexpression of DDC Worsens Tau-Induced Phenotypes

Because loss of DDC gene *bas-1* suppresses tau toxicity in our tau transgenic *C. elegans*, we investigated whether overexpression of biogenic amine aynthesis related (BAS-1) protein would modulate tau toxicity. We crossed tau transgenic *C. elegans* with two different strains overexpressing BAS-1 protein fused to GFP under the *bas-1* promoter (*Pbas-1::bas-1::gfp).* Overexpression of BAS-1::GFP fusion protein has been shown to rescue age-dependent decreases in serotonin and dopamine levels in *C. elegans*, suggesting that the GFP fusion does not interfere with BAS-1 protein activity (18). We found significant enhancement of the tau-induced behavioral dysfunction with overexpression of BAS-1 (Figure 3A–C). However, overexpressing BAS-1 impaired the behavior of wild-type *C. elegans* to a similar extent. This suggests that

Table 2. Effect of *bas-1* Alleles on Tau Levels

Comparison to tgT337 Levels	tgT337;*bas-1(ad446)*	tgT337;*bas-1(tm351)*
Total Tau/Tubulin (n = 6)	88% (SEM = 9%; p = .88)	125% (SEM = 23%; p = .88)
RAB (Soluble) Tau/Total Tau (n = 5)	99% (SEM = 14%; p = 1)	107% (SEM = 29%; p = 1)
RIPA (Detergent Soluble) Tau/Total Tau (n = 5)	43% (SEM = 10%; p = .01)	76% (SEM = 16%; p = .26)
FA (Detergent Insoluble) Tau/Total Tau (n = 5)	44% (SEM = 13%; p = .07)	39% (SEM = 20%; p = .05)
CP13 Tau/Total Tau (n = 5)	52% (SEM = 8%; p = .02)	55% (SEM = 7%; p = .01)
PHF-1 Tau/Total Tau (n = 5)	71% (SEM = 8%; p = .04)	76% (SEM = 14%; p = .10)
pS422/Total Tau (n = 4)	31% (SEM = 8%; p = .04)	53% (SEM = 15%; p = .39)

For each replicate, the measured chemiluminescence signals were normalized to that for tau transgenic worms alone (tgT337). Four to six replicates were used for each analysis. Data were analyzed by Kruskal-Wallis test with post hoc Dunn's multiple comparisons test used to determine significance.

FA, formic acid; RAB, reassembly; RIPA, radioimmunoprecipitation assay.

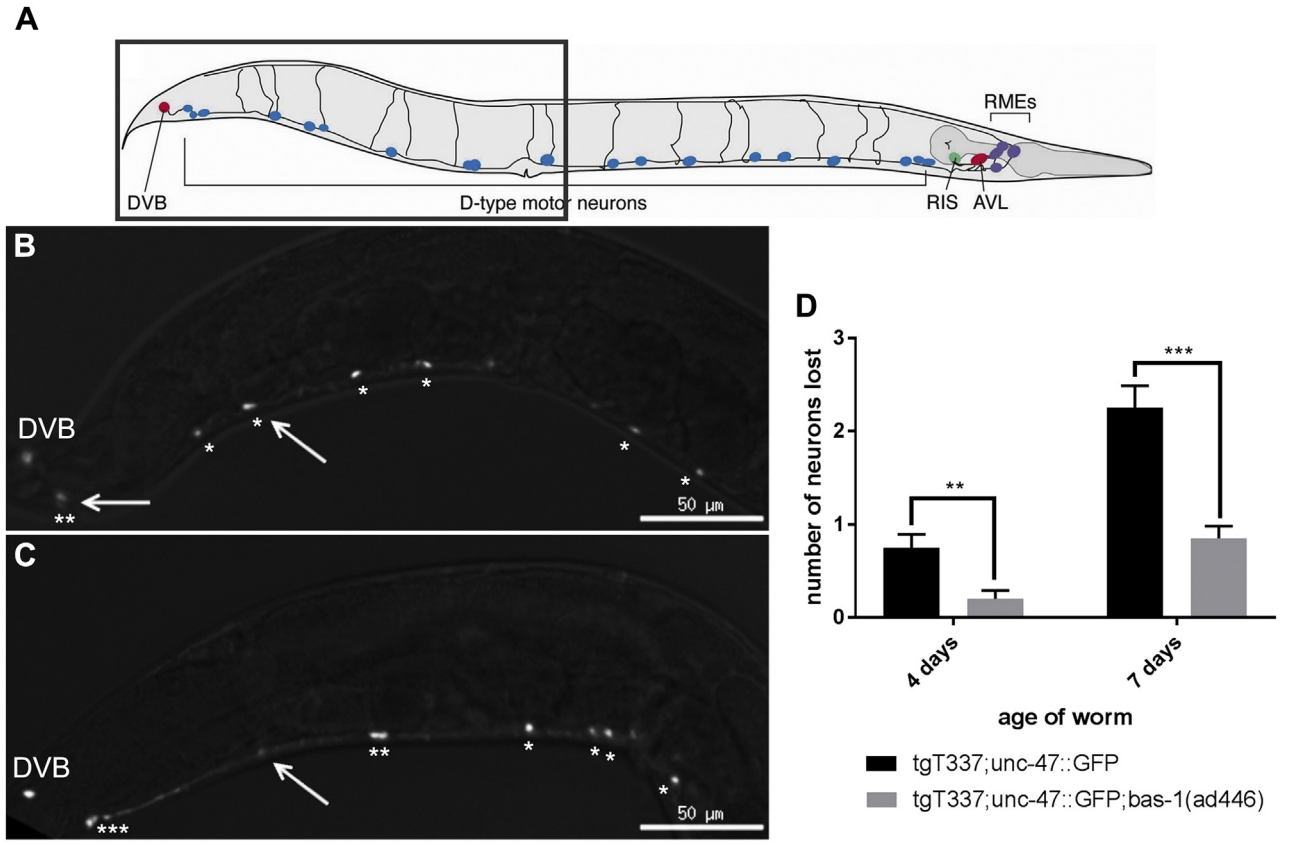

Figure 2. Loss of *bas-1* function ameliorates tau-induced neuron loss. Tau transgenic (tgT337) and *bas-1* mutant (tgT337;*bas-1(ad446)*) *Caenorhabditis elegans* were crossed with EG1285, a reporter transgenic strain expressing green fluorescent protein (GFP) in gamma-aminobutyric acidergic neurons (*unc-47::GFP*). **(A)** Schematic showing the 19 D-type motor neurons, adapted from Schuske *et al.* (36). The gray box indicates the approximate region of the worm imaged in panels **(B, C)**. **(B)** Representative image of a tgT337;*unc-47::GFP* worm (tail end) at 7 days. The stars indicate the neurons that were counted. The arrows indicate missing neurons. **(C)** Representative image of tgT337;*unc-47::GFP;bas-1(ad446)* at 7 days. **(D)** Quantification of gamma-aminobutyric acidergic neuron loss in tgT337;*unc-47::GFP* and tgT337;*unc-47::GFP;bas-1(ad446)* worms. The D-type gamma-aminobutyric acidergic motor neurons were counted in 4-day-old and 7-day-old worms. A total of 20 worms were analyzed for each group at each time point. Data are displayed as mean ± SEM. Student's *t* test was used to compare tgT337;*unc-47::GFP* to tgT337;*unc-47::GFP;bas-1(ad446)* at each time point. $^{**}p$ = .003, $^{***}p$ = 1.8E-5. AVL, DVB, RIS, and RMEs are gamma-aminobutyric acidergic neuron subtypes.

overexpression of BAS-1 may induce behavioral dysfunction independently of tau.

Enzymes Upstream of DDC Are Required for Suppression of Tau-Induced Phenotypes

DDC catalyzes the second step in the dopamine and serotonin synthesis pathways. Loss of DDC gene *bas-1* significantly reduces dopamine and serotonin levels in *C. elegans* (18–20). Loss of function in the genes encoding tyrosine hydroxylase (*cat-2*) or tryptophan hydroxylase (*tph-1*) causes significant reduction in dopamine or serotonin levels, respectively (20,21), but neither had an effect on tau-induced behavior dysfunction (Supplemental Table S2). In addition, loss of *cat-4* or *cat-1*, two other genes that regulate dopamine and serotonin levels in *C. elegans* (22), did not suppress tau-induced behavior dysfunction (Supplemental Table S2). One way loss of *bas-1* could suppress tau-induced toxicity is via accumulation of dopamine and serotonin synthesis intermediates L-DOPA and 5-hydroxytryptophan (5-HTP). Accumulation of these intermediates requires the activity of upstream enzymes tyrosine hydroxylase and tryptophan hydroxylase. Thus, to test whether suppression of tau by loss of DDC is modulated by upstream enzymatic activity, we generated tau transgenic *C. elegans* carrying the *bas-1* null allele *ad446* and a loss-of-function allele in *cat-2*, *tph-1*, or *cat-4*. We compared the behavior of these strains to that of tau transgenic *C. elegans* (tgT337) and to tau transgenic *C. elegans* carrying the *bas-1* null allele (tgT337;*bas-1(ad446)*) (Figure 3D; Supplemental Table S5).

Loss of *cat-2* should decrease L-DOPA synthesis but not affect 5-HTP synthesis. We found that tau transgenic *C. elegans* carrying null alleles in both *bas-1* and *cat-2* still had significantly improved behavior compared with tau transgenic *C. elegans* but were significantly worse than tau transgenic *C. elegans* only carrying a null allele in *bas-1*, suggesting that loss of *cat-2* partially blocked suppression of tau by loss of *bas-1*.

Similarly, loss of *tph-1* should decrease 5-HTP synthesis but not affect L-DOPA synthesis. We found that tau transgenic

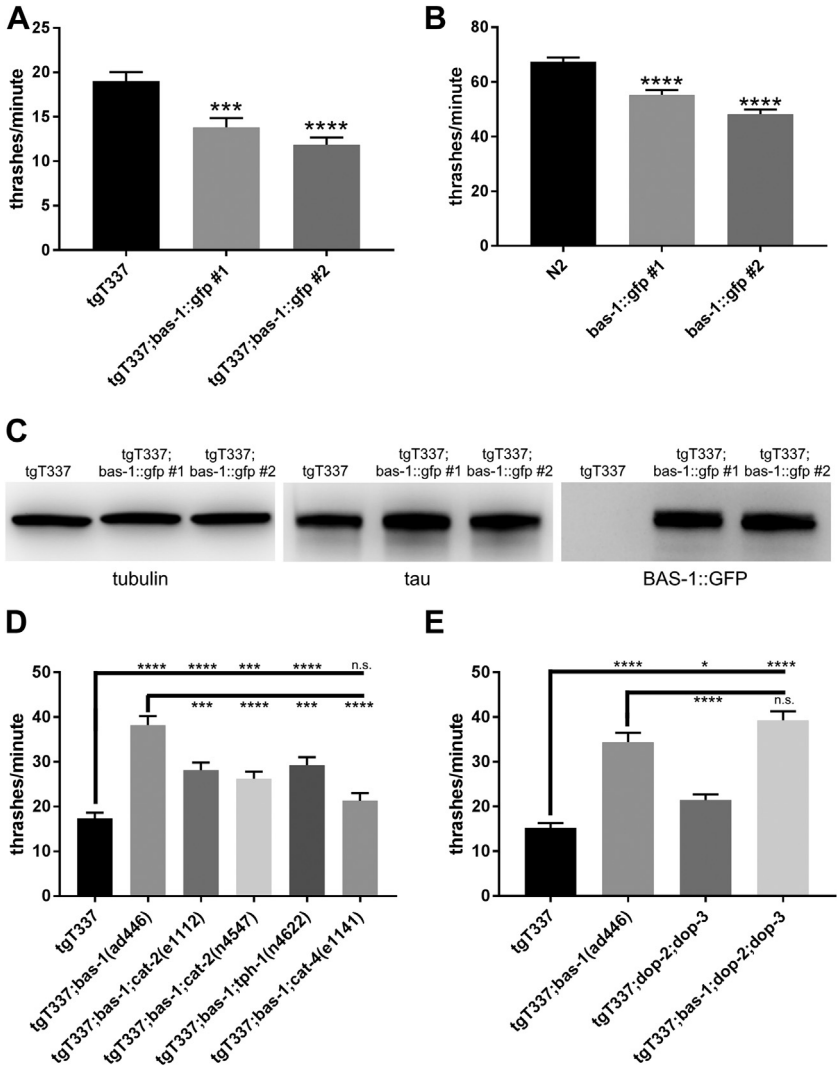

Figure 3. Overexpression of BAS-1::green fluorescent protein (GFP) increases behavioral dysfunction in tau transgenic *Caenorhabditis elegans*. **(A)** Effect of BAS-1::GFP overexpression on liquid thrashing rate of tau transgenic (tgT337) worms. **(B)** Effect of BAS-1::GFP overexpression on liquid thrashing rate of wild-type (N2) worms. For panels **(A, B)**, 4-day-old *C. elegans* worms were placed in M9 buffer and thrashes were counted for 1 minute. The experiment was performed 5 times for a total of 100 worms per group. Data are displayed as mean ± SD. One-way analysis of variance and multiple pairwise comparisons (Dunnett's test) were used to compare tgT337;*bas-1::GFP* worms with tgT337 worms and compare *bas-1::GFP* worms with N2 worms. ***p = .0004, ****p = .0001. **(C)** Western blot for total tau and *bas-1::GFP* for tau transgenic and double transgenic worms. The rabbit monoclonal antibody (Rockland Immunochemicals) was used to detect total tau protein. GFP antibody was used to detect BAS-1::GFP (~80 kDa). Tubulin was used as a load control. **(D)** Loss of function in *cat-2* or *tph-1* partially blocks *bas-1* suppression of tau-induced phenotypes while loss of function in *cat-4* completely blocks suppression, as measured by liquid thrashing assay. **(E)** Loss of function in *dop-2* and *dop-3* does not alter *bas-1* suppression of tau-induced phenotypes. Mutant worms had alleles *dop-2(vs105)*, *dop-3(vs106)*, and/or *bas-1(ad446)*. For panels **(D, E)**, 4-day-old *C. elegans* worms were placed in M9 buffer and thrashes were counted for 1 minute. Experiments were performed seven times (for **D**) or five times (for **E**) for a total of 100 worms per group. Data are displayed as mean ± SEM. Data were analyzed by two-way analysis of variance. Multiple pairwise comparisons (Tukey's test) were performed between tau transgenic *C. elegans* (tgT337) or tgT337;*bas-1(ad446)* and the other strains. Average, SEM, and p values are listed in Supplemental Table S5 for panel **(D)** and Supplemental Table S6 for panel **(E)**. *$p < .05$, ***$p < .005$, ****$p < .0001$. n.s., not significant.

C. elegans carrying null alleles in both *bas-1* and *tph-1* had significantly improved behavior compared with tau transgenic *C. elegans*, but were significantly worse than tau transgenic *C. elegans* only carrying a null allele in *bas-1*, suggesting that loss of *tph-1* also partially blocks tau suppression by loss of *bas-1*.

Loss of *cat-4* should decrease both L-DOPA and 5-HTP synthesis. Tetrahydrobiopterin is a cofactor for aromatic amino acid hydroxylases such as tyrosine hydroxylase and tryptophan hydroxylase and *cat-4* encodes the *C. elegans* gene for guanosine-5′-triphosphate cyclohydrolase, the first enzyme in tetrahydrobiopterin synthesis (23). We found that tau transgenic *C. elegans* carrying loss-of-function alleles in both *bas-1* and *cat-4* had similar behavior dysfunction as tau transgenic *C. elegans*. This suggests that loss of *cat-4* completely blocks the suppression of tau toxicity by loss of *bas-1*. Because loss of *cat-4* reduced the L-DOPA accumulation in *bas-1* null *C. elegans* to the same extent as loss of *cat-2* (Supplemental Table S3), loss of *cat-4* does not block suppression of tau toxicity by *bas-1* solely through lowering L-DOPA but must involve other molecular mechanisms.

Loss of DDC and Loss of D_2-like Dopamine Receptors Suppress Tau-Induced Behavior via a Shared Mechanism

We initially tested *bas-1* and other dopamine-related genes due to the fact that loss of D_2-like dopamine receptor genes suppressed tau-induced toxicity in tau transgenic *C. elegans* (3). To see if loss of DDC and loss of D_2-like dopamine receptors suppress tau via a shared mechanism, we created tau transgenic *C. elegans* with loss-of-function alleles in *bas-1*, *dop-2*, and *dop-3*. We compared the behavior of tau transgenic *C. elegans* with loss of *bas-1* only, tau transgenic *C. elegans* with loss of *dop-2* and *dop-3*, and tau transgenic *C. elegans* with loss of *bas-1*, *dop-2*, and *dop-3* (Figure 3E; Supplemental Table S6). We found that all three strains exhibited significantly suppressed tau-induced toxicity, but

there was no significant additive suppression of tauopathy in animals carrying all three dopamine pathway mutations. This indicates that *bas-1* and *dop-2/dop-3* suppression of tauopathy share a common signaling mechanism.

DISCUSSION

We have used our transgenic *C. elegans* model of tau toxicity to evaluate dopamine-related genes in the genesis of tauopathy phenotypes. In this screen, we found several candidate enhancers of tau-mediated behavioral phenotypes, but none appeared to specifically modify tauopathy. We identified *bas-1*, the *C. elegans* homolog of DDC, as a suppressor of tau toxicity in *C. elegans.* We found that loss of *bas-1* ameliorated the behavioral deficits caused by tau, reduced the phosphorylation level of tau, reduced the accumulation of insoluble tau, and reduced neurodegeneration.

Interestingly, no suppressors were identified among the screened genes that encode proteins downstream of the dopamine receptors in signaling pathways. Whether this is due to the essential nature of downstream genes or their redundant functions in signaling remains unclear. Alternatively, there may be signaling pathways downstream of dopamine receptors that mediate suppression that were not screened, such as the pathway from dopamine receptors to adenosine monophosphate–activated protein kinase inactivation (8).

Regardless, the enhancers we identified included genes whose human homologs have been reported to modulate tau including *Tax-6* (human homolog PPP3CC) and *unc-43* (human homolog CAMK2D) (24,25). In addition, all of the enhancer genes caused profound behavioral deficits in *C. elegans* even in the absence of tau transgenic expression. The enhancement in behavioral dysfunction observed when these genes are lost may be due to an independent, additive effect on behavior. For instance, both loss- and gain-of-function alleles of *unc-43* caused significant behavioral dysfunction with or without tau transgenic expression. Therefore, it is difficult to interpret the effect of identified enhancers on tau. However, tau suppression by *bas-1* is interpretable because loss of *bas-1* on its own does not overtly impact motor function. Interestingly, overexpression of BAS-1 affected behavior in both wild-type and tau transgenic *C. elegans* to a similar extent. This suggests the possibility that any behavioral enhancement seen with BAS-1 overexpression in tau transgenic *C. elegans* is due to two independent mechanisms. Perhaps we did not observe a synergistic effect between BAS-1 and tau overexpression on behavior because the effect of BAS-1 activity on motor phenotypes is already maximal.

DDC catalyzes the second step in the two-step synthesis process for both dopamine and serotonin. Loss of DDC gene *bas-1* causes a reduction in dopamine and serotonin as well as the accumulation of precursors L-DOPA and 5-HTP (18–20) (see Supplemental Table S3). Our results indicate that loss of dopamine and serotonin cannot be the mechanism of suppression because loss-of-function mutations in other genes required for their biosynthesis did not suppress tau-induced toxicity.

Our results support a possible mechanism for suppression of tau toxicity by the combined accumulation of L-DOPA and 5-HTP (Figure 4). Loss of *cat-2*, the *C. elegans* gene for tyrosine hydroxylase, reduces levels of only L-DOPA and not 5-HTP. Conversely, loss of *tph-1*, the *C. elegans* gene for tryptophan hydroxylase, reduces the levels of only 5-HTP and not L-DOPA. Loss of either *cat-2* or *tph-1* partially blocked the suppression of tau toxicity by loss of *bas-1*. Loss of *cat-4*, the *C. elegans* gene for guanosine-5′-triphosphate cyclohydrolase, reduces the synthesis of tetrahydrobiopterin, an essential cofactor for both tyrosine hydroxylase and tryptophan hydroxylase. Loss of *cat-4* function, which reduces both L-DOPA and 5-HTP levels, completely blocked the suppression of tau toxicity by *bas-1* loss of function. Because loss of *cat-2* and loss of *cat-4* caused similar reductions in L-DOPA levels in *bas-1* null *C. elegans*, accumulation of L-DOPA cannot solely explain the mechanism of suppression. Similarly, because loss of *tph-1* only partially blocked suppression of tau toxicity by *bas-1*, accumulation of 5-HTP also cannot solely explain the mechanism of suppression. The combined loss of tyrosine hydroxylase and tryptophan hydroxylase activity and subsequent reduction in both L-DOPA and 5-HTP levels may explain how loss of *cat-4* blocks suppression of tau toxicity by loss of *bas-1*. Tetrahydrobiopterin is also a cofactor for phenylalanine hydroxylase and alkylglycerol monooxygenase (23). Phenylalanine hydroxylase activity is necessary for melanin synthesis in *C. elegans*, while alkylglycerol monooxygenase activity is necessary for cuticle integrity. Neither of these enzymes is expressed in neurons, and loss of these enzymes does not affect serotonin or dopamine levels. It seems unlikely that loss of *cat-4* would block suppression of tau toxicity by *bas-1* via enzymes that are not expressed in the same cells or modulate the same pathways.

The mechanism of suppression of tauopathy by *bas-1* loss of function must be cell nonautonomous because GABAergic neurons are protected despite the fact they do not normally express *bas-1*. At the molecular level, L-DOPA and 5-HTP could mediate tauopathy suppression by directly activating receptors (26), or derivate compounds such as tetrahydroisoquinolines could mediate neurotoxicity (27). Alternatively, loss of *bas-1* could suppress tauopathy by reducing consumption of its coenzyme, pyridoxal 5′-phosphate, a limiting cofactor for many pyridoxal 5′-phosphate–dependent enzymes (28). This hypothetical mechanism explains why overexpression of BAS-1 exacerbates tauopathy phenotypes, but does not reconcile how loss of tyrosine hydroxylase or tryptophan hydroxylase activity could reduce tau suppression by loss of *bas-1*.

Interestingly, exogenous L-DOPA administration increased phosphorylation of tau in mice (29–31). This is the opposite of what we observed in tau transgenic *C. elegans* lacking the DDC gene *bas-1.* Multiple mechanisms could explain this discrepancy. First, exogenous L-DOPA can be converted to dopamine in any cell with DDC expression, including serotonergic neurons (26). The metabolism of L-DOPA may also differ when DDC activity is lost compared with administration of exogenous L-DOPA. Second, the manner in which L-DOPA was administered affected tau phosphorylation; pulsatile administration, but not continuous infusion, was found to increase tau phosphorylation (29,30). Third, exogenous L-DOPA administration in mice increased plasma homocysteine and decreased S-adenosylmethionine, the methyl donor needed for methylation of the major tau phosphatase, PP2A (31). It is possible that this pathway is not conserved in *C. elegans*.

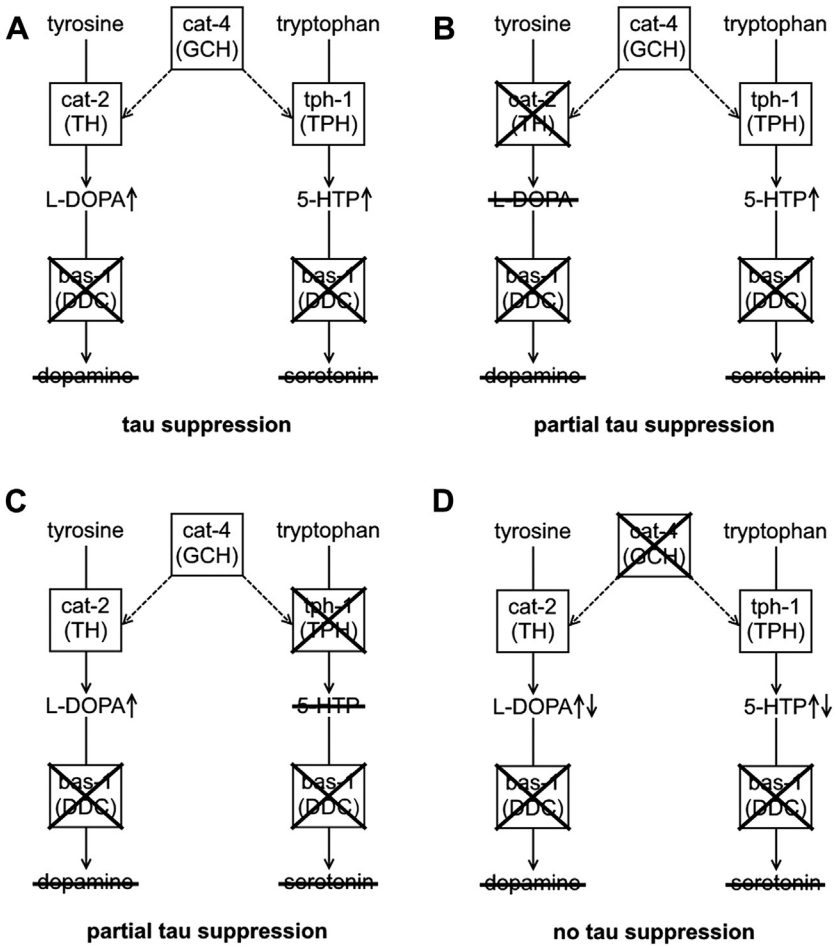

Figure 4. A possible model for suppression of tau by loss of *bas-1*. **(A)** Loss of *bas-1* (DOPA decarboxylase [DDC]) leads to the accumulation of levodopa (L-DOPA) and 5-hydroxytryptophan (5-HTP). L-DOPA is synthesized from tyrosine by *cat-2* (tyrosine hydroxylase [TH]) and 5-HTP is synthesized from tryptophan by *tph-1* (tryptophan hydroxylase [TPH]). *cat-4* (guanosine-5′-triphosphate cyclohydrolase [GCH]) synthesizes the cofactor needed for *cat-2* and *tph-1* activity. **(B)** Additional loss of *cat-2* reduces L-DOPA levels without affecting 5-HTP and partially blocks suppression of tau by loss of *bas-1*. **(C)** Additional loss of *tph-1* reduces 5-HTP levels without affecting L-DOPA and partially blocks suppression of tau by loss of *bas-1*. **(D)** Additional loss of *cat-4* reduces both L-DOPA and 5-HTP levels and completely blocks suppression of tau by loss of *bas-1*. Altogether this suggests that the accumulation of both L-DOPA and 5-HTP is necessary to see the full effect of loss of *bas-1* on tau toxicity.

Another possible mechanism for suppression of tau toxicity by loss of DDC gene *bas-1* could be modulation of expression levels of other related genes. For instance, loss of the dopamine transporter has been shown to change the expression of tyrosine hydroxylase (32). However, the expression levels of dopamine-related proteins, except for monoamine oxidase A, were unchanged in mice with significantly reduced DDC expression (33). This suggests that a change in transcription of related genes is not likely to be the mechanism of *bas-1* suppression.

Instead, our results support the possibility that loss of D_2-family dopamine receptors suppresses tau by decreasing DDC activity. There is some evidence that D_2-like dopamine receptors are expressed in serotonin neurons in *C. elegans* (34), which suggests that these receptors could modulate DDC in both dopamine and serotonin neurons. Suppression of tau-induced behavior by loss of DDC gene *bas-1* was not altered by additional loss of D_2-like dopamine receptor genes *dop-2* and *dop-3*, indicating that DDC and D_2-family dopamine receptors share a mechanism for suppression of tau. Interestingly, pharmacological agents that inhibit D_2 receptors or antisense RNA against D_2 receptors have been shown to increase DDC activity in rodents (35). This is the opposite of what we would expect based on our data. However, constitutive loss of D_2-family dopamine receptors may have different effects on DDC activity in *C. elegans* compared with short-term or pharmacological loss of D_2-family dopamine receptor activity in rodents.

In conclusion, we have identified *bas-1*, the *C. elegans* homolog of DDC, as a suppressor of tau toxicity in our transgenic *C. elegans* model of tauopathy. While we have determined that upstream enzymatic activity by tyrosine hydroxylase and tryptophan hydroxylase are necessary for observing the full suppression by DDC, the mechanism of tau suppression is still unclear. Because loss of *bas-1* reduced phosphorylation of tau, there are likely kinases or phosphatases modulated by loss of *bas-1* function; further study will be needed to identify them. In addition, further translational studies exploring whether loss of DDC suppresses tau toxicity in the mammalian brain will be required to understand whether DDC could serve as a point of intervention in authentic human tauopathy disorders.

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