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Morphological Analysis of Dopaminergic Neurons with Age Using *Caenorhabditis elegans* GFP Reporter Strains

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[Abstract] This protocol describes how to quantify different morphological defects as observed in the dopaminergic neurons using *C. elegans* GFP reporter strains with age.
Keywords: Dopaminergic neurons, Neurodegeneration, Aging, GFP Reporter strains, *C. elegans*

Materials and Reagents

1. Plastic plates, 60 x 15 mm (Olympus Plastics, catalog number: 32-105G)
2. Microscopic slides (Denville Scientific, catalog number: M1002)
3. Micro cover glass, 22 x 22 mm (VWR, catalog number: 48366-067)
4. OP50-1 Bacteria (*E. coli* https://cgc.umn.edu/strain/OP50-1)
5. *C. elegans* reporter strains
   BY200 - *Pdat-1*::GFP*(vtIs1) V* (Michael Aschner, Albert Einstein College of Medicine, NY, USA)
   BY250 - *Pdat-1*::GFP*(vtIs7) V* (Randy Blakely, Florida Atlantic University, FL, USA)
   UA44 - *[baInl1; Pdat-1::α-syn high, Pdat-1::gfp]* (Guy Caldwell, University of Alabama, AL, USA)
   UA57 - *baIs4 [dat-1p::GFP + dat-1p::CAT-2]* (CGC, University of Minnesota, MN, USA)
6. Sodium hypochlorite solution (Avantor Performance Materials, J.T. Baker, catalog number: 9416-01)
7. M9 buffer
8. NGM agar
9. Agarose LE (Apex Chemicals and Reagents, catalog number: 01132-34)
10. Levamisole hydrochloride (MedChemExpress, catalog number: HY-13666)
11. Immersion Oil (Immersol 518F, Carl Zeiss, catalog number: 444960-0000-000)

Equipment

1. Centrifuge
2. Shaker
3. Fluorescent Microscope (Olympus, model: BX51, TRF)
4. Digital camera (Hamamatsu ORCA-ER, version: C4742-80)

Software

1. ImageJ
2. GraphPad Prism

Procedure

1. Embryos were obtained by hypochlorite treatment of gravid adults as stated in He, 2011.
2. After incubate the embryos for 17-24 h in M9 buffer to obtain synchronized L1s, wash the worms once in 10 ml dH_2O, spread on OP50-1 containing plates and incubate at 20 °C during the whole assay time period.
   Note: The assay was conducted at different stages of worms' life cycle: L4 larvae, day 5 and day 10 adults (using 40 to 50 worms per biological replicate). Also, during the assay, from L4 till day 10 worms were transferred every day till day 5 and from then on every alternate day till day 10, to separate the hermaphrodites from their progenies and also to avoid any risk of potential contamination in the plate or being the OP50-1/food to run out.
3. On the day of the microscopy, immobilize worms with 3 mM levamisole on 4% agarose pads and score immediately.
   a. For quantitative analyses of dopaminergic neurodegeneration, examine approximately 50 worms under a fluorescent compound microscope (Olympus, Hamburg, Germany) using filters for excitation wavelengths from 455 to 490 nm at 60x oil objective.
   b. For dopaminergic neuron analyses using the *dat-1* promotor, examine all four cephalic sensilla (CEP) dendrites and GFP fluorescence within the dendrites followed from the nerve ring to the tip of the nose along with the two anterior dierids (ADE) neurons (Figure 1).

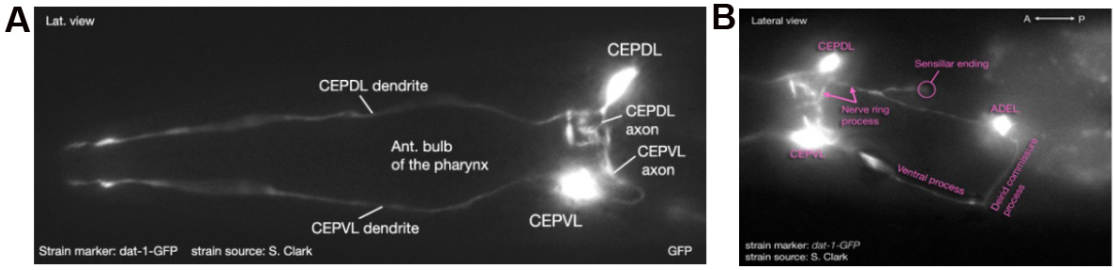


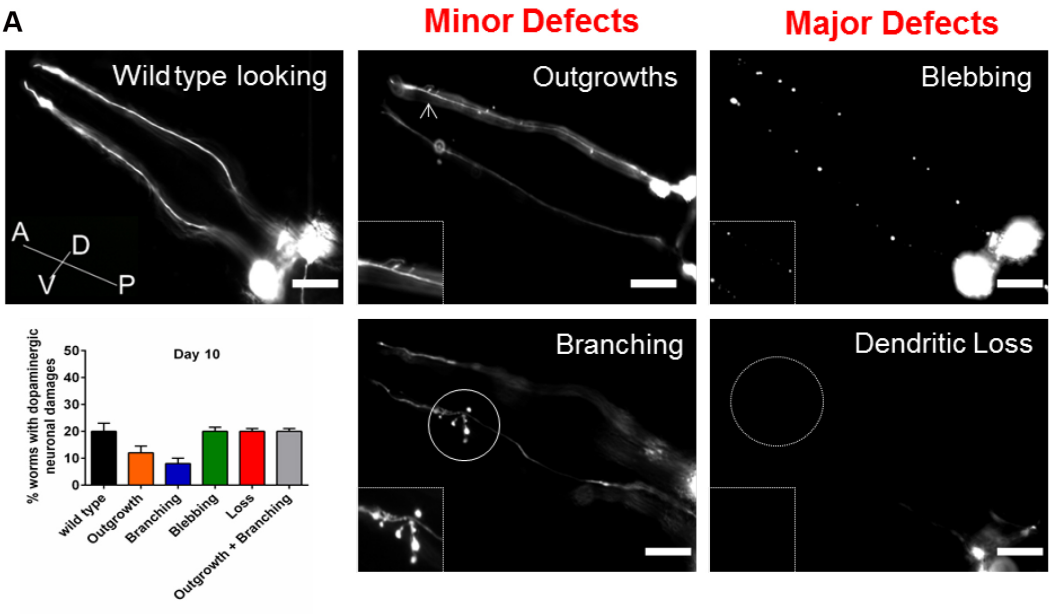
Figure 1. Anterior *C. elegans* dopaminergic neurons as visualized by the *dat::GFP* reporter strain. A. Image depicting the CEP dendrites and axons (dorsal and ventral). B. Image depicting of one of the ADE neurons with its process in the worm strain carrying GFP to label these dopaminergic neurons. Images taken as a reference from Wormatlas.

4. If any part of the dendrite was absent, the worm is considered to have altered dopaminergic neurons and is scored as positive for dopamine neural loss. Other major phenotypes such as

axonal blebbing (broken neurites) & minor phenotypes such as axonal branching and outgrowth are counted likewise (Figure 2A). Normally, a worm displays a blebbing phenotype or a neuronal loss phenotype only, but in some cases it can show multiple phenotypes such as branching and/or outgrowth simultaneously, indicating that particular neuron is getting damaged progressively (Nass *et al.*, 2002; Cao *et al.*, 2005).

5. Count and sum up the number of worms having those specified defects (mentioned in Step 4) and then divide it by the total number of worm used in this assay. Finally multiply with 100 to express the percentage (Figure 2B).

A



B

	wt looking	Outgrowth	Branching	Blebbing	Loss	Outgrowth+Branching	Total # of worms
# of worms	10	6	4	10	10	10	50
percentage of worms	20	12	8	20	20	20	

Figure 2. Different stages of Dopaminergic (DA) neurodegeneration using the BY250 (*dat-1p::GFP*) *C. elegans* reporter strain. A. From left to right, representative temporally staged images depicting CEP DA neuron morphology of wild type and its two kinds of defects observed (minor–outgrowth and branching; major–blebbing and dendritic loss) specifically in the later stages of their life cycle, starting from Day 10. Scale bars = 20 μm. Left bottom shows a representative graph showing quantification of the neuro degeneration assay. Scale bars in right bottom images are 10 μm. B. A sample scoring sheet depicts how the quantification is inputted. Basically, count the number of worms carrying each particular defect, sum it up, divide by the total worm population used in the assay and then multiply by 100 to express as a percentage. In rare cases, a single worm can carry multiple neuron defects, and they are counted likewise.

Acknowledgments

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Conflict of Interest: The authors declare that they have no conflict of interest.

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