Genetic analysis of age-dependent defects of the Caenorhabditis elegans touch receptor neurons

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Although many genes have been implicated in the pathogenesis of common neurodegenerative diseases, the genetic and cellular mechanisms that maintain neuronal integrity during normal aging remain elusive. Here we show that Caenorhabditis elegans touch receptor and cholinergic neurons display age-dependent morphological defects, including cytoskeletal disorganization, axon beading, and defasciculation. Progression of neuronal aging is regulated by DAF-2 and DAF-16 signaling, which also modulate adult life span. Mutations that disrupt touch-evoked sensory activity or reduce membrane excitability trigger accelerated neuronal aging, indicating that electrical activity is critical for adult neuronal integrity. Disrupting touch neuron attachment to the epithelial cells induces distinct neurodegenerative phenotypes. These results provide a detailed description of the age-dependent morphological defects that occur in identified neurons of C. elegans, demonstrate that the age of onset of these defects is regulated by specific genes, and offer experimental evidence for the importance of normal levels of neural activity in delaying neuronal aging.

extracellular matrix | insulin signaling | ion channels | mechanosensory neurons | neurodegeneration

In humans, aging is accompanied by the progressive decline in behavioral and cognitive functions. Studies have shown that the numbers of neurons in most cortical regions of the human brain are relatively preserved during aging (reviewed in ref. 1). Although reduced synaptic numbers or white matter density has been proposed as the cellular basis for age-related behavioral decline (reviewed in ref. 1), descriptions of neuronal morphology during aging remain inconsistent, and a detailed record of normal neuronal aging is largely lacking.

In the nematode Caenorhabditis elegans, age-dependent morphological changes are widespread in somatic tissues (2–4). However, there is little evidence for neuronal aging in C. elegans (3). The observations by Herndon et al. (3) suggest that neuronal loss or axon guidance defects do not occur in the aging C. elegans nervous system. It was also shown that whereas nuclear membranes of other somatic cell nuclei undergo drastic age-dependent deterioration, those of neuronal nuclei remain relatively intact in aging animals (4).

There is, however, a clear age-dependent behavioral decline in C. elegans, including decrease in pharyngeal pumping, locomotion and chemotaxis (5). Evidence suggests that failure in neuronal activity could play a direct role in age-dependent behavioral deterioration. Cai and Sesti (6) showed that age-dependent oxidation of the C. elegans potassium channel KV-1 causes sensory loss and that protection of neuronal KV-1 from oxidation rescues age-dependent decline in chemotaxis behavior. Electrical activity has been shown to be important for neuronal development (7) and was recently implicated in the survival or maintenance of adult mammalian and Drosophila neurons (8, 9). However, it is unclear how electrical activity promotes the integrity of adult neurons.

In this paper, we address whether more subtle, subcellular changes occur in the aging C. elegans nervous system. Our results indicate that C. elegans neurons do develop age-dependent changes. Moreover, we show that electrical activity and normal attachment to the neighboring epithelial cells are required for the maintenance of adult touch receptor neurons.

Results

Age-Dependent Neuronal Defects in C. elegans. To further characterize age-dependent changes in C. elegans neurons, we focused on the ALM and PLM touch receptor neurons, two classes of bilaterally symmetric mechanosensory neurons that respond to light touch. These neurons extend a long anterior process that makes synaptic contact with other neurons and a short posterior process with no known function (Fig. L4). The ALM process extends a synaptic branch into the nerve ring, the major C. elegans neuropil, and the PLM process extends a collateral branch to make synaptic contact in the ventral nerve cord (10). Whereas the ALM soma was typically round or oval in young adult animals, it became progressively irregularly shaped in old animals and often elaborated aberrant protrusions (Fig. L1 B and C). Immunostaining for acetylated α-tubulins revealed that these aberrant protrusions contain acetylated microtubules (Fig. L1B). In young animals, microtubule bundles were oriented in parallel, whereas in old animals with irregular ALM somas, microtubule bundles appeared more disorganized (Fig. L1B). We also observed age-dependent defects in the ALM and PLM processes (Fig. L1 E–K and Table S1). Bubble-like lesions could occur along the length of the touch neuron processes (Fig. L1E). Beading represented focal enlargement along the nerve process (Fig. L1F). Blebs, triangular-shape protrusions from the touch neuron processes, often led to distortion of the gross structure (Fig. L1H). In extremely old animals [days of adulthood 20 (D20) or older], slender branches developed from the blebs (Fig. L1I). Beading and bubble-like lesions occurred in both the ALM and the PLM, whereas blebs and branching occurred most frequently in the PLM. These age-dependent defects were also seen in the AVM and PVM touch neurons. We confirmed these observations with a second GFP reporter and also by acetylated α-tubulin staining (Fig. S1 A and B). Axon beading or blebs were not labeled by antibodies against Rab7, a marker for late endosome and lysosome (Fig. S1 E–J). This leads us to speculate that some of the blebs or bubble-like lesions may represent an early phase of axon splitting. The frequency of these abnormalities increases with age (Fig. 1 C, J, and K). Importantly, even in touch neurons with marked somatic and axonal defects, the nuclear DAPI staining was intact and never appeared fragmented (Fig. S1 K and L), suggesting that these aged neurons were not in the process of apoptosis or necrosis.

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The authors declare no conflict of interest.

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Longitudinal Imaging of *C. elegans* Touch Neurons During Aging. To understand how age-dependent defects evolve in touch neurons over time, we performed longitudinal imaging on individual ALM neurons over the animal's life span (Fig. 2). The onset of growth of aberrant protrusions is variable, and they may persist or retractor from the cell body briefly after their appearance (Fig. 2A). The posterior process of ALM sometimes branched in middle age (Fig. 2B). We also documented the formation of bubble-like lesions (Fig. 2A). A segment of the ALM process first became distended, and several foci devoid of axoplasmic GFP fluorescence subsequently coalesced to form a single large bubble-like structure, which could continue to expand (Fig. 2A). We observed disappearance of the bubble-like lesion 1 day before the animal died, accompanied by a thinning and disassembly of the ALM process (Fig. 2A). These observations highlight the transient and dynamic nature of morphological defects in aging touch neurons. In contrast to axon elongation after laser axotomy (reviewed in ref. 11), the age-dependent morphological decline of *C. elegans* neurons seen in our longitudinal studies was slow, and we did not observe neuronal behaviors indicative of regeneration.

Fig. 1. Age-dependent defects occur in *C. elegans* touch receptor neurons. (Scale bar: 5 μm.) (A) Diagram of *C. elegans* paired ALM and PLM touch receptor neurons (lateral view). Only neurons on the left are shown. (B) Immunostaining of the wild-type ALM neuronal cell bodies with the anti-acetylated α-tubulin antibody 6-11B-1. The age of the animals (days of adulthood) is indicated. (C) Quantification of ALM neurons with irregularly shaped soma in the wild type and the mutant representatives. Representative images of different wild-type ALM neuronal shapes are shown, with number of neurons scored (n) at each time point. GFP is from *zdsIs(Pmec-4::gfp)*. (D–I) Epifluorescence images of age-dependent defects of the ALM (D and E) and the PLM (F–I) processes in wild type. Anterior is to the left. (E) A bubble-like lesion in the ALM process. *Inset* is enlarged on the right. Beading (F, arrows), blebbing (H, arrows), and branching (I) of the PLM process are shown. Branching appears to develop from the sites of blebbing (l, arrow). (J and K) Quantification of the ALM (J) and the PLM (K) process abnormalities in wild-type and mutant adult animals. Error bars are SEs of proportions. The number of neurons scored (n) at each time point is provided. *P < 0.05, **P < 0.01, and ***P < 0.001 (Fisher's exact test or two-proportion test).

To test whether age-related defects are specific to touch neurons, we examined cholinergic axons in the ventral nerve cord (VNC), a pair of longitudinal nerve tracts that extend from the head to the tail. VNC cholinergic axons of young adult animals travel along the antero-posterior axis in two discrete fascicles on the right side, and individual axons are tightly packed into either fascicle (Fig. S2A). In middle age (D6–D8), however, occasionally three, instead of two, cholinergic nerve fascicles were observed in the right VNC (Fig. S2B). The length of the supernumerary processes and the invariant anterior–posterior orientation suggest that these were defasciculated axons rather than aberrant neuronal sprouts. VNC defasciculation became more pronounced, and axon beading developed in animals of advanced age (D14–D17) (Fig. S2B and E). These results indicate that age-dependent morphological defects occur in multiple classes of *C. elegans* neurons.
Age-Dependent Neuronal Defects Are Modified by Genes That Control the Life Span. Genes that control *C. elegans* life span also control age-dependent tissue degeneration. Tissue degeneration is delayed in the long-lived *daf-2* mutant and is accelerated in the short-lived *daf-16* mutant (2, 4). The FOXO transcription factor DAF-16 mediates the longevity effects of mutations in *daf-2*, which encodes the sole insulin-like growth factor receptor homolog in *C. elegans* (12). We hypothesize that age-dependent neuronal defects are also regulated by mutations that affect life span.

We first examined the long-lived *daf-2* mutants and found that age-dependent defects of touch neurons and VNC cholinergic axons were significantly delayed (Fig. 1 C, J, and K and Fig. S2). By contrast, the frequency of touch neuron defects was significantly increased in the short-lived *daf-16(mu86)* mutants (Fig. 1 I and K). The development of touch neurons in *daf-16* mutants was normal, indicating that *daf-16* neuronal phenotypes represent premature aging rather than developmental abnormalities. These results indicate that insulin signaling regulates morphological aging of touch neurons in *C. elegans*.

We next examined mutations that affect aging independently of insulin signaling. Mutations in the *eat-2* nicotinic cholinergic receptor reduce pharyngeal pumping rate and promote moderate life-span extension (13), and the *eat-2(ad465)* mutant did not display premature neuronal aging. Mutations in *hsf-1*, which encodes the heat-shock transcription factor that mediates cellular responses to heat stress, shorten life span (14). We found that the loss-of-function *hsf-1(sy441)* mutation accelerated neuronal aging (Fig. 1 C, J, and K and Fig. S2 C–E). Age-dependent defects of touch neurons were not enhanced in the *daf-16 hsf-1* double mutants, suggesting that these two genes largely act in the same pathway for neuronal aging (Fig. S3 A). Similarly, mutations in the nuclear lamin gene *bnn-1* shortened life span and triggered neuronal aging (SI Results).

The morphological features of neuronal defects in the short-lived *daf-16* and *hsf-1* mutants were indistinguishable from those in aged wild-type worms, indicating that the observed neuronal defects in the *daf-16* and *hsf-1* mutants represented accelerated aging, which is supported by our longitudinal imaging (Fig. S4). Restoration of DAF-16 functions specifically in the neurons or in the body-wall muscles did not rescue the age-dependent touch neuron defects of the *daf-16(mu86)* mutant, indicating that DAF-16 acts outside the nervous system to regulate neuronal aging in *C. elegans* (Fig. S3 B).

**Touch Neuron Defects in Mutants Defective for Touch Sensitivity.** To test whether evoked activity is required for the maintenance of touch neurons, we examined mutants in which touch-evoked sensory transduction in these neurons was absent (also known as DAF-16 acts outside the nervous system to regulate neuronal aging in *C. elegans* (Fig. S3 B).

![Progressive touch neuron defects in the mec mutants. (A)](image-url) **Summary of the mec genes and their encoded proteins. (B)** Representative live GFP images (except for that of the mec-12 mutant, which is immunofluorescence image-labeled by 6–11B-1) of the ALM processes in the wild type (D16) and the mutants (D9). (Scale bar: 5 μm.) Arrows and arrowheads indicate branching and bubble-like lesions, respectively. (C) Quantification of defective ALM processes. Total numbers of ALM neurons scored (D1/D9/D12): wild type, 29/25/30; mec-1, 25/23/20; mec-2, 17/28/19; mec-4, 24/18/31; mec-5, 21/23/19; mec-6, 19/25/27; mec-9, 20/16/27; mec-10, 23/20/23; mec-12, 19/27/25. (D) Percentages of bubble-like lesions, beading, or blebbing/branching in all of the abnormal events found in the ALM neurons of wild type and the mutants. The age of the animals is specified. (E) Defects of the ALM and the PLM processes of D3 mec-1(e1066) mutants are enhanced by a daf-16 (mu86) mutation. (F) Overexpression of DAF-16 from the endogenous daf-16 promoter significantly suppresses ALM and PLM defects of D9 mec-1(e1066) mutants. Error bars are SEs of proportions. **P < 0.01 or ***P < 0.001 (Fisher’s exact test or two-proportion test).
the Mec phenotype) (10, 15) (Fig. 3A), while responses to harsh mechanical stimulation are retained. We found that touch neurons in the mec mutants developed normally, but displayed significant defects starting from mid-adulthood and increasing with age (Fig. 3B and C). Although mutations in mec-2, mec-4, mec-5, mec-6, mec-9, and mec-10 caused a shortened life span, those in mec-1 and mec-12 did not (Fig. S5). This indicates that mec-1 and mec-12 mutations specifically induce age-dependent neuronal defects without affecting the general aging process. In support of this hypothesis, we found that a daf-16 mutation significantly increased touch neuron defects in mec-1 animals (Fig. 3F). Overexpression of DAF-16, by contrast, significantly rescued the neuronal aging phenotypes of mec-1 mutants (Fig. 3F). These results indicate that activities of DAF-16 and MEC-1 act synergistically to maintain the integrity of touch neurons.

Age-dependent neuropathology of the mec mutants could be further divided into two types. Type 1 pathology was characterized by blebbing and bubble-like lesions of the ALM processes, which were similar to the aging phenotypes in wild-type animals (Fig. 3B and D). Mutations in genes that encode the touch mechanosensory channel components MEC-2, MEC-4, MEC-6, and MEC-10 (10, 16–18), the extracellular matrix (ECM) protein MEC-9 (19), and the α-tubulin MEC-12 (10) led to type 1 pathology (Fig. 3A, B, and D). By contrast, type 2 pathology was characterized by blebbing, branching, and distortion of the nerve processes. Mutations in genes that encode the unique ECM proteins MEC-1 and MEC-5 (19, 20) led to type 2 pathology (Fig. 3A, B, and D). These abnormal branches contained acetylated microtubules (Fig. S6 A and B).

Disruption of Nerve Attachment Leads to Blebbing and Branching of the Touch Neuron Processes. To our surprise, not all mec-1 mutations led to type 2 pathology. mec-1(e1066) and mec-1(e1292), which are predicted to truncate significant portions of the N-terminal region containing multiple EGF and Kunitz domains (20), caused type 2 pathology (Fig. 4B and G and Fig. S6B). By contrast, mec-1(e1526), which eliminates the C-terminal domain and the last Kunitz domain (20), caused type 1 pathology (Fig. 4B and H and Fig. S7B).

The aforementioned differences in neuronal defects could not be explained by a disruption in sensory-evoked activity, as these mutants were similarly impaired for touch sensitivity. In wild type, the processes of touch receptor neurons are engulfed by the neighboring epithelial cells and are separated from the body-wall muscles (20). Subsequently, hemidesmosome-like structures form in the squamified epithelial cell cytoplasm that overlies the touch neuron process, which completes the process of nerve attachment. In the mec-1(e1066) mutant, touch neuron processes failed to separate from body-wall muscles, and normal nerve attachment did not form (10, 20) (Fig. 4A–D). We found that nerve attachment was also disrupted in the mec-1(e1292) mutant. By contrast, the mec-1(e1526) mutant had intact nerve attachment (Fig. 4E). These results suggest that disruption of nerve attachment leads to type 2 pathology in the touch receptor neurons.

To test this hypothesis, we examined nerve attachment in representative mutants leading to type 1 or type 2 pathology. We found that, in the mec-4 and the mec-9 mutants, which showed type 1 pathology, nerve attachment was intact (Fig. S7D and E). By contrast, in the mec-5 mutant, which displayed type 2 pathology, nerve attachment was disrupted (Fig. S7C). Some mec-5 mutants retained partial nerve attachment, consistent with the fact that branching phenotypes of the mec-5 mutant were less severe than those of the mec-1(e1066) mutant (Fig. 3C).

We further tested two mutants with normal touch sensitivity but defective nerve attachment, him-4 and fbl-1, which encode the ECM proteins hemicentin and fibulin, respectively (21, 22). The him-4(rk319) null mutants phenocopied the mec-1(e1066) and the mec-1(e1292) mutants, displaying characteristic blebbing and branching defects of ALM and PLM (Fig. S6 C–F and I). Because the fbl-1(hd43) homozygotes were almost completely sterile, we examined fbl-1 homozygotes that segregated from the fbl-1 heterozygotes and thus retained the maternal FBL-1 components (fbl-1 M+Z–). In the fbl-1(hd43) (M+Z–) mutants, early onset of adult touch receptor neuron defects was found, with comparable frequency of type 1 and type 2 pathology (Fig. 3D and Fig. S6 G, H, and J). Taken together, these results indicate that disruption of nerve attachment induces blebbing and branching in the adult touch neurons.

Fig. 4. Disruption of nerve attachment or neuronal activity causes distinctive degeneration phenotypes. (Scale bar: 10 μm.) (A) Diagram of touch neuron attachment (transverse section). In wild-type L1 larvae, the process of the touch receptor neuron lies adjacent to the body-wall muscles. The nerve process later adopts the adult position where it is attached to the overlying cuticle and is thus separated from the muscles. In mutants with defective attachment, the touch neuron process remains in the juvenile position and is not attached to the epithelium. (B) MEC-1 protein structure. e1066, splice junction mutation; e1292, nonsense mutation; e1526, missense mutation. Orange, EGF domains; green, Kunitz domains; blue, the C terminus domain. Percentages of the predominant type defects (branching/blebbing or bubble-like/beading) of all axonal defects are provided. (C–E) Attachment of the ALM process in wild-type (C) and mec-1 mutants (D and E). (Upper panels) Differential interference contrast (DIC) images. (Lower panels) Overlay of DIC and GFP images. The lower borders of body-wall muscles are indicated by arrowheads. (F–H) Representative pathology of aging ALM neurons in wild-type (F) and mec-1 mutants (G and H). Single arrows: bubble-like lesions. Double arrows: branching of the nerve processes.

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Evoked Activity Is Required for the Maintenance of Adult Touch Neurons. Our mutant analysis suggests that sensory-evoked activity is required for the maintenance of adult touch neurons. To test this hypothesis further, we inactivated touch neurons by using a hyperpolarizing ion channel. The outward hyperpolarizing BK potassium channel SLO-1 is broadly expressed in the C. elegans neurons (23). In the slo-1 gain-of-function mutants slo-1(ky389) and slo-1(ky399) (24, 25), touch sensitivity was partially defective, suggesting that touch-evoked sensory transduction was impaired. Early onset, progressive touch neuron defects were found in both slo-1(ky389) and slo-1(ky399) mutants, including the formation of bubble-like lesions and axon beading (Fig. 5). Touch neuron somas in these mutants began to degenerate in early adulthood, characterized by a disorganization in shape, breakdown of microtubule bundles, and eventually complete disappearance of cell bodies (Fig. 5). Overexpression of wild-type SLO-1 in the neurons using the snb-1/synaptobrevin promoter also induced similar phenotypes (Fig. 5). To show that the requirement for evoked activity in neuronal maintenance is not a unique property of the touch neurons, we examined the requirement for evoked activity in neuronal maintenance is neither necessary nor sufficient for touch sensitivity, as attachment-defective him-4 and fbl-1 mutants show nearly normal touch response, and the attachment-intact mec-1(e1526) mutant is touch-insensitive (20–22). Our results are consistent with a model where HIM-4, MEC-1, and MEC-5 function in a common pathway for touch neuron attachment, which in turn is required for the maintenance of adult touch neuron integrity. Attachment of the nerve processes may protect them from mechanical strains during movements. Alternatively, ECM proteins may function to stabilize the neuronal membrane and the cytoskeleton via their interactions with membrane receptors and cytoskeleton adaptors in the neurons. Although no MEC-1 homologs have been identified in species other than C. elegans and Caenorhabditis briggsae, mutations in human hemicentin, the C. elegans homolog of which is HIM-4, have been associated with age-dependent macular degeneration, consistent with their functions in the maintenance of adult tissue integrity (27).

Discussion

A recent report shows that, although the numbers of neurons are preserved, loss of neuronal and dendritic architectures are likely responsible for the cortical thinning and brain atrophy in the normally aging human brain (26). Together with the findings presented in the current study, we conclude that alterations in neuronal cytoarchitecture are hallmarks of aging in both the vertebrate and the invertebrate nervous systems.

Our observation that nerve attachment is essential for the maintenance of adult touch receptor neurons reveals a previously unidentified role for this developmental process. Nerve attachment is neither necessary nor sufficient for touch sensitivity, as attachment-defective him-4 and fbl-1 mutants show nearly normal touch response, and the attachment-intact mec-1(e1526) mutant is touch-insensitive (20–22). Our results are consistent with a model where HIM-4, MEC-1, and MEC-5 function in a common pathway for touch neuron attachment, which in turn is required for the maintenance of adult touch neuron integrity. Attachment of the nerve processes may protect them from mechanical strains during movements. Alternatively, ECM proteins may function to stabilize the neuronal membrane and the cytoskeleton via their interactions with membrane receptors and cytoskeleton adaptors in the neurons. Although no MEC-1 homologs have been identified in species other than C. elegans and Caenorhabditis briggsae, mutations in human hemicentin, the C. elegans homolog of which is HIM-4, have been associated with age-dependent macular degeneration, consistent with their functions in the maintenance of adult tissue integrity (27).

We demonstrated that disrupted evoked activity or reduced neuronal excitability accelerates aging of the touch neurons. Disrupting the mechanosensory channel components abolished the evoked touch current in the neurons, and a mutation in the α-tubulin MEC-12, e1605, also significantly reduced this current (15, 28). Overactivation of the hyperpolarizing potassium channel SLO-1 impaired neuronal excitability and triggered progressive neuronal defects. Because slo-1 is broadly expressed in the nervous system, the effects of excessive SLO-1 activity on the maintenance of touch neurons could be cell-autonomous, or it could act in other neurons and impact touch neurons indirectly. We also noted that, in animals with excessive SLO-1 activity, acetylated microtubule immunoreactivity sometimes became discontinuous in the process or was significantly diminished in the soma of touch neurons. These findings were rare in aging wild-type touch neurons, suggesting that SLO-1 activity may impair neuronal structure via a hyperpolarization-independent mechanism. Nonetheless, together with the observation that altered synaptic transmission affects adult neuronal integrity, these data support the hypothesis that a reduction in neuronal activity likely contributes to morphological alterations during neuronal aging. unc-13 and unc-18 mutations did not trigger premature aging in the touch neurons, probably because they are not postsynaptic to other neurons, and their evoked activity is insufficient to maintain neuron integrity.

![Suppression of electrical activity by slo-1 gain-of-function mutations induces progressive touch receptor neuron defects.](image)

[Fig. 5. Suppression of electrical activity by slo-1 gain-of-function mutations induces progressive touch receptor neuron defects. (Scale bar: 5 μm.) (A) Immunofluorescence images of ALM neurons in the wild-type, slo-1(ky389), slo-1(ky399), and slo-1(s118); Pnb-1::slo-1 animals at D3 and D7 of adulthood, with touch receptor neurons labeled by the 6–11B-1 antibody. Ky389 and ky399 are gain-of-function mutations, and s118 is a recessive loss-of-function mutation. Acetylated tubulin was decreased and disorganized in the neuronal cell bodies in slo-1(ky389), slo-1(ky399), and slo-1(s118); Pnb-1::slo-1 mutants. Single arrows: bubble-like lesions; single arrowheads: discontinuity of the acetylated tubulin immunoreactivity; double arrows: ALM cell bodies; double arrowheads: branching from the nerve processes. (B) Quantification of ALM defects in wild type, slo-1(ky389), slo-1(ky399), and slo-1(s118); Pnb-1::slo-1 animals at D1, D3, and D7. Error bars are SEs of proportions. The number of cells scored is indicated. N/A, not available.]
dependent of synaptic release. Interestingly, a dominant, gain-of-function mec-4 mutation [mec-4(d)], which constitutively activates the MEC-4 Na\(^{+}\) channel, causes the touch neurons to develop enormous cytoplasmic vacuoles and die rapidly (18), probably caused by massive ion influx and subsequent activation of proteolytic enzymes (29). These results indicate that the overall level of activity is critical for the survival or maintenance of neurons. Identification of the transcriptional targets of electrical activity in the maintenance of adult neurons during aging may facilitate the development of therapeutics that effectively modify the progression of age-dependent neurodegenerative diseases.

Materials and Methods

C. elegans strains were cultured under established conditions. Information on alleles and integrated arrays, FUDR treatment, and immunofluorescence microscopy are in SI Materials and Methods.

Longitudinal Fluorescence Microscopy. Individual animals were immobilized with levamisole and imaged under a Zeiss AxioSkop microscope. Animals were rescued from the slides after imaging, placed on plates with bacterial food, washed with several drops of physiological M9 buffer, and allowed to recover. The imaging procedure took less than 2 min and was well tolerated by the animals. The same sides were repeatedly imaged for individual animals at defined time points along the animal’s life span. A total of 17 wild-type animals were recorded, and the maximal ages of recorded animals are the following: D17, \(n = 3\); D16, \(n = 1\); D15, \(n = 4\); D14, \(n = 4\); D12, \(n = 1\), and D9, \(n = 3\). One animal died after two imaging sessions on D1 from an unknown cause. In sum, 13 of 17 (76%) of the animals could be repeatedly imaged until D12 or older.

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Supporting Information

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SI Results

Elimination of C. elegans nuclear membrane components shortens lifespan (1). Because animals lacking both the maternal and zygotic components of the nuclear membrane protein LMN-1/ lamin are not viable, we analyzed mutants lacking the zygotic but retaining the maternal LMN-1, lmn-1 (M+Z-). Similar to daf-16 and hsf-1 mutants, lmn-1 (M+Z-) animals had shortened lifespan (1) and accelerated touch neuron aging (D1, no touch receptor neuron defects, n = 15; D4, ALM defects, 16%, n = 19; PLM defects, 11%, n = 19; D6, ALM defects, 52%, n = 21; P = 0.0000068, PLM defects, 57%, n = 23, P = 0.00009, Fisher’s exact test).

SI Materials and Methods

The following Caenorhabditis elegans alleles and transgenes were used in the current study: LGI: daf-16(mu86) (2), hsf-1(sy441) (3), mec-6(e1342) (4), lmn-1(tm1502)/hfl2 (1), unc-13(e1091), zdIs5[Pmec-4::gfp, lin-15(+)]; LGII: eat-2(ad465) (6), mls32[Pmec-7::gfp, lin-15(+)]; LGIII: daf-2(e1368), daf-2(e1370) (7), mec-12(e1605) (8); LGIV: fbl-1(hd43)/dpy-20(e1282) unc-24(e138) (9), zls356[Palph-16::daf-16::gfp, rol-6(su1006)] (10); LGV: mec-1(e1532), mec-1(e1594) (all from ref. 8), slo-1(js118) (11), slo-1(ky389), slo-1(ky399) (all from ref. 12), Punc-17::rpf (13); LGX: mec-2(e75) (8), mec-4(u253) (14), mec-5(e340) (8), mec-10(e1515) (8), him-4(rh319) (9), unc-18(e234), dkg-1(sy428); linkage group undetermined: mlsIs126[Pmyo-3::

gfp::daf-16, rol-6(su1006)], mlsIs131[Punc-119::gfp::daf-16, rol-6(su1006)] (15).

FUDR Treatment

slo-1(ky389) and slo-1(ky399) mutants are strongly egg laying-defective (Egl) and mostly die of internal larval hatching by D2. him-4(rh319) adult animals usually die of explosion from the vulva, a consequence of egg laying through a weakened uterine-vulval structure in this mutant. To circumvent these problems and obtain adult animals at older ages, we treated these mutants with 5-fluoro-2-deoxyuridine (FUDR) to prevent progeny production (16). Animals were placed as L4 on regular bacterial feeding plates with 20 mM FUDR. FUDR at this concentration effectively eliminated all progeny production and did not affect animals’ lifespan or neuronal morphology in wild type controls.

Immunofluorescence Microscopy

Immunostaining was performed as described previously (17). Primary antibodies were mouse monoclonal anti-acetylated a-tubulin antibody 6-11B-1 (1: 100, Santa Cruz Biotech) (18) or rabbit polyclonal Rab7 antibody (1: 200, a gift from Dr. Barth Grant, Rutgers University, Piscataway, NJ), and secondary antibodies were 1% Alexa488- and Alexa568-conjugated goat anti-rabbit or goat anti-mouse antibodies (Molecular Probes). Animals were counter-stained with DAPI for better cell recognition. Images were acquired under the Zeiss Axioskop or Zeiss LSM510 confocal microscope.

Fig. S1. Age-dependent morphological defects of touch receptor neurons in wild-type animals. Ages (days of adulthood) are indicated. (Scale bar: 5 μm.) (A and B) 6–11B-1 staining shows bubble-like lesions (A, arrowheads) and blebs (B, arrows) in the PLM processes. (C and D) Signs suggesting splitting of touch neuron processes in the PLM (C, epifluorescence) and the ALM (D, 6–11B-1 staining). Arrows indicate sites of splitting. (E–J) Confocal images of double immunostaining for acetylated α-tubulin (E and H, 6–11B-1) and lysosomes (F and I, anti-Rab7). Arrows indicate a beading in the ALM process (E–G) or blebs in the PLM process (H–J). These lesions are not colabeled with anti-Rab7, as shown in the merged images (G and J). Robust Rab7 immunoreactivity could be seen in the body-wall muscles (F and G). (K and L) Double labeling for acetylated α-tubulin (Left panels, 6–11B-1) and nuclear DNA (Center panels, DAPI) shows that even in aged ALM neurons with marked cytoskeletal defects, the nuclei seem to be intact. The arrowhead in K indicates a bubble-like lesion in the ALM process.
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Fig. S2. Age-dependent defects of ventral nerve cord (VNC) axons and touch neurons in wild type and mutants. (Scale bar = 5 μm.) (A–D) Epifluorescence images of wild-type (A and B) and hsf-1(sy441) mutants (C and D), in which VNC cholinergic axons were labeled with the reporter Punc-17::rfp. Anterior is to the left, with the right side down and the left side up. Arrows indicate defasciculated axons. (E) Quantification of VNC defasciculation in wild-type, hsf-1(sy441), and daf-2(e1368) mutants. (F and G) Quantification of age-dependent ALM (F) and PLM (G) defects in wild type, daf-2(e1368), and daf-2(e1370) mutants. daf-2(e1370) is a stronger allele than daf-2(e1368) in terms of life-span extension and constitutive dauer formation. Error bars: SEs of proportions. *P < 0.05; **P < 0.01; n.s., nonsignificant (Fisher’s exact test).
Fig. 53. (A) Age-dependent touch neuron defects in the daf-16(mu86) hsf-1(sy441) double-mutant animals. Numbers are neurons scored for each genotype at defined ages. P is calculated using the two-proportion test. (B) DAF-16 functions outside the nervous system to regulate neuronal integrity during normal aging. Bars indicate the percentages of defective neuronal processes for D1 (gray) or D9 (black) animals. Numbers are neurons scored for each genotype at defined time points. P is calculated using the two-proportion test. The integrated transgene muIs131(Punc-119::daf-16) expresses DAF-16 in the nervous system, and the integrated transgene muIs126(Pmyo-3::daf-16) expresses DAF-16 in the body-wall muscles. Both transgenes were kindly provided by Cynthia Kenyon (University of California San Francisco, San Francisco, CA).
Longitudinal imaging of progressive ALM and PLM defects in the *hsf-1* mutants. Epifluorescence images of an ALM (A and B) and a PLM (C) touch neuron in the *hsf-1*(*sy441*) animals at different time points over the animal’s life span (lateral view; anterior is up and ventral side to the right). GFP is *zdls5 (Pmec-4::gfp)*. Age of the animal is indicated as days of adulthood. [Scale bar: 5 μm (A and C) or 2.5 μm (B).] (A and B) Progressive ALM defects. The ALM process became thinner on D3, compared with that at L4 stage. Irregularity with a bubble-like lesion developed on D6 (Insets), and the process gradually disintegrated following D8. The animal died on D10. (B) Enlarged images of the Insets in the Upper panels. (C) Progressive PLM defects. Only the most distal part of the PLM process is shown. Similar to what occurred in the ALM process, the PLM process also became progressively thinner. Beading developed following D1, followed by irregularity of the process caliber. Arrows indicate a varicosity that became progressively enlarged after D6.
Fig. S5. Life-span assays of the wild-type and various mec mutants. Animals were cultured at 20 °C. The number of animals qualified for the life-span assay are in parentheses following each genotype.
Fig. S6. Blebbing and branching of the ALM processes in the mec-1, him-4, and fbl-1 mutants. Immunofluorescence (A–F) or epifluorescence (G and H) images of touch neuron processes in the mec-1(e1066), him-4(rh319), and fbl-1(hd43)(M+Z−) mutants labeled by the 6–11B-1 antibody. Arrows and arrowheads indicate branching and blebbing, respectively. (Scale bar: 10 μm.) (A and B) ALM in D9 mec-1(e1066). (C–F) him-4(rh319) on D1 (C and E) and on D5 (D and F). The phenotypes of ALM (C and D) and PLM (E and F) are highly reminiscent of those in mec-1. (G and H) fbl-1(hd43)(M+Z−) on D6, with ALM (G) and PLM (H) developing blebbing and beading. (I) Quantification of touch neuron defects in him-4 mutants. (J) Quantification of touch neuron defects in fbl-1(M+Z−) mutants. Error bars are SEs of proportions.
**Fig. S7.** Touch neuron defects in mutants with defective nerve attachment. (Scale bar: 10 μm.) (A) Quantification of defective ALM processes in wild-type and various **mec-1** mutant alleles at D1 and D12. (B) Percentages of bubble-like lesions, beading, or blebbing/branching formation in all of the abnormal events found in D12 wild-type and various **mec-1** mutant alleles. (C–E) Attachment of the ALM process in **mec-5** (C), **mec-4** (D), and **mec-9** (E) mutants. (Upper panels) Differential interference contrast (DIC) images. (Lower panels) Overlay of DIC and GFP images of ALM touch neurons. The inferior borders of body-wall muscles are indicated by arrowheads.
Fig. S8. Altered synaptic transmission influences the integrity of adult GABAergic motor neurons. (A) Schematic of the DD and VD GABAergic motor neurons and their axons in the ventral and dorsal nerve cords. The blue and the red boxes mark the regions of morphological assessment in the ventral and dorsal nerve cords, respectively. (B and C) Epi-fluorescence images of ventral (B) and dorsal nerve cords (C) in wild type and mutants with altered synaptic transmission. GFP is from *juIs76(Punc-25::gfp)*. Arrows indicate the cell bodies of the DD or VD neurons, and arrowheads indicate axon beading. (Scale bar: 10 μm.) (D and E) Quantification of ventral (D) and dorsal nerve cord (E) beading in wild type and mutants with altered synaptic transmission. Numbers are animals scored. N/A, not assessed.
Table S1. Age-dependent defects of touch receptor neuron processes in wild type and mutants

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