Chapter 8
Nervous System Ageing

Claire Bénard and Maria Doitsidou

Abstract In the face of ever-changing cellular environments during life and ageing, the nervous system ensures the coordination of behaviour and physiology. Over time, however, the nervous system declines structurally and functionally, leading to age-related cognitive and behavioural decline in humans. Aspects of nervous system ageing are being studied using *C. elegans* as a model system. Here we review the age-related neuronal changes that occur at the structural, cellular and functional levels in normally ageing animals, as well as how these changes relate to lifespan in healthy ageing and in neurodegenerative conditions. Understanding the cellular mechanisms that result in neuronal decline in *C. elegans* will help identify cellular factors that protect the nervous system structure and function during normal ageing and in disease states. Ultimately, elucidating the molecular networks and cellular processes underlying the ageing of the nervous system will fuel research and design of interventions to improve human life at old age.

Keywords *C. elegans* • Ageing • Aging • Neuronal • Neuron • Nervous system • Lifespan • Longevity • Behaviour • Decline • Memory • Learning • Axon regeneration • Neurodegeneration • Insulin signalling • Dietary restriction • Mitochondria • Proteostasis • Protein aggregation

8.1 Introduction

Ageing precipitates alterations in the physiology of the nervous system, including age-related cognitive decline and an increased incidence of neurodegenerative diseases. Whereas age is known to be a strong determinant of these conditions, the

---

C. Bénard (✉)
Department of Neurobiology, University of Massachusetts Medical School,
Worcester, MA, USA

Department of Biological Sciences, University of Québec at Montréal,
Canada
e-mail: claire.benard@umassmed.edu; benard.claire.2@uqam.ca

M. Doitsidou (✉)
Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK
e-mail: maria.doitsidou@ed.ac.uk

A. Olsen, M.S. Gill (eds.), *Ageing: Lessons from C. elegans*, Healthy Ageing
and Longevity 5, DOI 10.1007/978-3-319-44703-2_8

aetiology and molecular mechanisms leading to natural age-related neuronal deterioration are not well understood. Maintaining physiological functions with age depends on a continuous response to cellular stresses. General hallmarks of cellular ageing include DNA damage, loss of proteostasis, mitochondrial dysfunction, autophagy impairment, loss of cytoskeletal integrity, nutrient sensing dysregulation, among others. The molecular pathways that regulate cellular ageing are under intensive investigation and are reviewed elsewhere ([1, 2], Chaps. 6, 7, 8, 9, 10, 11, and 12 of this volume). The nervous system is inevitably impacted by universal cellular processes that lead to cellular ageing, as well as by neuronal-specific factors.

C. elegans is a powerful system in which to elucidate the genetic networks and molecular pathways underlying neuronal ageing. Its life cycle is fast, reaching adulthood in 3 days, generating its progeny in the following 5 days, and senescing in the following 2 weeks. Major conserved genetic determinants of lifespan have been elucidated [3, 4], enabling age manipulation in multiple ways. The worm's nervous system is simple, composed of exactly 302 identified neurons, which can be examined in exquisite detail in living animals at any point of their lives thanks to the worm's transparency and the ability to label specific neurons with fluorescent reporters. Further, the worm's entire neural circuitry has been defined, allowing one to probe neuronal structure and function in ageing animals. Importantly, the ease of genetic manipulation in the worm will fuel the identification of the genetic and molecular basis of neuronal ageing. Given the extensive evolutionary conservation of the development and function of neurons between *C. elegans* and mammals, the worm offers the possibility to efficiently figure out fundamental principles by which the nervous system ages. Recent studies in *C. elegans* have started to decipher the neuronal changes that accompany ageing and the factors that influence them, as we review below and summarize key findings in Fig. 8.1 and Table 8.1

8.2 Age-Related Structural and Cellular Changes in the Nervous System

Similar to the healthy ageing human brain, the nervous system of *C. elegans* shows no neurodegeneration or gross deterioration during normal ageing [5–9]. Furthermore, the overall architecture of the nervous system is preserved throughout life ([5, 6, 8, 9], Bénard C, unpub.). However, like in humans, more subtle morphological neuronal changes do occur in ageing *C. elegans*. Hermaphrodites have been used in the studies reviewed here (except in the case of male mating behaviour in Sect. 8.4). Neuronal soma and axon diameter shrink with age [5], and some neurons exhibit specific morphological changes, such as new branches along neuronal processes, axon swelling, axon waviness, defasciculation, new neurite-like extensions from the soma, and soma distortion ([6–9] and Bénard C, unpub.). These changes in neuronal morphology arise early during adulthood, progressively worsening in mid- (days 4–7) and old-aged animals ([6–9] and Bénard C, unpub.). The type of

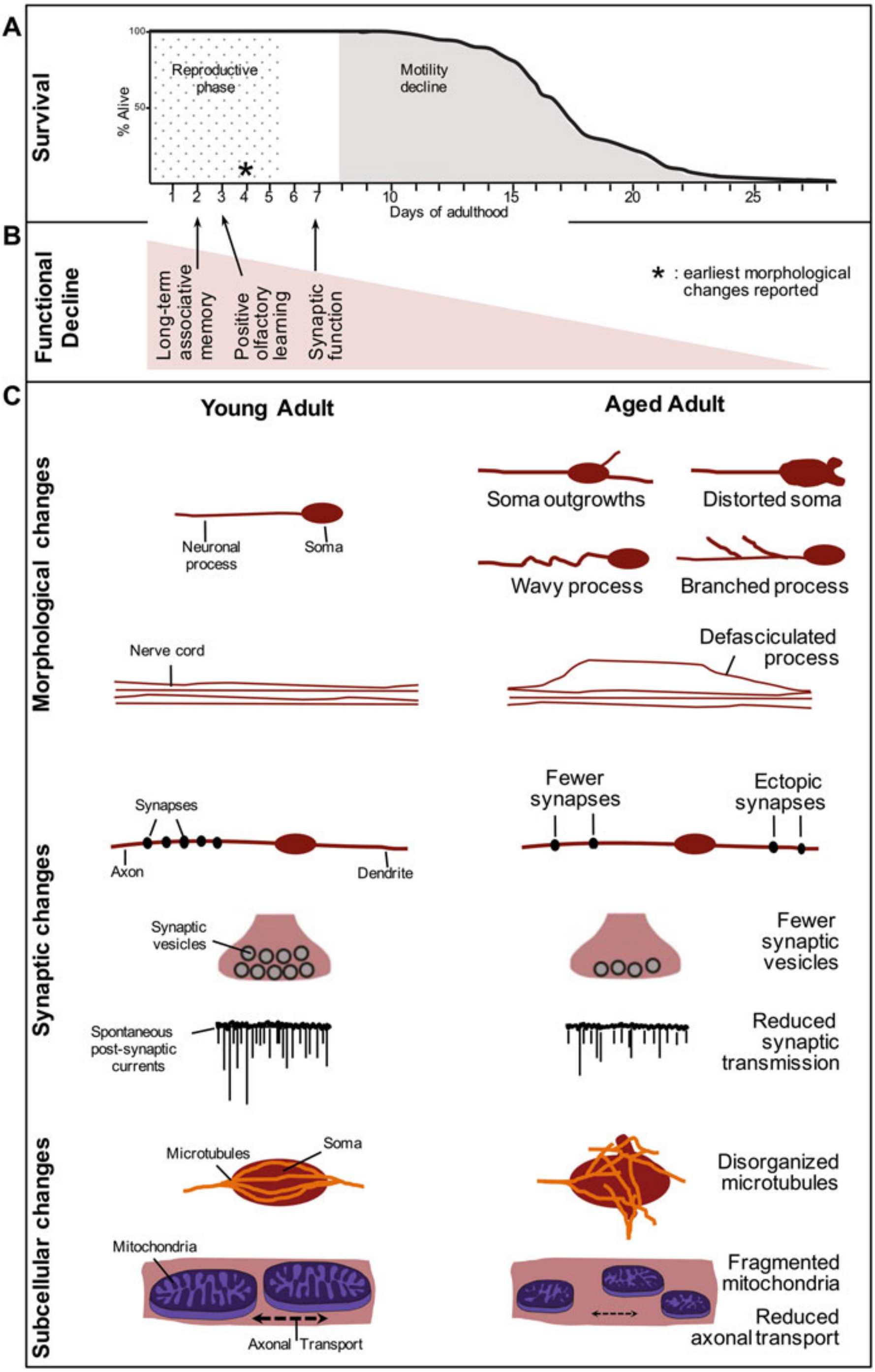

Fig. 8.1 Age-related changes in the *C. elegans* nervous system. (**a**) Schematic depiction of a survival curve for wild-type *C. elegans* self-reproducing hermaphrodites at 20 °C. The peak of self-progeny reproduction is during days 2–5 of adulthood. Motility declines from around day 8 onwards. (**b**) Timeline of selected manifestations in nervous system functional decline. Arrows indicate the age at which decline is first observed early in adulthood. Synaptic transmission is measurably reduced as early as day 7 of adulthood (in motor neurons). (**c**) Diagrams represent examples of age-related neuronal changes at the morphological, synaptic, and subcellular levels. See text for details on specific types of neurons affected, age of onset and rate of change

Table 8.1 Effect of longevity mutants on age-related changes in the nervous system

Genotype / Phenotype	Wild type	Insulin signalling			Dietary restriction	Mitochondria
		daf-2	*daf-16*	*hsf-1*	*eat-2*	*clk-1*
Neuronal morphology (Touch neurons)Abnormal branching, shape, wavy axons defasciculation	Appearance of neuron-specific changes with age [6-8]	In some cases decreased appearance of morphological changes with age [6-8] In other cases increased [7,9]	Increased appearance of morphological changes [7] Suppresses *daf-2* effects [8] In other cases, decreased [7,9]	Increased changes [6-8]	In some cases similar to WT [8] In other cases decreased [9]	Decreased morphological changes [8]
Synapses Number of synaptic vesicles and puncta	Vesicle and puncta decline (d15, 18) [6, 11]	Synaptic puncta maintained (d18, d30) [11]				
Amplitude of post-synaptic currents PSCs	PSC decline (starting d7) [13]	PSC maintenance (up to d27) [13]				
Mitochondria (in ALM) Mitochondrial load	Increases (up to d4) Maintained (d4-8), Decreases (after d8) [36]	Lower load than WT, but steady (d1 to 25) [36]	Similar to WT [36]		Lower than WT (d4-8), and steady (d4 to d11) [36]	
Mitochondrial transport	Decreases after d1 [36]	Steady (d1 to 25) [36]	Similar to WT [36]		Steady (d1 to 11) [36]	
Resistance to oxidative stress	Increases (up to d4) Decreases after d4 [36]	Higher resistance, lower rate of decline until d22 [36]				
Axon regeneration GABA motor neurons	Declines from d1 Abolished by d5 [5]	Delayed decline (no decline on d5, decline by d10) [60]	Suppresses increased regeneration of *daf-2* [60]		Similar to WT [60]	
Learning and Memory Thermotaxis learning	Declines (d6) Absent (d11) [97]	Enhanced learning in young Delayed decline in old [97]	Suppresses *daf-2* delayed decline [96, 97]		Increased learning in young. Delayed decline with age [97]	Enhanced learning in young. Delayed decline in old [97, 97]
LTAM (positive olfactory)	Declined (d2) Abolished by d5 [106]	Longer in young animals (40 vs 24 hr in the WT) [11] Not extended in aged animals [104]	Defective in LTAM [104]		Impaired in young adults Improved in older animals [104]	
STAM (positive olfactory)	Massed learning: decline begins d3, abolished by d6. Spaced learning lasts until d7 [104]	3x longer STAM in young adults Maintained in older worms (no loss in d5) [104]	Defective in STAM, suppresses *daf-2* STAM extension [11, 104]		Similar to WT in young adults [104] Improved in older animals (after spaced learning) [104]	
Neurodegeneration, proteotoxicity	Increased aggregation/proteotoxicit	Reduced aggregation and proteotoxicity [142, 146–150]	Suppresses *daf-2* protective effect [142, 146–150]	Required for *daf-2* and dietary	Protects from proteotoxicity [152]	

WT wild type, *d* day of adulthood, *PSC* post-synaptic currents, *LTAM* long-term associative memory, *STAM* short-term associative memory, *IT* isothermal tracking, *Aβ* Amyloid beta. *Green boxes* indicate that neuronal phenotype is improved (=more youthful, delay of aging phenotype) relative to the wild type. *Red boxes* indicate that neuronal phenotype is deteriorated (=less youthful, stronger aging phenotype) relative to the wild type. *Yellow boxes* in the lifespan mutants indicate no change relative to the wild type. Days indicate observation points reported in the cited papers morphological change, age of onset, and frequency are highly neuron-type specific. Furthermore, the incidence and severity of these morphological changes vary among individual worms in isogenic populations that have been age-synchronized and co-cultured, suggesting that stochastic factors may influence these age-related neuronal changes.

Structural changes have been most extensively characterized in “gentle touch” mechanosensory neurons (ALM, PLM, AVM, PVM), each of which displays specific types of morphological changes. For instance, ectopic outgrowths appear from the soma of ALM by day 4 of adulthood, and new branches along the axon of PLM are frequent by day 8. Ectopic neurites sprouting from neuronal processes extend and retract dynamically [6, 7, 10]. Microtubule networks are disorganized in mechanosensory neurons with misshapen soma (ALM [6]), and mitochondria are often located at the sites of ectopic neurites and swellings along the process [7]. The functional implications of these changes are unknown.

Other neurons also display age-related morphological changes, including branching from the soma of the dopaminergic neuron PDE from early adulthood onwards [7], defasciculation of cholinergic axons in the ventral nerve cord starting at day 6 of adulthood [6], axon beading of GABAergic neurons [6], and ectopic branches from GABAergic axons by day 5 [8]. Characterization of ageing in additional neuron types (e.g. other dopaminergic neurons, chemosensory neurons, interneurons, and motor neurons) extends the observation that age-related morphological changes are neuron-type specific and widespread across the nervous system, but not ubiquitous ([9], Bénard C, unpub). It will be important to study a variety of neuronal types in mechanistic detail to forge a deeper understanding of the neuronal responses to age and elucidate the factors underlying the differential susceptibility of neurons to ageing. Such analyses will provide insights into the basis of the selective neuronal vulnerability in neurodegenerative conditions in humans.

8.2.1 Synaptic Deterioration in Ageing Neurons

As observed at the ultrastructural level, evidence of synaptic deterioration at day 15 of adulthood includes a decline in synaptic vesicle numbers and a reduction in the size of presynaptic densities in the nerve cord and the nerve ring, which are sites of major synaptic contacts [7]. Synaptic vesicle density, observed using the fluorescently labelled synaptic vesicle protein RAB-3 GTPase, is also reduced in the presynaptic region of the motor neuron DA9 at day 18. Moreover, synaptic vesicle proteins (e.g. SNB-1/synaptobrevin and RAB-3 GTPase) ectopically accumulate in the dendritic and asynaptic axonal regions in ageing animals at day 12 and older (DA9 motor neurons, [11]). In addition, early endosomal membrane compartments (e.g. followed by RAB-5 GTPase), which are required for the formation and recycling of synaptic vesicles, are disorganized in ageing GABAergic motor neurons at day 10 [12]. Importantly, presynaptic release declines in motor neurons as early as day 7, and progressively worsens thereafter [13] (see also Sect. 8.4). Age-related deterioration of synaptic organization, including an altered number of dendritic spines, has also been observed in mammals [14–16].

Axonal transport is key for synaptic maintenance during ageing. At the molecular level, genetic screening revealed two molecules that affect synaptic ageing: the anterograde molecular motor UNC-104/KIF1A that transports synaptic vesicles and its regulator, the small GTPase ALR-1. Reduced function of UNC-104 accelerates synaptic deterioration and motor circuit dysfunction with age, whereas upregulation of UNC-104 improves synaptic function [11]. This highlights the importance of axonal transport in the maintenance of synaptic structural integrity throughout life.

8.2.2 Genetic Factors That Influence Morphological Ageing of the Nervous System

Multiple conserved signalling pathways, including insulin signalling (Chap. 4), dietary restriction (Chap. 16), and mitochondrial function (Chap. 5), modulate the worm's lifespan. The insulin and insulin-like growth factor (IGF1) signalling pathway (IIS) is defined by *daf-2*, a homologue of the IGF-1 receptor (IGF1R) [17], which acts through the phosphatidylinositol 3-kinase PI3K kinase cascade. *daf-2* mutations increase lifespan [18] through changes in gene expression via activation of the downstream *daf-16* Forkhead box O (FOXO)-transcription factor, mutations in which shorten lifespan [19, 20]. *eat-2* encodes a subunit of a nicotinic acetylcholine receptor that functions in the pharynx [21]. Loss of function of *eat-2* serves as a genetic model of dietary restriction as it causes the worms to pump more slowly [21] and reduce their food intake, leading to a moderate increase in lifespan [22]. *clk-1* encodes the respiratory chain CoQ biosynthesis enzyme [23], and mutations in *clk-1* reduce respiration and extend lifespan [24].

As neurons undergo morphological changes with age, a fair expectation could be that long-lived mutants would delay neuromorphological ageing, and conversely, short-lived mutants might accelerate neuronal changes. However, the relationship between lifespan pathways and age-related neuronal changes is complex, as only particular types of changes in certain neurons are affected by some but not all of the lifespan-altering mutations ([6–9], Bénard C, unpub.). For instance, studies show that whereas both *clk-1* and *eat-2* mutants have prolonged lifespans, neurite branching of mechanosensory neurons is delayed in *clk-1* mutants, but not in *eat-2* mutants [8]. Also, *daf-2* mutants exhibit a delayed appearance of some of the branching defects [7, 8], but not of other age-related neuronal alterations ([7, 9], Bénard, unpub.). For example, ~8 % of *daf*-2 mutants exhibit novel defects at day 10 (e.g. branching from the ALM and PVM neurons), which is not seen in same-age or older wild-type animals ([7, 9] and Bénard, unpub.). Similarly, in the short-lived mutants *daf-16*, ALM soma outgrowth and PLMs with wavy axons are increased in early adulthood (day 2), but other aspects of neuronal morphology are unaffected and remain wild type ([7, 9] and Bénard, unpub.). Thus, lifespan genes differentially impact distinct types of neuronal changes, in a neuron-specific manner. Consistent with this notion, the organismal healthspan, as measured by locomotion, stress resistance, fat accumulation, muscle frailty, etc, does not always correlate with lifespan [25]. The separation of age-related morphological changes and lifespan is further revealed by tissue-specific manipulations of the *daf-2*/IGF1R pathway [8].

Other pathways that influence age-related morphological changes are the MAP kinase, heat shock stress response, and neuronal attachment pathways. The c-Jun terminal kinase JNK-1 and upstream kinases, JKK-1 and MEK-1, prevent the formation of ectopic neurite branching during ageing in a cell-autonomous manner [8]. In addition, the heat shock transcription factor HSF-1, which is under the control of the IIS pathway, is also required cell autonomously for maintaining neuronal integrity of ALM and PLM neurons. Finally, age-related defects in mechanosensory neurons are increased in the *mec-1* and *mec-5* mutants, in which the normal attachment of the touch neurons to the neighbouring hypodermal cells is disrupted [6].

8.2.3 Maintenance of Adult Nervous System Architecture

A number of genes of the immunoglobulin superfamily function to maintain neuronal architecture in *C. elegans* [26]. Some genes, such as the two-immunoglobulin domain containing proteins ZIG-3 and ZIG-4, act in early larval development to preserve the precise positioning of axons along the nerve cord. Other maintenance factors such as SAX-7, a homologue of L1CAM, and DIG-1, a large secreted protein required for basement membrane maintenance, play roles not only during larval development, but also during adulthood where they maintain ganglia and nerve ring organization [27–32]. For instance, ganglia become disorganized in late larvae and adult *sax-7* mutants, in a way similar to the ganglia disorganization that occurs in normally ageing wild-type adult animals, albeit earlier and more severely ([9], Bénard, unpub.) Furthermore, the two-immunoglobulin domain protein ZIG-10 is required continuously, including during adulthood, to maintain synapse density [33]. Such neuronal maintenance molecules, especially those mediating maintenance of the nervous system in adults, are likely to be neuroprotective during ageing ([9], Bénard, unpub.)

8.2.4 Subcellular Changes in Ageing Neurons

As an organism ages, several features of senescence become apparent at the subcellular level, including alterations of organelle and cytoskeleton integrity, autophagic recycling, mitochondrial function and biogenesis, protein folding and homeostasis, telomere length, and transcriptional regulation, to name a few [2]. One of the challenges that neuronal cells face is to maintain an adequate energy supply in distal neuronal processes, which they achieve by distributing mitochondria along axons and dendrites through specialized transport and anchoring [34]. Thus, processes that disturb the cytoskeletal network or mitochondrial function and transport can potentially affect healthy ageing and lead to neurodegenerative disease [35]. As mentioned above (Sect. 8.2), such cellular events are affected in ageing *C. elegans* as microtubule networks become disorganized in neurons with age [6] and mitochondria localize at the base of age-related ectopic branches along neuronal processes [7].

The effect of ageing on *C. elegans* neuronal mitochondria in the cell body and processes of the mechanosensory neuron ALM was examined by Morsci et al. The frequency and distance of mitochondrial anterograde and retrograde transport progressively declines within the neuronal processes, starting already from the first day of adulthood, indicative of cytoskeletal transport decline [36]. Indeed, microtubules of mechanosensory neurons were shown to disorganize with age [6] and play a role in structural maintenance of neurons in the adult [37]. The size, density and stress resistance of mitochondria also change with age following a phasic pattern: first they increase during early adulthood (days 1–4), then they are maintained at high levels in mid-adulthood (days 4–8), and finally they decline in later adulthood (days 8–15) [36]. The mitochondrial filamentous network becomes more complex and expansive in mid-adulthood whereas at later stages mitochondria exhibit ultrastructural abnormalities, e.g. loss of cristae structures [36]. Mitochondrial fragmentation was also observed in mechanosensory neurons and the ADF neurons [38]. By day 9 of adulthood, 50 % of the ADF neurons exhibit fragmented mitochondria.

Mitochondrial changes are affected by lifespan mutations [36]. Mitochondrial fragmentation is attenuated in long-lived *daf-2*/IGF1R mutants, whereas it progresses more rapidly in short lived *hsf-1* mutants. *daf-2*/IGF1R mutants also have an elevated baseline oxidative stress level and do not exhibit decay in mitochondrial trafficking with age. Long-lived mutants *daf-2*, *eat-2* and overexpression of *sir-2.1* maintain a steady mitochondrial load during mid-adulthood, in contrast to the elevated levels of same age wild-type animals [36]. Since compared to the wild type, long-lived mutants in general maintain a higher level of nervous system function at old age (see Sect. 8.5), it appears that the mitochondrial profile of healthy neuronal ageing correlates with steady, rather than increased, mitochondrial content. How the interplay of mitochondrial biogenesis, degradation or fusion/fission dynamics brings about age-related mitochondrial changes and how these changes impact nervous system function, is under investigation in *C. elegans* and other models [35, 39].

8.2.5 Relevance to Cellular Changes in the Human Nervous System

In humans too, normal brain ageing is characterized by subtle changes in the morphology of specific neurons in selective brain regions [40, 41]. For instance, dendritic branching and length is enhanced in some hippocampal regions in aged individuals compared to young adults, and changes in dendritic spine and synapse number are observed in the ageing neocortex and hippocampus [40, 42, 43]. Despite the simplicity of its nervous system and the short life of *C. elegans*, its neurons -as described above- undergo age-related changes that parallel some neuronal changes in humans. Given the extensive evolutionary conservation of cellular processes between worms and humans, elucidating the mechanisms underlying the neuronal responses to ageing in *C. elegans* is expected to uncover conserved principles of neuronal ageing.

8.3 Axon Regeneration and Ageing

Damaged axons have the ability to repair, which helps the nervous system to remain functional throughout life. In *C. elegans*, axons can be injured by laser axotomy and their regeneration examined with single-cell resolution. Severed axons frequently form a growth cone and regrow [44]. Multiple types of neurons, including mechanosensory neurons (ALM, PLM, AVM) and GABAergic motor neurons can regenerate, and the regenerative capacity differs among neuron types [45–48]. Similar to mammals, regrowth of injured axons in *C. elegans* is often misguided; nonetheless, regenerated axons appear to rewire -at least partly- into proper circuits, as demonstrated in worms that regain mobility after regeneration of their GABA motor neurons [45, 49].

Several molecular pathways that promote or inhibit axon regeneration have been discovered in *C. elegans* through genetic screening [50, 51]. Mechanisms of axon regeneration [52–56] include the PTEN and DLK-1 MAP kinase pathway and other MAP kinase pathways [51, 57–62], Notch signalling [54], microtubule regulators [50, 63, 64], and the IIS pathway [60]. Genetic analysis of axon regeneration has revealed that different neuron types share some regeneration genes, but have striking neuron-type-specific dependencies on other genes for axon regeneration.

8.3.1 Age-Dependent Decline of Regeneration

Age is a strong determinant of a neuron's potential to drive axon repair. Young neurons regenerate damaged axons, but the regenerative ability of neurons quickly declines in early adulthood, worsening further with age [44]. Studies on the effect of age on axon regeneration have identified age-dependent mechanisms that regulate regenerative potential. In the mechanosensory neuron AVM, regeneration declines already during larval development and reaches stable levels that are sustained in adults. The pathway of miRNA *let-7* and its target gene *lin-41* regulates a switch from high capacity for axon regrowth in early larvae when AVM develops, to low capacity for axon regrowth shortly after the developmental outgrowth of AVM is complete [65]. In contrast, the axon regrowth capacity of GABA motor neurons is high throughout larval stages and up to day 1 of adulthood, but steeply declines during adulthood (severely reduced by day 5 and abolished by day 10) [57, 60]. This decline is a result of age-related deterioration in both axon initiation and axon elongation after injury. The insulin receptor DAF-2/IGF1R regulates this decline in GABA axon regeneration by inhibiting the *daf-16*/FOXO transcription factor and its downstream regulation of *dlk-1*/DLK and other genes of the DLK MAP kinase pathway [60]. Thus, *C. elegans* regulates the regenerative capacity of neurons in response to age.

The capacity of axons to regenerate in ageing *C. elegans* does not directly correlate with lifespan, as not all long-lived mutants maintain regenerative capacity at old age. For instance, long-lived *eat-2* mutants and animals overexpressing *sir-2.1* have the same rates of regeneration as the wild type [60]. In contrast, loss of DAF-2/IGF1R function enhances regeneration of aged axons but not of young axons [60]. Neuron-specific expression of DAF-16/FOXO, which does not rescue lifespan, rescues axon regeneration in aged animals. Conversely, intestine-specific expression of DAF-16/FOXO, which rescues lifespan, does not rescue axon regeneration phenotypes in aged *daf-2* mutant animals. Thus, the role of the *daf-2*/*daf-16* pathway on axon regeneration is intrinsic to the nervous system and is uncoupled from its roles in lifespan regulation. The *C. elegans* adult neuronal IIS/FOXO transcriptome revealed the forkhead transcription factor FKH-9 as a IIS/FOXO target [66]. Loss of *fkh-9* impairs axon regeneration in aged *daf-2* mutants, and pan-neuronal expression of FKH-9 in *daf-2*;*fkh-9* mutants restored the regeneration phenotype, confirming its neuronal site of action [66].

8.3.2 Relevance to Axon Regeneration in Ageing Humans

During axon regeneration in *C. elegans* both age and neuron type determine a neuron's regenerative potential, partly because of specific dependencies on molecular pathways mediating axon regeneration. Similarly, age and neuron type strongly influence the regenerative capacity in humans. In adults, axons in the peripheral nervous system regenerate, whereas axons in the central nervous system do not [67]. Intrinsic determinants of regeneration differ across the nervous system as well; for instance, removing PTEN greatly enhances optic and peripheral nerve regeneration, but has a modest effect on spinal cord axons [68–70]. These findings highlight the importance of studying diverse neuronal types in order to gain an understanding of regeneration, a goal that is achievable in the short term in *C. elegans* and that will inform research in mammals. Molecules identified in *C. elegans* to function in axon regeneration (e.g. PTEN and DLK), are conserved in mammals. Elucidating the mechanisms that regulate adult axon regeneration and the effect of age on neuronal regeneration will increase our understanding of how a neuron ages and inform approaches to treat injury and disease in humans.

8.4 Functional Decline of the Ageing Nervous System

C. elegans is capable of versatile behaviours: it performs locomotion, rhythmic contractions for feeding known as pharyngeal pumping, defecation, egg-laying, and mating [71]. It also senses and responds to environmental cues including touch, odorants, temperature, and oxygen levels, and responds through the execution of behaviours such as the escape response, chemotaxis, and thermotaxis, to name a few [72]. As worms age, however, there is widespread behavioural decline [73]. The rate of locomotion slows down and eventually worms stop moving completely; in fact, a worm is considered dead when it fails to move in response to prodding. Measures of spontaneous locomotion (e.g. body bends, speed, turns, net displacement, trashing), as well as locomotion in response to a stimulus (e.g. chemotaxis and response to gentle touch), all decline with age [5, 73–80].

As a first step towards elucidating the causes for this behavioural decline, the ageing of both the muscles involved in the behaviour and the neurons/neural circuits mediating the behaviour needs to be examined. Body wall muscles, which power locomotion, have been found to progressively deteriorate with age in *C. elegans* starting at around day 10, and there is a clear correlation in individual animals between the severity of sarcopenia and the decline in locomotion [5]. This suggests that some of the age-related behavioural decline can be attributed to muscular deterioration. Several studies have tried to tease apart muscle vs. neuronal contributions and although the primary cause has not yet been determined, there is clear evidence supporting a neuronal contribution to behavioural decline.

Clues about age-related decline in neuronal function came from pharmacological manipulations of the neuromuscular junction. Aged animals treated with the muscarinic agonist arecoline, which stimulates acetylcholine release from motor neurons, partially remedied age-related locomotion decline in day 8 and 10 animals. This raised the possibility that ageing affects neurotransmitter signalling at the neuromuscular junction [80]. Further pharmacological studies used the cholinergic agonist levamisole and the cholinesterase inhibitor aldicarb to stimulate body contractions throughout adulthood, including in very old worms that would otherwise be almost immobile (day 16); the findings indicated that presynaptic neuromuscular transmission declines after day 5 of adulthood [81]. Notably, 16-day old worms were capable of producing the same maximal contraction as young worms upon pharmacological stimulation of the neuromuscular junction, pointing to a neuronal synaptic contribution to the age-related locomotion decline [81]. Consistent with the notion that synaptic function deteriorates with age, synaptic terminal size and synaptic vesicle numbers decrease in old animals, and 18-day old animals that preserve higher synaptic integrity have better locomotion ability than same-aged animals with more deteriorated synapses [7].

Recently, electrophysiological studies using patch clamp recordings at the *C. elegans* neuromuscular junction provided direct functional evidence that the decline in presynaptic motor neuron function precedes muscle functional deficits [13]. The frequency of spontaneous neurotransmitter release from presynaptic motor neurons, measured as spontaneous post-synaptic currents, declines as early as day 5 of adulthood and progressively worsens with age, coinciding with locomotory decline. On the other hand, the amplitude of the postsynaptic currents recorded in muscles in response to presynaptic release did not change until day 11. Moreover, consistent with earlier pharmacological studies, the capacity of muscle contraction in response to levamisole did not decline before day 9. These experiments suggest that ageing of the motor nervous system is an earlier underlying reason for locomotory deterioration with age. Future studies will address the impact of the neuronal function decline on muscle deterioration, and the cellular and molecular basis leading to these functional changes in neurons.

C. elegans males perform mating behaviour, which is a series of sensory and motor sub-behaviours to achieve copulation. The spicule intromission step of male copulation quickly deteriorates in adults and wild-type males' mating potency significantly decays by day 3 of adulthood (before age-related muscle deterioration), becoming impotent by day 5 [82]. Calcium imaging, pharmacological tests, and genetic manipulations showed that the decay in male mating results from increased excitability of the male sex muscles [83]. SIR-2.1, an ortholog of yeast SIR2 [84], is required to maintain mating potency, possibly by impacting metabolism at the level of glycolysis, fatty acid oxidation and oxidative stress responses, which in turn affect the excitability of the sex muscles [83].

Sensory perception-based behaviours also decline with age. While decay in locomotion with age certainly impairs these behaviours, age-related defects in the sensation and integration of stimuli also play a major role. Calcium imaging techniques, microfluidics and tools for neural circuit dissection have facilitated the study of neuronal function [85, 86]. Monitoring neuronal activity illuminates fine aspects of age-related changes in neuronal function that would be difficult to discern through behavioural analysis alone. For example, monitoring responses to glycerol in the sensory neuron ASH revealed that calcium responses first increase in day 3–4 of adulthood, before they start decreasing in day 5 adults [87]. In a comprehensive analysis of odour-evoked neural signalling, Leinwand et al. identified a circuit of primary and secondary neurons that collectively encode benzaldehyde-evoked behavioural plasticity [88]. They find that the combinatorial circuit of odour sensing declines with age due to functional decline of the secondary but not the primary neurons, demonstrating that ageing differentially affects sensory neurons in the same circuit [88]. Whole-brain calcium imaging approaches in *C. elegans* [89, 90] will allow for the establishment of temporal hierarchies of functional decline within neural circuits during ageing.

8.5 Learning and Memory in Ageing

Learning and memory are fundamental biological processes that allow living organisms to respond and adapt to their environment. Memory decline in ageing is a well-documented phenomenon in humans [91] and a common feature across species [92].

Despite the simplicity of its nervous system, *C. elegans* exhibits behavioural plasticity and a range of well-characterized paradigms of short- and long-term memory [93]. These include examples of associative and non-associative memory, some of which have been studied in detail. Genetic pathways known to affect lifespan also affect learning and memory in different ways. In some cases, they play a role in the formation of memory itself; in other cases, they influence how fast memory declines during ageing. Here, we focus on some of the well-characterized models of learning and memory in *C. elegans* to review the consequences of ageing on neuronal plasticity and the influence of lifespan-altering mutations on age-related decline.

8.5.1 Thermotaxis Learning and Memory in Ageing

In a process known as thermotaxis, *C. elegans* has the ability to sense temperature and modify its behaviour according to previous experience. It associates a past cultivation temperature with food and moves towards that temperature in search for food. Also, when found on a temperature gradient, it moves isothermally within the cultivation temperature. This behaviour is called isothermal tracking and has characteristics of memory [94], for example it is CREB-dependent [95]. Thermotaxis learning declines with age, as isothermal tracking behaviour becomes reduced by day 6 of adulthood and absent by day 14 among worms that retain mobility [96].

The IIS pathway, which regulates lifespan [18], affects associative learning behaviour. In thermotaxis learning, aged animals of long-lived mutants *age-1*/PI3K and *daf-2*/IGF1R show enhanced isothermal tracking performance in young and old animals compared to the wild type. This enhancement consists of both a stronger association of temperature with food (as assessed by the number of animals performing isothermal tracking), as well as a delay in age-related decline of thermal learning [97]. This delay in age-related decline was not due to locomotion effects or simply a byproduct of increased lifespan: when Murakami et al. took into account the physiological age (instead of the chronological), *age-1* mutants still had higher isothermal tracking behaviour than the wild type [97]. In fact, there is a 210 % extension in the period of high thermotaxis learning behaviour in *age-1* mutants, compared to only 65 % extension of the lifespan [97]. Moreover, expression of AGE-1/PI3K in AIY neurons in *age-1* mutants suppressed the *age-1* learning phenotype but not its longevity effects [97], thus dissociating lifespan from its effects on learning. The positive effect of *daf-2*/IGF1R and *age-1*/PI3K mutations on associative learning is *daf-16*/FOXO dependent. Interestingly, insulin peptide INS-1, the closest ortholog of human insulin, is required for the formation of the food-temperature association in a mechanism that acts antagonistically to the *daf-2*/IGF1R pathway [98].

In addition to the IIS pathway, thermotaxis learning is also affected by other longevity pathways. *eat-2* mutants, which extend lifespan through a dietary restriction mechanism, have enhanced thermotaxis learning in young adults but also delayed isothermal tracking decline with age [97]. Mitochondrial dysfunction also affects lifespan. Despite the complex roles of mitochondrial metabolism and ROS production in ageing [99], specific mutants of the electron transport chain are known to alter lifespan. Both *isp-1* (coding for the iron sulphur protein of mitochondrial respiratory complex III [100]) and *clk-1* (coding for a central enzyme in ubiquinone synthesis) mutants show increased thermotaxis learning behaviour in young adult animals assessed by isothermal tracking [96]. The increased learning in *isp-1* mutants is *daf-16*/FOXO-dependent and it is abolished in *daf-16* mutants, despite the fact that the longevity effect of these mutants does not depend on *daf-16*/FOXO. Furthermore, *clk-1* mutants also delay age-related decline of isothermal tracking behaviour. In contrast, short lived *gas-1* and *mev-1* mutants, defective for respiratory complex I and II, are more sensitive to oxidative stress [101, 102] and show decreased thermotaxis behaviour, a phenotype rescued by treatment with antioxidants [96].

8.5.2 Positive Olfactory Associative Learning and Memory

C. elegans learns to associate volatile chemicals like butanone with food, and chemotaxes towards them [103]. When butanone is paired with food for a single training session (massed learning) it produces a short-term associative memory (STAM). In contrast, when worms are subjected to multiple training sessions (spaced learning) they form a long-term associative memory (LTAM) that lasts between 16 and 24 h [104]. LTAM formation declines with age, starting already at day 2 of adulthood and is abolished by day 5. LTAM deteriorates prior to the decline in olfactory learning, chemotaxis and motility [104], suggesting a higher sensitivity of LTAM in ageing. Massed learning begins to decline already by day 3 and is completely lost by day 6 of adulthood. Spaced learning, which begins to decline on day 3, is lost by day 7.

Lifespan mutants affect LTAM and STAM in *C. elegans* both in young and older age. In young adults, *daf-2*/IGF1R mutants show three times longer STAM, although their learning rate is similar to wild type. Moreover, *daf-2* mutants show significantly longer LTAM, which remains active past 40 h. Lastly, LTAM is established after fewer training sessions in *daf-2*/IGF1R mutants compared to wild-type animals. These memory improvements in young animals are *daf-16* dependent [104].

In older worms, *daf-2*/IGFR mutants retain their ability to learn for a longer time. At day 5 of adulthood, there is no significant loss in the formation of STAM. However, although learning is extended, LTAM is not improved in aged *daf-2*/IGFR mutants compared to the wild-type animals [104].

Dietary restriction affects positive olfactory associative memory differently than the IIS pathway. In young animals, *eat-2* mutants show no improvements in STAM compared to the wild type, whereas LTAM is reduced. In contrast, older *eat-2* animals show improved memory compared to wild type, and both STAM and LTAM persist for a longer period. Importantly, age-dependent memory loss can be alleviated if dietary restriction is imposed in adult worms [104].

8.5.3 Habituation (Non-associative Learning)

C. elegans also exhibits non-associative memory [93]. The best characterized examples are habituation to a mechanical stimulus and chemosensory habituation. These forms of adaptation can have short- or long-term memory timescales depending on the training regime. Age-related changes in habituation have been reported: worms in their sixth and eighth day of adulthood habituate more rapidly to mechanical stimulus and show slower recovery from habituation than younger adults [105]. Timbers et al. tested adaptation to mechanical stimulus in middle-aged worms and showed that changes in habituation started at the peak of their reproductive age, as early as the second day of adulthood [106]. This timeline is similar to the onset of changes in positive olfactory associative learning described above. In contrast to associative learning, the IIS pathway does not impact non-associative learning protocols involving chemosensory habituation [107].

8.5.4 Mechanisms of Age-Related Learning and Memory Decline

Several forms of memory in *C. elegans*, for example olfactory STAM and LTAM, decline before any morphological neuronal changes become apparent [104, 108]. As a cautionary note, an analysis of age-related morphological changes of neurons that mediate learning and memory is still lacking. Thus, it seems that changes at the molecular level, which precede obvious morphological defects, are responsible for memory decline [104, 108]. Indeed, LTAM deterioration with age in both the wild type and longevity mutants correlates tightly with *crh-1*/CREB expression levels [104]. This correlation appears to be conserved in mammals: the levels of CREB in the brain are predictive of spatial memory decline in aged rats [109] and overexpression of CREB in the hippocampus attenuates spatial memory impairment during ageing [110].

Parallels can be drawn between memory and synapse decline during ageing in the wild type and lifespan mutants. The complex synaptic machinery required for memory formation is well documented [111]. *C. elegans* research has revealed a correlation between synapse deterioration and STAM decline with age [11]. At the molecular level, the anterograde kinesin motor UNC-104/KIF1A, which transports synaptic vesicles along axons, is required for STAM maintenance in ageing. UNC-104/KIF1A levels are reduced with age in the wild type but maintained in *daf-2* mutants in a *daf-16*/FOXO-dependent manner [11].

The examples above demonstrate that a reduction in the IIS pathway promotes positive associative learning and memory in ageing *C. elegans*, through activation of the DAF-16/FOXO transcription factor. Neuron-specific transcriptome analysis of DAF-16/FOXO targets in *daf-2*/IGF1R mutants revealed a landscape of DAF-16/FOXO-dependent regulators of short-term memory extension, distinct from previously identified targets in other tissues. This analysis showed that some of the DAF-16/FOXO neuronal targets that extend memory in *daf-2*/IGF1R mutants also regulate memory in the wild type [66]. Thus, IIS pathway-dependent memory extension is due to augmentation or maintenance of the molecular machinery that regulates memory in the wild type, rather than the activation of an alternative mechanism. Similarly in mouse, FOXO6 is highly expressed in adult hippocampus and is required for memory consolidation by regulating the expression of genes responsible for synaptic function [112].

Among the DAF-16/FOXO targets that are upregulated in *daf-2*/IGF1R mutants at the whole worm level is FKH-9. It was shown that FKH-9 is required in the neurons for memory enhancement in *daf-2* mutants and in the somatic cells for lifespan extension [112]. Molecular characterization of the tissue-specific transcriptional programmes that regulate longevity or neuronal function, combined with an analysis of the conservation of these programmes across phylogeny, will facilitate a more complete understanding of nervous system ageing.

8.5.5 Relevance to Learning and Memory in Humans

As described above, learning and memory decline in *C. elegans* becomes apparent in ageing animals in early adulthood. Recent studies indicate that cognitive decline in humans might start as early as the fourth decade of life [113]. *C. elegans* findings demonstrated the positive role of IIS pathway reduction and dietary restriction in delaying the decline of learning and memory. In humans, IIS plays physiological roles in various regions of the central nervous system, regulating neuronal function, including learning and memory. However, impaired insulin signalling and resistance observed in age-related diseases complicates the role of IIS in the human nervous system [114]. Clarifying discrepancies will delineate its exact mechanisms of action in the ageing human brain.

Dietary restriction in humans has beneficial effects on brain structure and function [115]. Brain regions most vulnerable to the ageing process, namely frontal and medial temporal lobes, are negatively affected in obese individuals [116]. Excessive energy intake and elevated levels of blood glucose and fatty acids negatively impacts cognition [117]. In contrast, caloric restriction in elderly adults was shown to improve memory [118]. Recent evidence suggests that memory enhancement is a result of a negative energy balance (weight loss phase) rather than an effect of low weight maintenance [115]. Consequently, intermittent fasting or other interventions that mimic the effects of dietary restriction [119, 120] could prove more beneficial to healthy cognitive ageing than constant dietary restriction. *C. elegans* research has indeed demonstrated that intermittent fasting extends lifespan through the action of a small GTPase, RHEB-1, via the IIS pathway. Inhibition of RHEB-1 successfully mimicked the effects of caloric restriction. Thus, this and similar studies in *C. elegans* [121] can open the way to develop mimetics of dietary restriction to improve lifespan, health and cognitive function without the potential negative effects of caloric restriction.

8.6 Neurodegenerative Diseases

Age is the leading risk factor for neurodegenerative disease [41, 122, 123]. This suggests that cellular changes occurring during ageing increase the vulnerability of neurons to such conditions. Ageing cells show increased levels of oxidative, metabolic and ionic stress that result in the accumulation of dysfunctional organelles, damaged proteins and DNA. Failure of neurons to adapt to such stresses leads to neuronal dysfunction and susceptibility to neuronal degeneration. Understanding the molecular mechanisms underlying age-related neurodegenerative diseases and identifying neuroprotective strategies is a major focus of modern medical research.

8.6.1 C. elegans Models of Neurodegenerative Disease

Besides the general advantages of *C. elegans* as a model organism (discussed in Sect. 8.1 and Chap. 1 of this volume) additional characteristics make it particularly suitable for studying human neurodegenerative disease: *C. elegans* tolerates nervous system defects very well, as most of its neurons are dispensable for survival and reproduction in laboratory conditions [124, 125]. Furthermore, the majority of human genes implicated in monogenic forms of neurodegenerative disease have conserved *C. elegans* orthologs. When an ortholog does not exist, then expression of the human gene has often been used to reproduce disease phenotypes. Lastly, the power of genetic screens in *C. elegans* renders it ideal for rapid, genome-wide discovery of disease modifiers. Therefore, *C. elegans* has been extensively used to model and study neurodegenerative diseases [126–128].

Here we briefly summarize some examples of human neurodegenerative diseases studied in *C. elegans*, several of which are characterized by protein misfolding and aggregation (see also Chap. 12). Parkinson's disease (PD) is an age-related movement disorder, accompanied by loss of dopamine neurons. *C. elegans* models of PD include overexpression of human α-synuclein in muscles or neurons, mutations in orthologs of human Parkinsonism (PARK) genes, and environmental toxins (such as paraquat, rotenone, MPTP, MPP+ and 6-OHDA) [129, 130]. Alzheimer's disease (AD) results in a progressive loss of cognitive function and is characterized by extracellular deposits of amyloid β (Aβ) and intracellular aggregates of microtubule associated protein *tau* (*MAPT*). *C. elegans* models of AD include overexpression of human Aβ peptides or tau [131–133]. Huntington's disease (HD) is a fatal neurodegenerative disorder caused by polyglutamine (polyQ) repeat expansion in huntingtin (*HTT*), and the corresponding *C. elegans* models express fragments of the human protein with various lengths of polyQ repeats [134]. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting motor neurons, which is caused by diverse genetic mutations. Most models of ALS in *C. elegans* relay on overexpression of wild-type and mutated forms of the causative genes *superoxide dismutase 1* (*SOD1*), *TAR DNA binding protein 43* (*TDP-43*) and *Fused-in-sarcoma* (*FUS*)

[135]. Finally, a number of *C. elegans* models exist for studying neuronal channelopathies and excitotoxic cell death [136–138].

Modifier screens on the above models have contributed important insights into the understanding of neurodegenerative disease and revealed numerous general and disease-specific modifiers conserved in other organisms. Studies on *C. elegans* models of PD led to the discovery of several neuroprotective mechanisms; for instance, overexpression of the chaperone *TOR2/TorsinA*, the lysosomal P-ATPase *catp-6/ATP13A2*, or human Cathepsin D were shown to ameliorate aspects of α-synuclein toxicity [139–141] and the glycolytic enzyme GPI-1/GPI was identified as a conserved modifier of dopaminergic degeneration [142]. Genetic screens in *C. elegans* models of AD led to the discovery of many disease modifiers, e.g. orthologs of human kinases such as *kin-18/TAOK1* and *sgg-1/GSK3β*, and chaperone stress response molecules (such as *xbp-1/XBP1*, *hsp-2/HSPA2*, *hsf-1/HSF1* and *chn-1/CHN1*) were identified as key regulators of *tau* toxicity [143]. A long list of similar discoveries has confirmed the validity of *C. elegans* as a model to study neurodegenerative disease. Key discoveries stem not only from research on models of disease but also from studies that enhanced our understanding of the normal function of disease-associated genes in healthy situations. A comprehensive review of these findings is beyond the scope of this chapter.

8.6.2 Ageing Pathways and Neurodegenerative Disease

Although familial cases of neurodegenerative diseases in humans start earlier in life, the vast majority of cases for numerous neurodegenerative diseases are sporadic and manifest during the seventh decade or later, making ageing the major risk factor [144]. Is it then possible to prevent or delay such conditions by delaying ageing? Several studies in *C. elegans* show that manipulating longevity pathways affects pathological manifestations in models of neurodegenerative disease [145].

In *C. elegans* models of neurodegenerative disease, the IIS pathway modulates protein aggregation and toxicity. Reducing insulin signalling in *C. elegans* models of HD and AD alleviated polyglutamine [146, 147] and Aβ toxicity [148, 149], respectively. Similar protective effects of IIS reduction were reported in an ALS model of SOD-1 aggregation [150] and in an α-synuclein overexpression model [142]. These results of reduced proteotoxicity across disease models are consistent with findings that overall protein insolubility and aggregation (an inherent part of normal ageing in *C. elegans* and other animals) is also alleviated by reduction in IIS signalling [151]. The mechanism of the *daf-2* mediated protection depends on the action of DAF-16/FOXO and HSF-1. These transcription factors have in fact opposing protective effects in AD models, with HSF-1 promoting disaggregation of Aβ, and DAF-16 promoting the active aggregation of toxic oligomers to less toxic forms [148]. Longevity manipulations through dietary restriction also suppress both polyglutamine and Aβ toxicity in *C. elegans* [152] and protect dopaminergic neurons from degeneration in a 6-OHDA model [153]. Finally, consistent with major longevity pathways modulating neurodegenerative disease, germ-cell ablation also attenuated polyglutamine toxicity in a DAF-16/FOXO and HSF-1 dependent manner [154].

The protective link between reduced IIS signalling and neuronal proteotoxicity was shown to be conserved in mice [155, 156], validating the relevance of the *C. elegans* findings. It also extends to neurological conditions that are not based on proteotoxic aggregation, for example hypoxia-induced ischemic stroke [157]. Similarly, the protective role of dietary restriction is conserved in mouse models of β-amyloid neuropathy [158] and in non-human primate models of PD [159]. Importantly, the effects exerted by ageing manipulations can be, at least in some cases, uncoupled from the extension of lifespan. This was demonstrated in mice by reducing IIS later in life when it can no longer extend the lifespan, a manipulation that nevertheless protected from Aβ toxicity [160]. The interplay of longevity pathways and disease has obvious implications in medical and lifestyle interventions, which could potentially delay or ameliorate devastating age-related disorders.

8.7 Concluding Remarks

Studies in *C. elegans* have contributed to our understanding of the molecular machineries that protect the nervous system structure and function during normal ageing, following injury, and in disease states. One striking property of the ageing nervous system in *C. elegans*, which is also common in humans, is the differential susceptibility of neurons to age. Moreover, whereas the decline of overall nervous system function is delayed in lifespan-extending mutations, specific aspects of neuronal deterioration are not, and specific neuron types are impacted differently by these lifespan mutations. The precise descriptions of age-related nervous system changes reviewed here constitute the basis for future mechanistic studies of neuronal ageing. Longitudinal analysis and the development of more tools to measure diverse aspects of neuronal ageing simultaneously will help establish relationships between age-related changes and identify the genetic and environmental factors underlying ageing of the nervous system. Ultimately, what we learn about the mechanisms influencing neuronal ageing will facilitate the development of therapeutic interventions to help improve the human condition in old age.

Acknowledgements We thank Arantza Barrios, Emanuel K. Busch and Cassandra Blanchette for feedback on the manuscript. Research in the lab of Dr. Maria Doitsidou is supported by the Norwegian Research Council and the Wellcome Trust, UK. Research in the lab of Dr. Claire Bénard is supported by grant R01 AG041870-01 from the National Institutes of Health of the USA to C.B., the Ellison Medical Foundation New Scholar Aging Award to C.B., and the American Federation for Aging Research Award to C.B..

Dedicated to the memory of Muhammad Ali (January 17, 1942–June 3, 2016).

References

1. López-Otín C, Blasco MA, Partridge L et al (2013) The hallmarks of aging. Cell 153:1194–1217. doi:10.1016/j.cell.2013.05.039
2. DiLoreto R, Murphy CT (2015) The cell biology of aging. Mol Biol Cell 26:4524–4531. doi:10.1091/mbc.E14-06-1084
3. Kenyon CJ (2010) The genetics of ageing. Nature 464:504–512. doi:10.1038/nature08980
4. Hekimi S, Guarente L (2003) Genetics and the specificity of the aging process. Science 299:1351–1354. doi:10.1126/science.1082358
5. Herndon LA, Schmeissner PJ, Dudaronek JM et al (2002) Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. Nature 419:808–814. doi:10.1038/nature01135
6. Pan C-L, Peng C-Y, Chen C-H, McIntire S (2011) Genetic analysis of age-dependent defects of the *C. elegans* touch receptor neurons. Proc Natl Acad Sci U S A 108:9274–9279. doi:10.1073/pnas.1011711108
7. Toth ML, Melentijevic I, Shah L et al (2012) Neurite sprouting and synapse deterioration in the aging *C. elegans* nervous system. J Neurosci 32:8778–8790. doi:10.1523/JNEUROSCI.1494-11.2012
8. Tank EMH, Rodgers KE, Kenyon C (2011) Spontaneous age-related neurite branching in *C. elegans*. J Neurosci 31:9279–9288. doi:10.1523/JNEUROSCI.6606-10.2011
9. Khandekar A (2015) Age-related changes in the neuronal architecture of *C. elegans*. Doctoral thesis, Bénard Laboratory, University of Massachusetts Medical School, 2015
10. Peng C-Y, Chen C-H, Hsu J-M, Pan C-L (2011) *C. elegans* model of neuronal aging. Commun Integr Biol 4:696–698
11. Li L-B, Lei H, Arey RN et al (2016) The neuronal kinesin UNC-104/KIF1A is a key regulator of synaptic aging and insulin signaling-regulated memory. Curr Biol 26:605–615. doi:10.1016/j.cub.2015.12.068
12. Sann SB, Crane MM, Lu H, Jin Y (2012) Rabx-5 regulates RAB-5 early endosomal compartments and synaptic vesicles in *C. elegans*. PLoS One 7, e37930. doi:10.1371/journal.pone.0037930
13. Liu J, Zhang B, Lei H et al (2013) Functional aging in the nervous system contributes to age-dependent motor activity decline in *C. elegans*. Cell Metab 18:392–402. doi:10.1016/j.cmet.2013.08.007
14. Valdez G, Tapia JC, Kang H et al (2010) Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. Proc Natl Acad Sci U S A 107:14863–14868. doi:10.1073/pnas.1002220107
15. Chen S, Hillman DE (1999) Dying-back of Purkinje cell dendrites with synapse loss in aging rats. J Neurocytol 28:187–196. doi:10.1023/A:1007015721754
16. Rogers J, Zornetzer SF, Bloom FE, Mervis RE (1984) Senescent microstructural changes in rat cerebellum. Brain Res 292:23–32. doi:10.1016/0006-8993(84)90886-2
17. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G (1997) daf-2, an insulin receptor-like gene that regulates longevity and diapause in *C. elegans*. Science 277:942–946. doi:10.1126/science.277.5328.942
18. Kenyon C, Chang J, Gensch E et al (1993) A *C. elegans* mutant that lives twice as long as wild type. Nature 366:461
19. Lin K, Dorman JB, Rodan A, Kenyon C (1997) daf-16: an HNF-3/forkhead family member that can function to double the life-span of *C. elegans*. Science 278:1319–1322. doi:10.1126/science.278.5341.1319
20. Ogg S, Paradis S, Gottlieb S et al (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature 389:994–999. doi:10.1038/40194
21. McKay JP (2004) eat-2 and eat-18 are required for nicotinic neurotransmission in the *C. elegans* pharynx. Genetics 166:161–169. doi:10.1534/genetics.166.1.161

22. Lakowski B, Hekimi S (1998) The genetics of caloric restriction in *C. elegans*. Proc Nat Acad Sci U S A 95:13091
23. Ewbank JJ, Barnes TM, Lakowski B et al (1997) Structural and functional conservation of the *C. elegans* timing gene clk-1. Science 275:980–983. doi:10.1126/science.275.5302.980
24. Lakowski B, Hekimi S (1996) Determination of life-span in *C. elegans* by four clock genes. Science 272:1010–1013. doi:10.1126/science.272.5264.1010
25. Bansal A, Zhu LJ, Yen K, Tissenbaum HA (2015) Uncoupling lifespan and healthspan in *C. elegans* longevity mutants. Proc Natl Acad Sci U S A 112:E277–E286. doi:10.1073/pnas.1412192112
26. Bénard C, Hobert O (2009) Chapter 6 looking beyond development: maintaining nervous system architecture. In: Development of Neural Circuitry. Elsevier, Amsterdam, pp 175–194
27. Bénard CY, Boyanov A, Hall DH, Hobert O (2006) DIG-1, a novel giant protein, non-autonomously mediates maintenance of nervous system architecture. Development 133:3329–3340. doi:10.1242/dev.02507
28. Burket CT, Higgins CE, Hull LC et al (2006) The *C. elegans* gene dig-1 encodes a giant member of the immunoglobulin superfamily that promotes fasciculation of neuronal processes. Dev Biol 299:193–205
29. Johnson RP, Kramer JM (2012) Neural maintenance roles for the matrix receptor dystroglycan and the nuclear anchorage complex in *C. elegans*. Genetics 190:1365–1377. doi:10.1534/genetics.111.136184
30. Sasakura H, Inada H, Kuhara A et al (2005) Maintenance of neuronal positions in organized ganglia by SAX-7, a *C. elegans* homologue of L1. EMBO J 24:1477–1488. doi:10.1038/sj.emboj.7600621
31. Pocock R, Bénard CY, Shapiro L, Hobert O (2008) Functional dissection of the *C. elegans* cell adhesion molecule SAX-7, a homologue of human L1. Mol Cell Neurosci 37:56–68
32. Wang X, Kweon J, Larson S, Chen L (2005) A role for the *C. elegans* L1CAM homologue lad-1/sax-7 in maintaining tissue attachment. Dev Biol 284:273–291. doi:10.1016/j.ydbio.2005.05.020
33. Cherra SJ III, Jin Y (2016) A two-immunoglobulin-domain transmembrane protein mediates an epidermal-neuronal interaction to maintain synapse density. Neuron 89:325–336
34. Sheng Z-H (2014) Mitochondrial trafficking and anchoring in neurons: new insight and implications. J Cell Biol 204:1087–1098. doi:10.1083/jcb.201312123
35. Lionaki E, Markaki M, Palikaras K, Tavernarakis N (2015) Mitochondria, autophagy and age-associated neurodegenerative diseases: new insights into a complex interplay. Biochim Biophys Acta 1847:1412–1423. doi:10.1016/j.bbabio.2015.04.010
36. Morsci NS, Hall DH, Driscoll M, Sheng Z-H (2016) Age-related phasic patterns of mitochondrial maintenance in adult *C. elegans* neurons. J Neurosci 36:1373–1385. doi:10.1523/JNEUROSCI.2799-15.2016
37. Chew YL, Fan X, Götz J, Nicholas HR (2013) PTL-1 regulates neuronal integrity and lifespan in *C. elegans*. J Cell Sci 126:2079–2091. doi:10.1242/jcs.jcs124404
38. Jiang H-C, Hsu J-M, Yen C-P et al (2015) Neural activity and CaMKII protect mitochondria from fragmentation in aging *C. elegans* neurons. Proc Natl Acad Sci U S A 112:8768–8773. doi:10.1073/pnas.1501831112
39. Palikaras K, Lionaki E, Tavernarakis N (2015) Coordination of mitophagy and mitochondrial biogenesis during ageing in *C. elegans*. Nature 521:525–528. doi:10.1038/nature14300
40. Burke SN, Barnes CA (2006) Neural plasticity in the ageing brain. Nat Rev Neurosci 7:30–40. doi:10.1038/nrn1809
41. Yankner BA, Lu T, Loerch P (2008) The aging brain. Annu Rev Pathol 3:41–66. doi:10.1146/annurev.pathmechdis.2.010506.092044
42. Nimchinsky EA, Bernardo L, Sabatini A, Svoboda K (2003) Structure and function of dendritic spines. http://dx.doi.org/10.1146/annurev.physiol.64.081501.160008. 64:313–353. doi:10.1146/annurev.physiol.64.081501.160008

43. Hof PR, Morrison JH (2004) The aging brain: morphomolecular senescence of cortical circuits. Trends Neurosci 27:607–613
44. Hammarlund M, Jin Y (2014) Axon regeneration in *C. elegans*. Curr Opin Neurobiol 27:199–207
45. Yanik MF, Cinar H, Cinar HN et al (2004) Neurosurgery: functional regeneration after laser axotomy. Nature 432:822
46. Yanik MF, Cinar H, Cinar HN, Gibby A (2006) Nerve regeneration in *C. elegans* after femtosecond laser axotomy. IEEE J 12:1283
47. Wu Z, Ghosh-Roy A, Yanik MF et al (2007) *C. elegans* neuronal regeneration is influenced by life stage, ephrin signaling, and synaptic branching. Proc Natl Acad Sci U S A 104:15132–15137. doi:10.1073/pnas.0707001104
48. Gabel CV, Antoine F, Chuang C-F et al (2008) Distinct cellular and molecular mechanisms mediate initial axon development and adult-stage axon regeneration in *C. elegans*. Development 135:1129–1136. doi:10.1242/dev.013995
49. El Bejjani R, Hammarlund M (2012) Notch signaling inhibits axon regeneration. Neuron 73:268–278. doi:10.1016/j.neuron.2011.11.017
50. Chen L, Wang Z, Ghosh-Roy A et al (2011) Axon regeneration pathways identified by systematic genetic screening in *C. elegans*. Neuron 71:1043–1057
51. Nix P, Hammarlund M, Hauth L et al (2014) Axon regeneration genes identified by RNAi screening in *C. elegans*. J Neurosci 34:629–645. doi:10.1523/JNEUROSCI.3859-13.2014
52. Hilliard MA (2009) Axonal degeneration and regeneration: a mechanistic tug-of-war. J Neurochem 108:23–32. doi:10.1111/j.1471-4159.2008.05754.x
53. Wang Z, Jin Y (2011) Genetic dissection of axon regeneration. Curr Opin Neurobiol 21:189–196
54. Rachid El Bejjani MH (2012) Neural regeneration in *C. elegans*. Annu Rev Genet 46:499–513. doi:10.1146/annurev-genet-110711-155550
55. Chen L, Chisholm AD (2011) Axon regeneration mechanisms: insights from *C. elegans*. Trends Cell Biol 21:577–584. doi:10.1016/j.tcb.2011.08.003
56. Chisholm AD (2013) Cytoskeletal Dynamics in *C. elegans* Axon Regeneration. http://dx.doi.org/10.1146/annurev-cellbio-101512-122311 29:271–297. doi: 10.1146/annurev-cellbio-101512-122311
57. Hammarlund M, Nix P, Hauth L et al (2009) Axon regeneration requires a conserved MAP kinase pathway. Science 323:802–806. doi:10.1126/science.1165527
58. Nix P, Hisamoto N, Matsumoto K, Bastiani M (2011) Axon regeneration requires coordinate activation of p38 and JNK MAPK pathways. Proc Natl Acad Sci U S A 108:10738–10743. doi:10.1073/pnas.1104830108
59. Yan D, Wu Z, Chisholm AD, Jin Y (2009) The DLK-1 kinase promotes mRNA stability and local translation in *C. elegans* synapses and axon regeneration. Cell 138:1005–1018. doi:10.1016/j.cell.2009.06.023
60. Byrne AB, Walradt T, Gardner KE et al (2014) Insulin/IGF1 signaling inhibits age-dependent axon regeneration. Neuron 81:561–573. doi:10.1016/j.neuron.2013.11.019
61. Li C, Hisamoto N, Nix P et al (2012) The growth factor SVH-1 regulates axon regeneration in *C. elegans* via the JNK MAPK cascade. Nat Neurosci 15:551–557. doi:10.1038/nn.3052
62. Yan D, Jin Y (2012) Regulation of DLK-1 kinase activity by calcium-mediated dissociation from an inhibitory isoform. Neuron 76:534–548
63. Ghosh-Roy A, Goncharov A, Jin Y, Chisholm AD (2012) Kinesin-13 and tubulin posttranslational modifications regulate microtubule growth in axon regeneration. Dev Cell 23:716–728
64. Kirszenblat L, Neumann B, Coakley S (2013) A dominant mutation in mec-7/β-tubulin affects axon development and regeneration in *C. elegans* neurons. Mol Biol Cell 24:285
65. Zou Y, Chiu H, Zinovyeva A et al (2013) Developmental decline in neuronal regeneration by the progressive change of two intrinsic timers. Science 340:372–376. doi:10.1126/science.1231321
66. Kaletsky R, Lakhina V, Arey R et al (2016) The *C. elegans* adult neuronal IIS/FOXO transcriptome reveals adult phenotype regulators. Nature 529:92–96. doi:10.1038/nature16483

67. Chiu H, Alqadah A, Chuang C-F, Chang C (2011) *C. elegans* as a genetic model to identify novel cellular and molecular mechanisms underlying nervous system regeneration. Cell Adh Migr 5:387–394. doi:10.4161/cam.5.5.17985
68. Park KK, Liu K, Hu Y et al (2008) Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science 322:963–966. doi:10.1126/science.1161566
69. Christie KJ, Webber CA, Martinez JA et al (2010) PTEN inhibition to facilitate intrinsic regenerative outgrowth of adult peripheral axons. J Neurosci 30:9306–9315. doi:10.1523/JNEUROSCI.6271-09.2010
70. Geoffroy CG, Hilton BJ, Tetzlaff W, Zheng B (2016) Evidence for an age-dependent decline in axon regeneration in the adult mammalian central nervous system. Cell Rep 15:238–246. doi:10.1016/j.celrep.2016.03.028
71. Wood WB (1987) The nematode *C. elegans*. Cold Spring Harbour Laboratory, Cold Spring Harbor
72. Hart A (2006) Behavior. WormBook, ed. The *C. elegans* Research Community, WormBook, doi:10.1895/wormbook. 1.7. 1. doi:10.1895/wormbook
73. Collins JJ, Huang C, Hughes S, Kornfeld K (2008) The measurement and analysis of age-related changes in *C. elegans*. WormBook 1–21. doi:10.1895/wormbook.1.137.1
74. Croll NA, Smith JM, Zuckerman BM (2007) The aging process of the nematode *C. elegans* in bacterial and axenic culture. Exp Aging Res 3:175–189. doi:10.1080/03610737708257101
75. Bolanowski MA, Russell RL, Jacobson LA (1981) Quantitative measures of aging in the nematode *C. elegans*. I. Population and longitudinal studies of two behavioral parameters. Mech Ageing Dev 15:279–295
76. Duhon SA, Johnson TE (1995) Movement as an index of vitality: comparing wild type and the age-1 mutant of *C. elegans*. J Gerontol A Biol Sci Med Sci 50A:B254–B261. doi:10.1093/gerona/50A.5.B254
77. Wolkow CA (2006) Identifying factors that promote functional aging in *C. elegans*. Exp Gerontol 41:1001–1006. doi:10.1016/j.exger.2006.06.033
78. Johnson TE (1987) Aging can be genetically dissected into component processes using long-lived lines of *C. elegans*. Proc Natl Acad Sci U S A 84:3777–3781
79. Huang C, Xiong C, Kornfeld K (2004) Measurements of age-related changes of physiological processes that predict lifespan of *C. elegans*. Proc Natl Acad Sci U S A 101:8084–8089. doi:10.1073/pnas.0400848101
80. Glenn CF, Chow DK, David L et al (2004) Behavioral deficits during early stages of aging in *C. elegans* result from locomotory deficits possibly linked to muscle frailty. J Gerontol A Biol Sci Med Sci 59:1251–1260
81. Mulcahy B, Holden-Dye L, O'Connor V (2013) Pharmacological assays reveal age-related changes in synaptic transmission at the *C. elegans* neuromuscular junction that are modified by reduced insulin signalling. J Exp Biol 216:492–501. doi:10.1242/jeb.068734
82. García LR (2014) Regulation of sensory motor circuits used in *C. elegans* male intromission behavior. Semin Cell Dev Biol 33:42–49. doi:10.1016/j.semcdb.2014.05.006
83. Guo X, García LR (2014) SIR-2.1 integrates metabolic homeostasis with the reproductive neuromuscular excitability in early aging male *C. elegans*. Elife 3:e01730. doi:10.7554/eLife.01730
84. Guarente L (2001) SIR2 and aging – the exception that proves the rule. Trends Genet 17:391–392
85. Ben-Yakar A, Chronis N, Lu H (2009) Microfluidics for the analysis of behavior, nerve regeneration, and neural cell biology in *C. elegans*. Curr Opin Neurobiol 19:561–567. doi:10.1016/j.conb.2009.10.010
86. Stirman JN, Brauner M, Gottschalk A, Lu H (2010) High-throughput study of synaptic transmission at the neuromuscular junction enabled by optogenetics and microfluidics. J Neurosci Methods 191:90–93. doi:10.1016/j.jneumeth.2010.05.019
87. Chokshi TV, Bazopoulou D, Chronis N (2010) An automated microfluidic platform for calcium imaging of chemosensory neurons in *C. elegans*. Lab Chip 10:2758–2763. doi:10.1039/c004658b

88. Leinwand SG, Yang CJ, Bazopoulou D et al (2015) Circuit mechanisms encoding odors and driving aging-associated behavioral declines in *C. elegans*. Elife 4, e10181. doi:10.7554/eLife.10181
89. Kato S, Kaplan HS, Schrödel T et al (2015) Global brain dynamics embed the motor command sequence of *C. elegans*. Cell 163:656–669. doi:10.1016/j.cell.2015.09.034
90. Nguyen JP, Shipley FB, Linder AN et al (2016) Whole-brain calcium imaging with cellular resolution in freely behaving *C. elegans*. Proc Natl Acad Sci U S A 113:E1074–E1081. doi:10.1073/pnas.1507110112
91. Hedden T, Gabrieli JDE (2004) Insights into the ageing mind: a view from cognitive neuroscience. Nat Rev Neurosci 5:87–96. doi:10.1038/nrn1323
92. Yeoman M, Scutt G, Faragher R (2012) Insights into CNS ageing from animal models of senescence. Nat Rev Neurosci 13:435–445. doi:10.1038/nrn3230
93. Ardiel EL, Rankin CH (2010) An elegant mind: learning and memory in *C. elegans*. Learn Mem 17:191–201. doi:10.1101/lm.960510
94. Kimata T, Sasakura H, Ohnishi N et al (2012) Thermotaxis of *C. elegans* as a model for temperature perception, neural information processing and neural plasticity. Worm 1:31–41. doi:10.4161/worm.19504
95. Nishida Y, Sugi T, Nonomura M, Mori I (2011) Identification of the AFD neuron as the site of action of the CREB protein in *C. elegans* thermotaxis. EMBO Rep 12:855–862. doi:10.1038/embor.2011.120
96. Murakami S, Murakami H (2005) The effects of aging and oxidative stress on learning behavior in *C. elegans*. Neurobiol Aging 26:899–905. doi:10.1016/j.neurobiolaging.2004.08.007
97. Murakami H, Bessinger K, Hellmann J, Murakami S (2005) Aging-dependent and -independent modulation of associative learning behavior by insulin/insulin-like growth factor-1 signal in *C. elegans*. J Neurosci 25:10894–10904. doi:10.1523/JNEUROSCI.3600-04.2005
98. Kodama E, Kuhara A, Mohri-Shiomi A et al (2006) Insulin-like signaling and the neural circuit for integrative behavior in *C. elegans*. Genes Dev 20:2955–2960. doi:10.1101/gad.1479906
99. Wang Y, Hekimi S (2015) Mitochondrial dysfunction and longevity in animals: untangling the knot. Science 350:1204–1207. doi:10.1126/science.aac4357
100. Feng J, Bussière F, Hekimi S (2001) Mitochondrial electron transport is a key determinant of life span in *C. elegans*. Dev Cell 1:633–644
101. Adachi H, Fujiwara Y, Ishii N (1998) Effects of oxygen on protein carbonyl and aging in *C. elegans* mutants with long (age-1) and short (mev-1) life spans. J Gerontol A Biol Sci Med Sci 53A:B240–B244. doi:10.1093/gerona/53A.4.B240
102. Ishii N, Fujii M, Hartman PS et al (1998) A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 394:694–697. doi:10.1038/29331
103. Bargmann CI (2006) Chemosensation in *C. elegans* (October 25, 2006), WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook. 1.123. 1
104. Kauffman AL, Ashraf JM, Corces-Zimmerman MR et al (2010) Insulin signaling and dietary restriction differentially influence the decline of learning and memory with age. PLoS Biol 8, e1000372. doi:10.1371/journal.pbio.1000372
105. Beck CD, Rankin CH (1993) Effects of aging on habituation in the nematode *C. elegans*. Behav Processes 28:145–163. doi:10.1016/0376-6357(93)90088-9
106. Timbers TA, Giles AC, Ardiel EL et al (2013) Intensity discrimination deficits cause habituation changes in middle-aged *C. elegans*. Neurobiol Aging 34:621–631. doi:10.1016/j.neurobiolaging.2012.03.016
107. Pereira S, van der Kooy D (2012) Two forms of learning following training to a single odorant in *C. elegans* AWC neurons. J Neurosci 32:9035–9044. doi:10.1523/JNEUROSCI.4221-11.2012
108. Stein GM, Murphy CT (2012) The intersection of aging, longevity pathways, and learning and memory in *C. elegans*. Front Genet 3:259. doi:10.3389/fgene.2012.00259

109. Brightwell J (2004) Hippocampal CREB1 but not CREB2 is decreased in aged rats with spatial memory impairments. Neurobiol Learn Mem 81:19–26. doi:10.1016/j.nlm.2003.08.001
110. Mouravlev A, Dunning J, Young D, During MJ (2006) Somatic gene transfer of cAMP response element-binding protein attenuates memory impairment in aging rats. Proc Natl Acad Sci U S A 103:4705–4710. doi:10.1073/pnas.0506137103
111. Kandel ER (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science 294:1030–1038. doi:10.1126/science.1067020
112. Salih DAM, Rashid AJ, Colas D et al (2012) FoxO6 regulates memory consolidation and synaptic function. Genes Dev 26:2780–2801. doi:10.1101/gad.208926.112
113. Singh-Manoux A, Kivimaki M, Glymour MM et al (2012) Timing of onset of cognitive decline: results from Whitehall II prospective cohort study. BMJ 344:d7622–d7622. doi:10.1136/bmj.d7622
114. Steculorum SM, Solas M, Brüning JC (2014) The paradox of neuronal insulin action and resistance in the development of aging-associated diseases. Alzheimers Dement 10:S3–S11. doi:10.1016/j.jalz.2013.12.008
115. Prehn K, Jumpertz von Schwartzenberg R, Mai K, et al (2016) Caloric restriction in older adults—differential effects of weight loss and reduced weight on brain structure and function. Cereb Cortex bhw008. doi:10.1093/cercor/bhw008
116. Bischof GN, Park DC (2015) Obesity and aging: consequences for cognition, brain structure, and brain function. Psychosom Med 77:697–709. doi:10.1097/PSY.0000000000000212
117. Miller AA, Spencer SJ (2014) Obesity and neuroinflammation: a pathway to cognitive impairment. Brain Behav Immun 42:10–21. doi:10.1016/j.bbi.2014.04.001
118. Witte AV, Fobker M, Gellner R et al (2009) Caloric restriction improves memory in elderly humans. Proc Natl Acad Sci U S A 106:1255–1260. doi:10.1073/pnas.0808587106
119. Kyriazis M (2009) Calorie restriction mimetics: examples and mode of action. Open Longev Sci 3:17
120. Witte AV, Kerti L, Margulies DS, Flöel A (2014) Effects of resveratrol on memory performance, hippocampal functional connectivity, and glucose metabolism in healthy older adults. J Neurosci 34:7862–7870. doi:10.1523/JNEUROSCI.0385-14.2014
121. Onken B, Driscoll M (2010) Metformin induces a dietary restriction–like state and the oxidative stress response to extend *C. elegans* healthspan via AMPK, LKB1, and SKN-1. PLoS One 5, e8758. doi:10.1371/journal.pone.0008758
122. de Lau LML, Giesbergen PCLM, De Rijk MC et al (2004) Incidence of parkinsonism and Parkinson disease in a general population: the Rotterdam Study. Neurology 63:1240–1244. doi:10.1212/01.WNL.0000140706.52798.BE
123. Niccoli T, Partridge L (2012) Ageing as a risk factor for disease. Curr Biol 22:R741–R752
124. Bargmann CI, Hartwieg E, Horvitz HR (1993) Odorant-selective genes and neurons mediate olfaction in *C. elegans*. Cell 74:515–527. doi:10.1016/0092-8674(93)80053-H
125. Avery L, Horvitzt HR (1989) Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of *C. elegans*. Neuron 3:473–485. doi:10.1016/0896-6273(89)90206-7
126. Dimitriadi M, Hart AC (2010) Neurodegenerative disorders: insights from the nematode *C. elegans*. Neurobiol Dis 40:4–11. doi:10.1016/j.nbd.2010.05.012
127. Li J, Le W (2013) Modeling neurodegenerative diseases in *C. elegans*. Exp Neurol 250:94–103. doi:10.1016/j.expneurol.2013.09.024
128. Markaki M, Tavernarakis N (2010) Modeling human diseases in *C. elegans*. Biotechnol J 5:1261–1276. doi:10.1002/biot.201000183
129. Harrington AJ, Hamamichi S, Caldwell GA, Caldwell KA (2010) *C. elegans* as a model organism to investigate molecular pathways involved with Parkinson's disease. Dev Dyn 239:1282–1295. doi:10.1002/dvdy.22231
130. Caldwell GA, Caldwell KA (2008) Traversing a wormhole to combat Parkinson's disease. Dis Model Mech 1:32–36. doi:10.1242/dmm.000257

131. Hannan SB, Dräger N, Rasse TM et al (2016) Cellular and molecular modifier pathways in tauopathies: the big picture from screening invertebrate models. J Neurochem. doi:10.1111/jnc.13532, n/a–n/a
132. Link CD (2006) *C. elegans* models of age-associated neurodegenerative diseases: lessons from transgenic worm models of Alzheimer's disease. Exp Gerontol 41:1007–1013. doi:10.1016/j.exger.2006.06.059
133. Lublin AL, Link CD (2013) Alzheimer's disease drug discovery: in vivo screening using *C. elegans* as a model for β-amyloid peptide-induced toxicity. Drug Discov Today 10:e115–e119. doi:10.1016/j.ddtec.2012.02.002
134. Pouladi MA, Morton AJ, Hayden MR (2013) Choosing an animal model for the study of Huntington's disease. Nat Rev Neurosci 14:708–721. doi:10.1038/nrn3570
135. Therrien M, Parker JA (2014) Worming forward: amyotrophic lateral sclerosis toxicity mechanisms and genetic interactions in *C. elegans*. Front Genet 5:85. doi:10.3389/fgene.2014.00085
136. Nikoletopoulou V, Tavernarakis N (2014) Necrotic cell death in *C. elegans*. Meth Enzymol 545:127–155. doi:10.1016/B978-0-12-801430-1.00006-8
137. Mano I, Driscoll M (2009) *C. elegans* glutamate transporter deletion induces AMPA-receptor/adenylyl cyclase 9-dependent excitotoxicity. J Neurochem 108:1373–1384. doi:10.1111/j.1471-4159.2008.05804.x
138. Nagarajan A, Ning Y, Reisner K et al (2014) Progressive degeneration of dopaminergic neurons through TRP channel-induced cell death. J Neurosci 34:5738–5746. doi:10.1523/JNEUROSCI.4540-13.2014
139. Hamamichi S, Rivas RN, Knight AL et al (2008) Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model. Proc Natl Acad Sci U S A 105:728–733. doi:10.1073/pnas.0711018105
140. Gitler AD, Chesi A, Geddie ML, Strathearn KE (2009) α-Synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. Nature 41:308
141. Qiao L, Hamamichi S, Caldwell KA, et al (2008) Lysosomal enzyme cathepsin D protects against alpha-synuclein aggregation and toxicity. Mol Brain 2008 1:1 1:1. doi:10.1186/1756-6606-1-17
142. Knight AL, Yan X, Hamamichi S et al (2014) The glycolytic enzyme, GPI, is a functionally conserved modifier of dopaminergic neurodegeneration in Parkinson's models. Cell Metab 20:145–157. doi:10.1016/j.cmet.2014.04.017
143. Kraemer BC, Burgess JK, Chen JH et al (2006) Molecular pathways that influence human tau-induced pathology in *C. elegans*. Hum Mol Genet 15:1483–1496. doi:10.1093/hmg/ddl067
144. Amaducci L, Tesco G (1994) Aging as a major risk for degenerative diseases of the central nervous system. Curr Opin Neurol 7:283–286
145. Volovik Y, Marques FC, Cohen E (2014) The nematode *C. elegans*: a versatile model for the study of proteotoxicity and aging. Methods 68:458–464. doi:10.1016/j.ymeth.2014.04.014
146. Morley JF, Brignull HR, Weyers JJ, Morimoto RI (2002) The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in *C. elegans*. Proc Natl Acad Sci U S A 99:10417–10422. doi:10.1073/pnas.152161099
147. Hsu A-L, Murphy CT, Kenyon C (2003) Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science 300:1142–1145. doi:10.1126/science.1083701
148. Cohen E, Bieschke J, Perciavalle RM et al (2006) Opposing activities protect against age-onset proteotoxicity. Science 313:1604–1610. doi:10.1126/science.1124646
149. Florez-McClure ML, Hohsfield LA, Fonte G et al (2007) Decreased insulin-receptor signaling promotes the autophagic degradation of beta-amyloid peptide in *C. elegans*. Autophagy 3:569–580
150. Zhang T, Mullane PC, Periz G, Wang J (2011) TDP-43 neurotoxicity and protein aggregation modulated by heat shock factor and insulin/IGF-1 signaling. Hum Mol Genet 20:1952–1965. doi:10.1093/hmg/ddr076

151. David DC, Ollikainen N, Trinidad JC et al (2010) Widespread protein aggregation as an inherent part of aging in *C. elegans*. PLoS Biol 8, e1000450. doi:10.1371/journal.pbio.1000450
152. Steinkraus KA, Smith ED, Davis C et al (2008) Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in *C. elegans*. Aging Cell 7:394–404. doi:10.1111/j.1474-9726.2008.00385.x
153. Jadiya P, Chatterjee M, Sammi SR et al (2011) Sir-2.1 modulates 'calorie-restriction-mediated' prevention of neurodegeneration in *C. elegans*: implications for Parkinson's disease. Biochem Biophys Res Commun 413:306–310. doi:10.1016/j.bbrc.2011.08.092
154. Shemesh N, Shai N, Ben-Zvi A (2013) Germline stem cell arrest inhibits the collapse of somatic proteostasis early in *C. elegans* adulthood. Aging Cell 12:814–822. doi:10.1111/acel.12110
155. Cohen E, Paulsson JF, Blinder P et al (2009) Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. Cell 139:1157–1169. doi:10.1016/j.cell.2009.11.014
156. Gontier G, George C, Chaker Z et al (2015) Blocking IGF signaling in adult neurons alleviates Alzheimer's disease pathology through amyloid-β clearance. J Neurosci 35:11500–11513. doi:10.1523/JNEUROSCI.0343-15.2015
157. De Magalhaes Filho CD, Kappeler L, Dupont J, et al (2016) Deleting IGF-1 receptor from forebrain neurons confers neuroprotection during stroke and upregulates endocrine somatotropin. J Cereb Blood Flow Metab 0271678X15626718. doi:10.1177/0271678X15626718
158. Wang J, Ho L, Qin W et al (2005) Caloric restriction attenuates beta-amyloid neuropathology in a mouse model of Alzheimer's disease. FASEB J 19:659–661. doi:10.1096/fj.04-3182fje
159. Maswood N, Young J, Tilmont E et al (2004) Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease. Proc Natl Acad Sci U S A 101:18171–18176. doi:10.1073/pnas.0405831102
160. Cohen E, Du D, Joyce D et al (2010) Temporal requirements of insulin/IGF-1 signaling for proteotoxicity protection. Aging Cell 9:126–134. doi:10.1111/j.1474-9726.2009.00541.x