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CURRENT ADVANCES IN THE STUDIES OF OXIDATIVE STRESS AND AGE-RELATED MEMORY IMPAIRMENT IN *C. ELEGANS*

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25.1 INTRODUCTION

Memory impairment is one of the diverse manifestations of the aging process. It can occur either as a part of normal aging or in association with a pathologic process. Age-related memory impairment (AMI) is a normal result of aging, which is observed in a wide variety of species from the nematode *Caenorhabditis elegans* to human. Recent studies suggest that aging causes heterogeneous effects: Some types of learning and memory are decreased, but other types are increased or show no change. AMI is not a gradual decay but rather an aspect of age-related alterations in the nervous functions. Recently, the studies of AMI in *C. elegans* have been emerging. However, there are misconceptions that remain to be clarified. The mechanism of AMI is thought to be related, at least in part, to oxidative stress, which is caused by an imbalance between the generation and removal of reactive oxygen species (ROS) and reactive nitrogen oxygen species (RNOS). Oxidative stress damages intracellular and extracellular components, including proteins, lipids, and DNA, and therefore it is predicted that aging and oxidative stress interfere with normal function of learning and memory. Detailing the mechanism of AMI will allow development of methods for identifying, delaying, and preventing both AMI and disease-associated memory impairment. It requires rigorous analysis of both aging neurons and the aging processes that affect learning and memory. This chapter describes the emerging field of cognitive aging and discusses current understanding of oxidative stress and AMI, while referencing the insights from mammalian systems.

25.2 MEMORY IMPAIRMENT DURING AGING

Age-related changes in learning and memory can be seen as a form of "forgetfulness" in our daily life. This section provides basic information about AMI that is applicable from *C. elegans* to humans. We define terms and clarify major misconceptions.

25.2.1 Terminology

Age-related memory impairment (AMI), also called age-associated memory impairment (AAMI), includes mild impairment of the ability to learn new information and to recall previously learned information. The term age-associated cognitive impairment (AACI), is also used to describe memory impairment during aging. Similar to humans, we define AMI (or AAMI) as memory impairment in comparison with young normal controls and AACI as in comparison with age-matched normal counterparts. In this chapter, we use AMI but not AAMI, since AAMI is often confused with the transition state, MCI.

There is also confusion about the definition of short-term memory (SST), long-term memory (LTM), and

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working memory. The confusion is mainly due to various definitions from different investigators [1]. In this chapter, we define the terms as follows: Short-term memory (SST) is a transient memory, which lasts for a short time (ranging from seconds to hours) and decays rapidly; long-term memory (LTM) lasts days to decades. How long do SST and LTM last in *C. elegans*? In a typical associative learning assay, SST typically lasts for several hours. LTM lasts more than a day in *C. elegans*. Additionally, SST is sensitive to cold shock and other conditions. More details are described in Section 25.3.2, "Associative Learning and Memory." Importantly, SST and working memory are not totally different [1]. Working memory uses SST as a buffer of memory for maintaining and manipulating memory or for using to affect behavioral outputs [1, 2]. Typically, working memory uses multiple SST buffers. Working memory is more complex than SST as working memory assumes more functions. The term working memory was originally described in Miller *et al.* (1960) [3]. An early model of working memory has been developed in order to describe STM more accurately [4] than the classical modal model [5].

Memory can be classified into two classical forms, including explicit memory (or declarative memory) and implicit memory (or nondeclarative memory) [summarized in Ref. 2]. When you can determine whether you remember or not, memory is called "explicit" (you know that you know) [2]. Explicit memory includes memory for event (episodic memory) and memory for fact (semantic memory). In contrast, memory other than explicit memory is generally classified as implicit memory. Implicit memory includes memory for skills, habits, and behaviors (procedural memory) and classical conditioning. Classical conditioning associates a stimulus to predict important events. and therefore it is a form of associative learning and memory. Recent studies suggest that associative learning and memory are vulnerable to aging from *C. elegans* to humans (see below).

25.2.2 Age-Related Memory Impairment

AMI creates an increasing number of concerns in elderly patients [6, 7]. AMI is the first step toward mild cognitive impairment (MCI; transition state) and dementia (disease state) [8]. Alzheimer disease (AD) is the major type of dementia. Progression to dementia occurs at a high rate in patients with AMI (42% within 3 years) [9]. In humans, it appears likely that memory impairment occurs in the order of:

$$\text{AMI} \rightarrow \text{MCI} \rightarrow \text{Dementia}$$

The current understanding of AMI is that a wide variety of associative learning and memory are altered (impaired or increased) during aging from *C. elegans* to humans [7, 10–14] (also see Section 25.3.2). Aging selectively affects working memory and some tasks of short-term memory and long-term memory [10, 13, and this Chapter]. It seems to be generally agreed that implicit memory is reduced in learning new information and procedures (procedural learning) and remains intact in old memory and well-learned procedures (procedural memory for long term) during aging [2]. Other types of implicit memory, including classical conditioning, are differentially reduced in a wide variety of species, including *C. elegans* [reviewed in Ref. 13; also see this chapter], in fruit flies [15], in bees [16], in snails [17]. in rodents [18–20] and in humans [21]. Similar to implicit memory, explicit memory shows a decline in learning new information but retains existing memory well. It appears that plasticity to new environments is reduced. Our current model is that aging causes a reduced ability to refresh the memory previously formed. More details of mammalian AMI are described elsewhere [2, 22]. Cognitive aging in *C. elegans* is described below.

A key question in cognitive aging is how aging affects cognitive functions. It has been suspected that aging does not occur at the same speed [2] (also see Section 25.7.1). Cognitive aging is presumably caused by age-related processes and by the results of aging (i.e., age-related changes) in neurons and by modifiers of learning and memory. The model systems, including *C. elegans*, are useful to reveal the mechanisms underlying cognitive aging. Excitingly, the study of cognitive aging has recently begun in *C. elegans*.

25.2.3 Common Misconceptions

In the field of AMI, it is useful to know misconceptions that remain to be or have been clarified. We list up some examples we have encountered through communication with the experts. First, AMI is not a simple decrease in learning and memory. Recent studies suggest that aging causes not only declines but also increases in learning and memory [e.g., Refs. 10, 23]. AMI is not a gradual decay but rather an aspect of age-related alterations in the nervous functions. A good example is seen in visual memory. Older patients require briefer stimulus time than younger patients in motion discrimination [23]. Aging may reduce the function of the interneurons, which is inhibitory to motion discrimination, and improve motion discrimination in senescent neurons [23]. In humans, some but not all forms of short-term memory and procedural memory show age-related declines [10]. Therefore, it is essential to describe which type of learning and memory is altered and how it is altered.

Secondly, AMI is not a disease. AMI is a normal state prior to the disease state. What distinguishes AMI from

dementia besides cognitive deficits? A few lines of evidence from functional magnetic resonance imaging (fMRI) analysis suggest that reduced metabolic activity may be a hallmark of AMI in two hippocampal subregions (the subiculum and the dentate gyrus) [24]. More detailed analysis of aging neurons and aging processes is essential for the understanding the mechanism, prevention, and treatment of AMI.

Finally, AMI cannot be independent of aging. There is an argument that aging should be separated from AMI. However, aging affects learning and memory, which results in AMI. More precisely, AMI is caused by age-related processes that affect learning and memory. The age-related processes should include age-related changes in neurons as well as elsewhere, including modifiers and pathways for learning and memory. In fact, a type of AMI can be suppressed by serotonin inhibitors [25], which are good examples of such modifiers. It is unlikely that aging neurons are the sole cause of AMI. Thus, focusing only on neurons would miss important aspects of the mechanisms for AMI. Although the study of aging neurons has been emphasized, it is equally essential to investigate the aging processes.

25.3 COGNITIVE AGING IN *C. ELEGANS*

C. elegans shows non-associative and associative learning and memory. Both forms of memory show alterations during aging. This section overviews the studies of aging of learning and memory that have been emerging recently.

25.3.1 Non-associative Learning and Sensory Functions

Several studies have investigated aging of learning and memory in *C. elegans*. Similar to mammals, not all functions of learning and memory decline with increasing age. A form of non-associative learning, habituation (i.e., a simple reduced response after a repeated stimulus) [26], increased in old animals, while recovery from habituation was slower in the old compared to the young [27]. Glenn *et al*. (2004) showed that chemotaxis, including attraction to benzaldehyde and avoidance of octanol, declines during aging [28]. However, it is not clear whether the declines were caused by reduced motor activity or by reduced sensory functions.

25.3.2 Associative Learning and Memory

C. elegans shows implicit memory that includes classical conditioning. By classical conditioning, non-associative can learn to associate food [unconditional stimulus(US)] with a stimulus [conditional stimulus(CS)] and use the stimulus to look for food. The stimulus is associated with food (US), forming US-CS association. Thus it is a form of associative learning and memory. CS can be temperature or a chemical stimulus (i.e., smell and taste); US can be starvation (absence of food) or noxious stimulus (e.g., electric shock, heat, some chemicals and heavy metals). There are three types of associative learning behaviors known in *C. elegans*. They include thermotaxis, chemotaxis (with conditioning). and basal/enhanced slowing response.

25.3.3 Thermotaxis Learning and Memory

The study of thermotaxis has provided the first evidence for aging of associative learning and memory in *C. elegans* [11]. In thermotaxis, *C. elegans* can learn to associate a temperature with food (food-temperature association) [29, 30]. Food-temperature association leads to the tracking of the given temperature, using short-term memory (STM). It has been shown that peak performance of temperature-food association declines with increasing age [11]. It is likely that STM for temperature-food association is declined during aging, since old animals that have normal locomotion show impaired thermotaxis.

Thermotaxis can be assessed either in a single-animal assay (isothermal tracking) or in a thermotaxis population assay. The single-animal assay uses a thermal gradient as shown in Figure 25.1A. Worms can learn to move toward a given temperature (16 °C) when conditioned with food (temperature-food association). When a worm is placed in a thermal gradient created on an assay plate, it moves toward the given temperature and tracks the isotherm. Thermotaxis can also be assessed with multiple worms. In this case, conditioned worms are placed on a thermal gradient plate. The fraction of worms in the area that covers the given temperature is assessed. In contrast, when worms are conditioned with no food (starvation), they avoid the temperature (temperature-starvation association). Worms conditioned with starvation are placed on thermal gradient near the area of the given temperature.

25.3.4 Chemotaxis/Olfactory Learning and Memory

Naive *C. elegans* moves to an attractant odor. When *C. elegans* is conditioned with the attractant, such as benzaldehyde, in the absence of food, it is no longer attracted by the odor and exhibits avoidance behaviors (starvation-odor association). The type of chemotaxis avoidance is a function of associative learning and is not related to exhaustion of sensory or motor functions [31]. STM and LTM can be assessed with a single

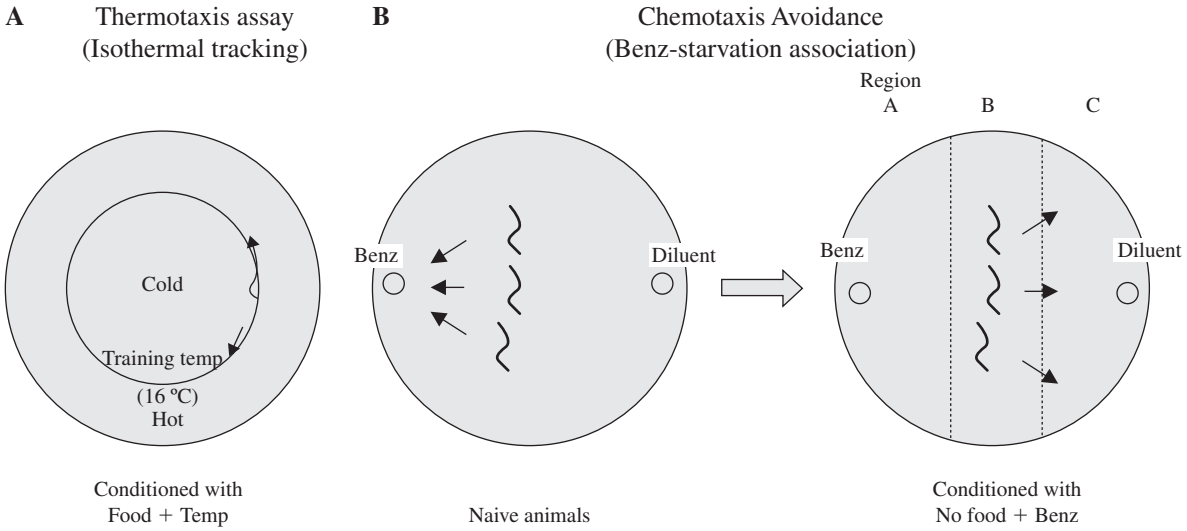

Fig. 25.1 Assay for learning and memory in *C. elegans*. **A**, Assay for thermotaxis learning and memory. The diagram represents an assay plate with a thermal gradient. A learning animal moves to and tracks the given temperature (16 °C) created on the plate. **B**, Assay for chemotaxis learning and memory. Shown is a classical assay system for chemotaxis assay. Animals are placed in B and allowed to crawl. The chemotaxis index (CI) is calculated as follows:

$$CI = (A - C)/(A + B + C)$$

where A, B, and C are the numbers of the animals in each area. The learning index (LI) for avoidance assay is calculated as follows:

$$LI = (CI\ naive - CI\ conditioned)/CI\ naive$$

When CI conditioned is less than 0, LI becomes more than 1. To avoid this overestimation, CI conditioned = 0 when CI conditioned has a negative value Benz, benzaldehyde.

conditioning and repeated conditionings, respectively. It has been shown that starvation-odor association declines during aging [32, 33] (Murakami *et al*., unpublished). Although both STM and LTM decline, it appears that age-related decline in LTM occurs slightly earlier than that of STM (Murakami *et al*., unpublished). Since LTM uses repeated conditioning with starvation, LTM may be more sensitive to age-related changes in starvation response than STM. Therefore, the results should carefully be validated.

There are several systems for chemosensory avoidance and attraction using different combinations of odor and taste [e.g., Refs. 11, 33–35]. Figure 25.1B describes a typical example of the assay system. Naive animals move to the attractant (benzaldehyde) (Figure 25.1B, left). When animals experience starvation with benzaldehyde, they avoid and move away from benzaldehyde (Figure 25.1B, right). The chemotaxis index (CI) and the learning Index (LI) are defined in the Figure 25.1B legend.

25.3.5 Experience-Dependent Modulation of Locomotory Rate

The earliest change in associative learning behavior has been observed in another type of associative learning and memory, called basal and enhanced slowing response [25]. Basal and enhanced slowing responses are experience-dependent locomotion behavior with associative learning properties [36]. When well-fed animals enter the presence of food, their movements slow to minimize the risk of moving away from the food area. This is called basal slowing response, and is regulated by dopamine signal [37]. Starved animals have a greater slowing response, called enhanced slowing response, than that of well-fed animals. This response is dependent on serotonergic signal [37, 38]. In normal aging, basal slowing response is increased, leading to a diminished difference between basal and slowing response [25]. It has been shown that the age-related change in slowing response is regulated by the serotonin pathway [25].

25.3.6 Neurons and Genes Relevant to Associative Learning and Memory

The neuronal circuits for thermotaxis associative learning consist of several types of neurons (Fig. 25.2). A pair of interneurons, AIY and AIZ, mediate thermal inputs from the thermosensory neuron AFD. These AIY and AIZ interneurons are required for associative learning; the thermosensory circuit shows conservation with the

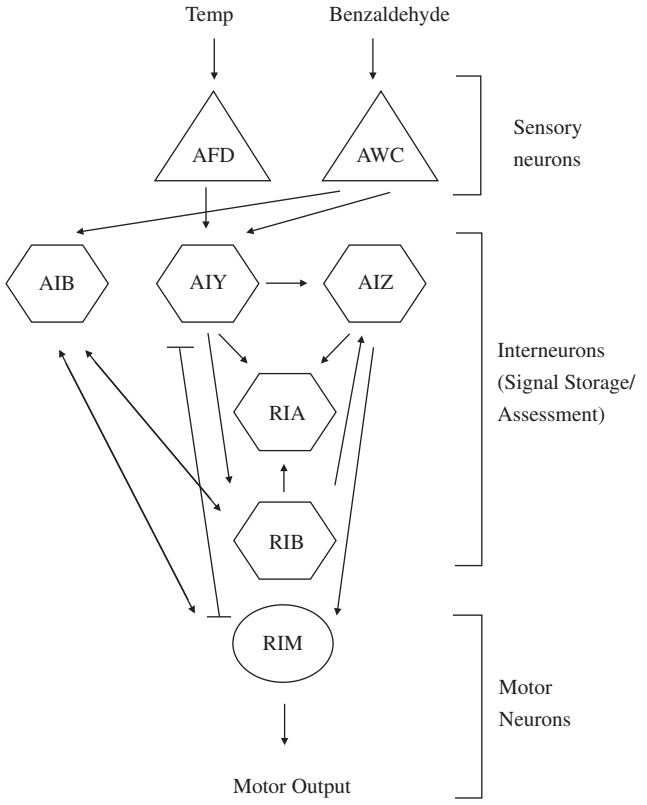

Fig. 25.2 Neurons relevant to thermotaxis learning and memory. Arrows, synapses; H-shaped bars, Gap junctions.

visual sensory circuit in vertebrates [39]. Thermotaxis learning requires the *ncs-1* neuronal calcium sensor gene [40] and the genes in the insulin/IGF-1 pathway (Fig. 25.3). A few modulators of thermotaxis learning are known. Serotonin mediates food responses associated with thermotaxis and olfactory associative learning [41, 42]. The *ncs-1* gene encodes an EF hand-containing calcium sensor protein, which is well conserved in a variety of species [40]. Expression of a neuronal calcium sensor protein gene, *ncs-1*, in AIY can increase isothermal tracking performance, while *ncs-1* knockout reduces the performance [40]. Another modulator, *hen-1*, encodes a secretory protein with an LDL receptor motif gene and is expressed in AIY and a chemosensory neuron ASE [43]. Mutations in *hen-1* abolish thermotaxis and chemotaxis learning behavior.

Olfactory learning requires sensory neurons for odors and some genes including *glr-1* (AMPA-like glutamate receptor gene). Benzaldehyde is primarily sensed by the AWC sensory neurons, which are chemosensory neurons for volatile odors (Fig. 25.2). Also of interest, mutations in *glr-1* impair olfactory associative learning when diacetyl is paired with acetic acid [44]; AMPA-type glutamate receptor mediates fast excitatory neurotransmission in the vertebrate brain. The *hen-1* LDL receptor gene is also involved in chemotaxis learning behavior when Cu^{2+} and diacetyl are paired [43]. There are four mutants that affect chemotaxis associative learning, including *lrn-1, lrn-2*,

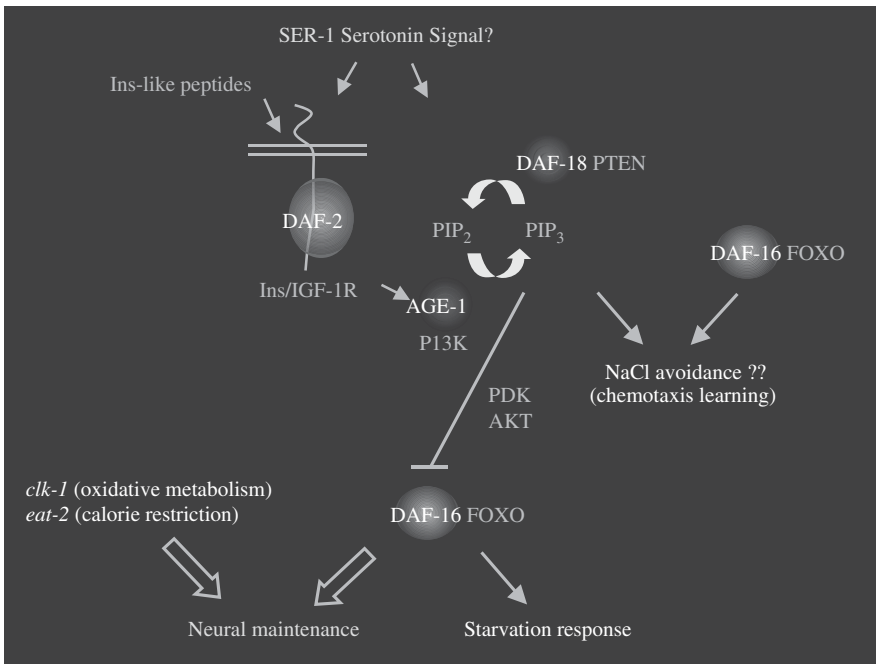

Fig. 25.3 A model for memory regulation by the insulin/IGF-1 pathway. It appears likely that the effects of life extension, or improved neuronal maintenance, lead to an increase in temperature-food association. *(See color insert.)*

JN603, and JN683 [34, 35]. Associative learning and memory are known to involve cAMP, calcium signaling and CREB in model systems including fruit flies, *Aplysia*, and rodents [45–47]. Locomotory associative-learning behaviors require dopaminergic neurons and serotonin-secreting neurons [37]. Basal slowing response uses the dopaminergic neurons, including CEP, ADE, and PDE, to sense mechanosensory stimuli. In contrast, enhanced slowing response uses serotonin-secreting neurons, including NSM.

25.3.7 *C. elegans* AD Models

The field is rapidly growing (196 publications by NCBI search, 2010) [reviewed in Refs. 48, 49]. Genes similar to those involved in human AD have been identified in *C. elegans*. They include App (*apl-1*), presenilin (*sel-12*), and Tau (*ptl-1*). *C. elegans* models of AD have been available as a form of transgenics expressing amyloid-β1-42 in muscle [50]. Recently an improved version has been generated that express A-β in neurons [51]. Importantly, the life-extending mutants of the insulin/IGF-1 pathway can delay behavioral deficits in *C. elegans* and mouse models of AD [52, 53]. AD appears to be caused by proteotoxicity rather than aggregation of the proteins.

25.4 WHAT CAUSES AMI?

This section and the next section summarize age-related changes (and processes) that affect AMI. Important questions are how the changes are related to AMI and which changes are causes of AMI. This area of the study still remains to be explored rigorously.

25.4.1 Functional Alterations in Normal Aging

Age-related changes that affect AMI include (but are not limited to) increased oxidative stress, physiological alterations, neuroinflammation, reduced metabolic functions, and changes in endocrine function. Interestingly, microarray analysis has revealed two well-conserved processes, including reduced mitochondria metabolism (see Section 25.5.3, Reduced Mitochondrial Functions) and stress response in mice, monkeys and humans [reviewed in Ref. 54]. Stress response may be triggered by increased oxidative stress, which is consistent with the multiplex stress resistance model of aging [55]. Since microarray analysis determines relative abundance of mRNA, functional analysis should be performed to confirm the results. In addition, a large-scale functional analysis should be performed in order to overview the genetic basis of AMI.

25.4.2 Limited Neural Loss in Normal Aging

It was thought that cognitive aging is caused by a loss of neurons. However, it is becoming clear that a loss of neurons plays a relatively minor role. The number of principal neurons remains relatively intact during aging in *C. elegans*, rodents, and humans [56, 57]. *C. elegans* has a simple nervous system with a small number of neurons compared to other model animals [58], providing an ideal system to keep track of the fate of neurons. The structures of *C. elegans* neurons are relatively well preserved during aging, including the number of synapses [59].

In humans and monkeys, there is reduced brain volume that may be linked to cognitive aging [60, 61]. MRI studies suggest that healthy brains show a shrinkage in volume in humans [e.g., Ref. 61] and in monkeys [62, 63]. Neuronal loss plays a limited role in the shrinkage of the brain. The brain shrinkage is probably caused by shrinkage of neurons, reductions of synaptic spines, and lower numbers of synapses [61]. It seems consistent that brain shrinkage is observed in the region called white matter, which is rich in myelinated nerve fibers [64]. The integrity of myelin may be important for cognitive aging. The most affected areas in the brain include the frontal cortex and the temporal cortex. The frontal cortex is required for working memory, which is sensitive to aging. In contrast, the hippocampus remains relatively intact in the numbers of neurons and synapses. The hippocampus is critical for storing memory in classical conditioning; neural loss in the hippocampus is a warning sign of a disease state, including early AD [65]. Although the volumetric variable has been frequently linked to cognitive aging, there is little evidence for volume-cognition relations because of technical difficulties [22].

It is known that spatial memory can be impaired without neural loss [66]. In primates, neural loss occurs in the frontal cortex and in the cerebellum, including Purkinje neurons [67]. Purkinje neuron loss is well correlated with eye-blink conditioning deficits [66]. However, Purkinje neuron loss can be induced by various factors not necessarily specific to aging, including development, toxins, and autoimmune diseases [66, 68, 69]. Taken together, neural loss appears to play a limited role in AMI.

25.4.3 Oxidative Stress in Normal Aging

Oxidative stress occurs *in vivo* when ROS generated by normal metabolism are not fully scavenged. The free radical theory of aging, also called the oxidative stress theory of aging, assumes that there is an accumulation of oxidative damage in macromolecules during aging, which eventually results in dysfunction of biological functions [70, 71]. Although the role of oxidative stress

in life span specification has been challenged (see Section 25.8, Role of Oxidative Stress in Aging and AMI), it remains convincing that increased oxidative stress has an impact on age-related functional declines in biological processes. It has been shown that an indicator of oxidative damage on DNA [8-oxo-2-deoxynucleotide (oxo8dG)] increases with aging in rodent strains including F344 rats, B6D2F1 mice, and C57BL/6 mice [72]. Another indicator of oxidative damage, protein carbonyl, increases sharply in the last third of life span in a variety of species from *C. elegans* to humans [73, 74].

The central nervous system consumes a great deal of oxygen and is rich with components susceptible to oxidative stress, such as polyunsaturated fatty acids (PUFA, often referred to as "fish oil") [75, 76]. In the rodent strains, brain is one of the tissues that show the greatest increases in oxo8dG levels (ranging from 167% to 340%) in 24-month-old compared to 6-month-old animals [72]. The increases in oxo8dG may be caused by increased sensitivity to oxidative stress.

In humans, gene expression is reduced in the frontal cortex, including the genes involved in synaptic plasticity, including NMDA and AMPA receptor function, calcium-mediated signal, and synaptic vesicle release and recycling [77]. The reduced expression occurs in the relatively early phase of normal aging (after the age of 40; mid-preproduction period).

25.4.4 Oxidative Stress in Neurodegenerative Diseases

There are extensive studies that suggest an association between increased oxidative stress and neurodegenerative diseases, including AD, Parkinson disease, and Huntington disease [e.g., Refs. 78–80]. Although the mechanism is to be determined, oxidative stress has been implied as a factor that promotes the initial phase of neurodegenerative diseases; other factors include glutamate signaling, altered calcium homeostasis, decreased growth factors, and genetic mutation [81]. For example, AD patients show increased levels of protein carbonyls, oxo8dG, and lipid peroxidation, especially in the brain area with amyloid plaques and neurofibrillary tangles [82]. In cultured neurons and in rat synapses, lipid peroxidation has been observed after exposure to amyloid-β, a component of amyloid plaques [e.g., Refs. 81, 83].

25.4.5 Mutations with Increased Oxidative Stress in *C. elegans*

Experimental evidence for the role of oxidative stress in AMI comes from a study using two mutational defects in the mitochondrial respiratory chain. The *mev-1* mutation has a defect in a cytochrome *b* large subunit (SDHC) in complex II in the mitochondrial electron transport chain) [84]. The *gas-1* mutation has a defect in a subunit of the mitochondrial NADH:ubiquinone-oxidoreductase in complex I of the respiratory chain [85]. Both the *mev-1* SDHC mutation and the *gas-1* mutation cause overproduction of superoxide (O_2^-) and precocious aging [reviewed in Ref. 84].

It has been shown that thermotaxis is impaired by *mev-1* and *gas-1* mutations with increased oxidative stress [86]. Treatment of a natural pro-antioxidant, lipoic acid, can partially restore impaired thermotaxis in the *mev-1* mutant. It is plausible that oxidative stress plays a role in the performance of thermotaxis. Perhaps more convincing evidence for the role of oxidative stress has been provided by the studies of mutants and drugs that can reduce oxidative stress or oxidative metabolism (see Section 25.6.2).

25.5 OTHER FACTORS THAT MAY CAUSE AMI

This section continues on from Section 25.4, which describes potential causes of AMI. Listed below here are the factors that may affect AMI in *C. elegans* and mammals.

25.5.1 Physiological Alterations

AMI is in part caused by physiological changes in normal aging of the nervous system in mammals [7, 14]. It seems that AMI is caused by impairment of the ability to renew recent acquired information [87; this chapter]. Although neural loss is minimal, there is disruption of myelinated fibers, which may affect physiology of synapsis and communication among the brain regions in normal aging [88]. fMRI studies suggest that coordination between brain regions is significantly weakened in normal aging [88]. The elderly use a broader region of the brain (i.e., delocalization) than younger counterparts, which may be a compensatory response in the elderly.

25.5.2 Neuroinflammation

In normal aging, there is an imbalance between inflammation and antiinflammation, shifting toward a proinflammatory state. In humans and rodents, there is an increase in reactivity of microglia and astrocytes, which is indicative of increased innate immunity [89]. However, in humans, upregulation of genes involved in inflammatory is seen in the disease state, AD, while upregulation is weak in normal patients [90]. It is known that neuroinflammation is involved in an early phase toward AD and the diseases of aging [91] rather than AMI. It appears that patients with AMI are in a reactive but pre-inflammation state. Upon exposure to an inflammatory stimulus, including infection and stress, the

reactive state should trigger an overreaction of inflammation, leading to increased decrements in learning and memory [92]. Interestingly, anti-inflammatory drugs, including nonsteroidal anti-inflammatory drugs (NSAIDs), can reduce age-related cognitive decline in the elderly [93].

25.5.3 Reduced Mitochondrial Functions

The central nervous system relies on mitochondrial metabolism for energy production. Organismal aging in *C. elegans* shows reduced metabolic function [94], although energy production is uncoupled from life span extension in the Clk mutations that reduce oxidative metabolism [Ref. 94; also see Section 25.6.2, "Update"]. Microarray analysis has shown that mitochondrial genes show progressive declines during aging in humans, monkeys, and rats [reviewed in Ref. 95]. Importantly, proteomics analysis identified oxidatively modified proteins involved in energy metabolism and ATP production [96]. The modified proteins showed reduced activity. Oxidative stress also modifies the promoters of the mitochondrial genes [95], possibly leading to reduced expression of the genes. More detailed discussion about energy metabolism is available elsewhere [95].

25.6 IMPROVED LEARNING AND MEMORY

An exciting aspect for the studies of AMI is to identify genetic and pharmacological manipulations that rescue deficits in learning and memory. This section explores possibilities from previous findings.

25.6.1 Exploring Genetic and Pharmacological Interventions

Understanding of AMI will include neurological variables: (1) variables that cause AMI (e.g., modifiers) and (2) variables that are secondary or compensatory changes in response to the factors that causes AMI. The variables that cause AMI are reasonable targets for intervention, which is being explored. In *C. elegans*; such intervention includes mutations and drugs that reduce oxidative stress, alter neuroendocrine function (the insulin/IGF-1 pathway and the serotonin/octopamine pathway), and cause calorie restriction.

25.6.2 Mutants and Drugs that Can Reduce Oxidative Stress or Oxidative Metabolism

Mutants with deficits in mitochondrial transport chains are known to alter oxidative metabolism. The *isp-1* (iron sulfur protein gene) mutant decreases oxygen consumption by half and therefore reduces oxidative metabolism [97]. Mutations in *clk-1* (mitochondrial di-iron carboxylase gene) lack an endogenous isoform of a redox-active lipid, coenzyme Q (Q) [98. 99]. The *clk-1* mutant accumulates a biosynthetic intermediate, demethoxy-Q_9 (DMQ_9) [99], that has a strong ROS scavenging activity. The mutants are Clk mutants and show increased longevity [100], stress resistance [101], delayed development, and extended rhythmic behavior cycles [97, 98].

A metabolic antioxidant, lipoic acid (LA), can improve the performance of thermotaxis [86]. LA is reduced to a potent antioxidant, dihydrolipoic acid, which can recycle other antioxidants such as vitamins C and E [103]. LA can decrease oxidative damage in the brains of older rats and partly restore age-related declines in nervous functions [104]. LA, along with statins (inhibitors of HMG-CoA reductase), can reduce LDL oxidation, which is also a risk factor of neurodegenerative disease and cardiovascular disease [105]. In mice, SOD/catalase mimetics (EUK-189 and EUK-207) can reduce oxidative damage (lipid peroxidation, nucleic acid oxidation, and ROS levels) and improve age-related decline in performance in the fear-conditioning task [106].

Update: During the review process of this chapter, a manuscript has been published that reverses the original claim that the *clk-1* and *isp-1* mutations reduce oxidative stress [97, 98, 100] to a new claim that the mutants modestly increase mitochondrial superoxide [102]. Importantly, the mutants show reduced mitochondrial functions, including oxidative metabolism, and, therefore, it is still reasonable to claim that the *clk-1* and *isp-1* mutations show reduced oxidative metabolism. Although the manuscript provides an interesting model that mitochondrial superoxide plays a role as a protective signal to mediate life extension [102], no data are presented to support the model, which remains to be tested.

25.6.3 Serotonin Pathway

Increased serotonin appears to alter behaviors during aging [25]. It is suggested that mRNA and expression of the serotonin biosynthesis gene, *tph-1* (tryptophan hydroxylase-1), are increased during aging [Ref. 25 and Murakami *et al.*, unpublished]. Consequently, reducing serotonin signal by serotonergic inhibitors and by the serotonin/octopamine pathway genes rescues age-related changes in basal/slowing response and chemotaxis avoidance [Refs. 25, 107, and Murakami *et al.*, unpublished]. Importantly, serotonin pathways also regulate life span [108].

25.6.4 Insulin/IGF-1 Pathway

It has been shown that the insulin/IGF-1 pathway modulates the late phase of age-related changes in

thermotaxis (temperature-food association) [11]. Figure 25.3 summarizes the current model; alternative models have been discussed elsewhere [13]. The insulin/IGF-1 pathway includes *daf-2* (insulin-like receptor gene) and *age-1* (phosphatidylinositol-3 OH kinase gene), which negatively regulate the Forkhead transcription factor, encoded by *daf-16* (Fig. 25.3).The *age-1* gene functions in the AIY, AIZ, or RIA interneurons [11, 109], suggesting that interneurons may be the core site of modulation by the insulin/IGF-1 pathway. Of note is the fact that proteotoxicity in the AD models is also suppressed by the mutations in the insulin/IGF-1 pathway (See Section 25.3.7, *C. elegans* AD Models).

Update: Several groups have confirmed that the insulin/IGF-1 pathway controls associative learning and memory in various *C. elegans* systems in young adults and during aging [33, 110–112].

25.6.5 Calorie Restriction

Interpretation of previous studies on calorie restriction (CR) requires careful consideration. CR can improve associative learning and memory in thermotaxis (food-temperature association) [86] and in chemotaxis avoidance (starvation-odor association) [33] in *C. elegans*. In contrast, CR in fruit flies does not: CR extends life span but does not suppress aging of an aversive learning task (odor-mechanical shock association) [113]. This may suggest a difference between the species. Alternatively, it may suggest a difference in experimental assay systems. The *C. elegans* studies use food (absence or presence of food) as a UC, while the fruit fly study uses mechanical shock as a UC. Thus an alternative model is that CR improves responses to food or starvation.

25.7 WHEN DOES COGNITIVE AGING BEGIN?

Although each form of learning and memory shows alterations at a different speed, current studies indicate two major time points that alter learning and memory in *C. elegans*. These include the early-mid reproduction period and around the end of the preproduction period, which appear consistent with human studies.

25.7.1 Multiple Phases of AMI

The studies of *C. elegans* suggest at least two phases of AMI. The early phase of AMI includes age-related decline in basal and slowing response and olfactory learning and memory (STM and LTM) [33, 86, 112]. The timing is at the early to mid-reproduction period. The late phase, in contrast, is around the end of the reproduction period. In the late phase, AMI includes disturbances of various behaviors, including reduced thermotaxis associative learning behaviors and locomotion. It is unlikely that reduced locomotion at the late phase affects thermotaxis, as there is no correlation between reduced locomotion (by up to 40%) and thermotaxis in some mutations [86]. For example, the clk-1 and isp-1 mutations with reduced mitochondrial metabolism show reduced locomotion but an increased performance of thermotaxis [86].

Similarly, there are intriguing observations in humans. The cross-sectional studies suggest that some aspects of age-related cognitive decline begin in healthy, educated adults when they are in their 20s and 30s [114]. In contrast, the longitudinal data suggest around 60 years of age [115]. Although there seems to be a discrepancy, the two methods may be effective to reveal different phases of AMI in humans. It is notable that the standard cognitive tests use age-related adjustment, which artificially minimizes age-related changes [22]. The timing of the two AMI phases in humans is relatively similar to *C. elegans*. It is worth noting that there is an increase in oxidative damage around 40 years of age [77]. It is also possible that there are more than two phases of AMI. This line of experiments will be promising and should be performed for future studies.

25.8 ROLE OF OXIDATIVE STRESS IN AGING AND AMI: A THEORY

Oxidative stress increases with aging. However, the free radical theory of aging does not appear to explain aspects of aging. In this section, we propose a theory that incorporates evolutionary and biological aspects of aging.

25.8.1 Midlife Crisis Theory

Oxidative stress increases during aging and can damage macromolecules. The free radical theory of aging assumes that oxidative stress causes aging of biological functions [70]. Recent studies suggest that mutants in the superoxide dismutase (sod) genes show increased oxidative damage but fail to accelerate aging, providing evidence against the theory [116–118].

It is puzzling that ROS scavenging activities are relatively well retained, although oxidative damage increases during aging [73, 119]. In *C. elegans*, enzymatic activities of SOD (superoxide dismutase) and catalase do not decline until late in the life span (summarized in Fig. 25.4) [120]. This is consistent with mRNA levels of *sod* and *ctl* (catalase) genes, except for *sod-2*, declines with aging at mRNA levels [121].

Our midlife crisis theory assumes that oxidative stress is a part of an age-related crisis that occur in the middle

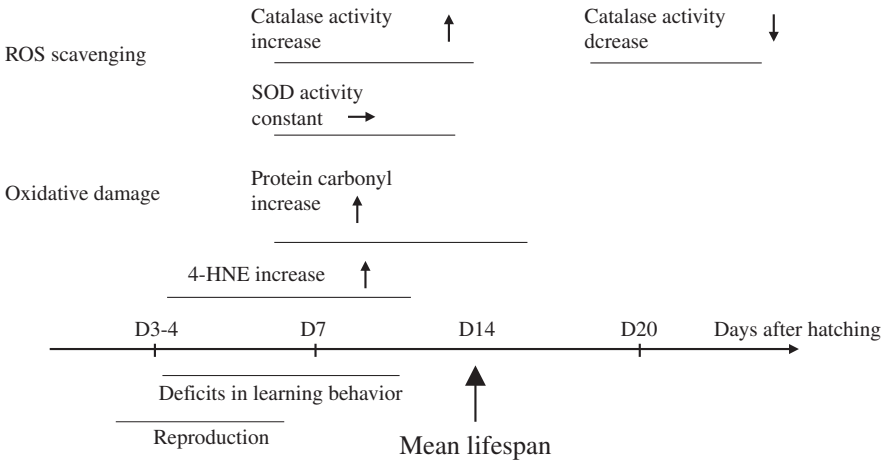

Fig. 25.4 A summary of *C. elegans* ROS and oxidative damage results during life span. Days are adjusted to the growth at 20 °C.

of the life span. It has been thought that normal metabolism in mitochondria is the primary source of oxidative stress [70]. Previous studies suggest that activities of mitochondria gradually change during aging [121]. If normal mitochondrial metabolism is the primary site of ROS generation during, ROS levels should gradually change during aging. However, an indicator of oxidative damage, protein carbonyl formation, increases in the middle of the life span [73]. The mechanism that causes age-related oxidative stress may be more complex than originally predicted by Harman (1956) [70].

The timing of increased oxidative stress is similar to that in which mortality increase, altered learning behaviors, and deteriorations of reproductive tissues are observed. We are inclined to propose a "midlife crisis" theory in which age-related increase in ROS is associated with the age-related deteriorations. In this theory, age-related ROS is associated with a wide variety of crises ranging from minor to major deficits. The midlife deficits occur around the end of reproduction, at which there is a diminishing force of natural selection. The deficits are unlikely to affect fitness of the species since they have a low impact on prosperity of the species. Therefore, the theory has an aspect of evolutionary and biological theory of aging. Importantly, in the theory, the *sod* mutations should have a limited impact on aging.

25.9 CONCLUSION AND PERSPECTIVE

AMI and neurodegenerative diseases impact more than 6.9 million people in the US [8, 123, 124]. Oxidative stress increases during aging and is a major risk factor for AMI and neurodegenerative diseases. Increased oxidative stress reduces expression of a specific set of neural genes during human brain aging [77] and is a component of the transition state prior to the onset of neurodegenerative diseases such as AD [81]. It appears that aging neurons are sensitive to stress, including infections, oxidative stress, and environmental toxins. Such intrinsic and environmental stress may be partially rescued by nutritional approaches that include foods rich in antioxidants or anti-inflammatory compounds (e.g., berries, nuts, and grapes) [125, 126].

Despite the recent investigations, the genetic basis of AMI still remains to be determined. How does aging cause AMI? Can general aging be genetically delineated from tissue-specific aging that occur in neurons? Model systems, such as *C. elegans*, will provide useful systems to overview the mechanisms of AMI. Current understanding of AMI and oxidative stress appears to point out that oxidative stress and other age-related deteriorations occur in the mid to late phase of AMI. In the early phase of AMI, it seems that there is endocrine disturbance, as mutations in the insulin/IGF-1 pathway and serotonin pathway can rescue early markers of AMI. The emerging field of AMI is waiting to reveal its mechanisms.

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