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NEURONAL INPUTS AND OUTPUTS OF AGING AND LONGEVITY

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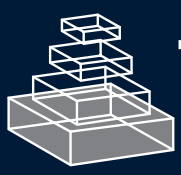
Joy Alcedo, Thomas Flatt and
Elena G. Pasyukova



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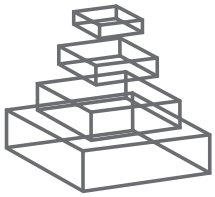

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NEURONAL INPUTS AND OUTPUTS OF AGING AND LONGEVITY

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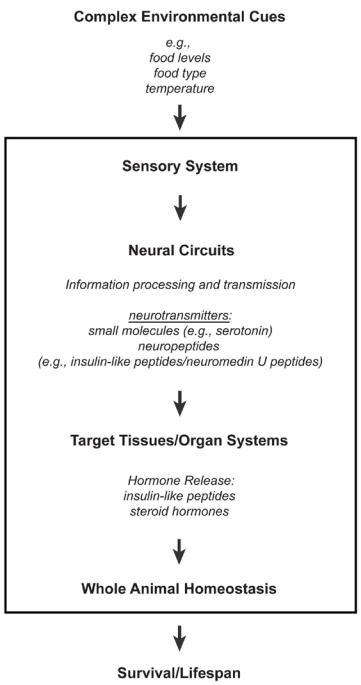

Alcedo, J., Flatt, T., and Pasyukova, E. (2013). Neuronal inputs and outputs of aging and longevity. *Front. Genet.* 4:71. doi: 10.3389/fgene.2013.00071.

An animal's survival strongly depends on its ability to maintain homeostasis in response to the changing quality of its external and internal environments. This is achieved through intercellular communication not only within a single tissue but also among different tissues and organ systems. Thus, alterations in tissue-to-tissue or organ-to-organ communications, which are under genetic regulation, can affect organismal homeostasis, and consequently impact the aging process. One of the organ systems that play a major role in maintaining homeostasis is the nervous system. Considering that the nervous system includes the sensory system, which perceives the complexity of an animal's environment, it should be no surprise that there would be a sensory influence on homeostasis and aging. To promote homeostasis, any given sensory information is transmitted through short-range signals via neural circuits and/or through long-range endocrine signals to target tissues, which may in turn be neuronal or non-neuronal in nature. At the same time, since homeostasis involves a number of feedback mechanisms, non-neuronal tissues can also modulate sensory and other neuronal functions.

Several genes that regulate signaling pathways known to affect homeostasis and aging have been shown

to act in neurons, in tissues that are likely downstream targets of the nervous system, or through feedback regulation of neuronal activities. These genes can have different temporal requirements: some might function early, e.g., by affecting neural development, while others may only be required later in adulthood. Some well-known examples of genes involved in the neuronal regulation of homeostasis and longevity encode components of the evolutionarily conserved nutrient-sensing insulin/insulin-like signaling pathway, the stress-sensing internal repair system, and the mitochondrial electron transport chain. Indeed, the genetic perturbation of these pathways has been found to lead to numerous diseases, many of which are age-related and involve the nervous system, such as neurodegeneration and the metabolic syndrome.

Despite much progress, however, many aspects of the neuronal inputs and outputs that affect aging and longevity are poorly understood to date. For example, the precise neuronal and non-neuronal circuitries and the details of the molecular mechanisms through which genes/signaling pathways maintain homeostasis and affect aging in response to the environment remain to be elucidated. Similarly, it is presently unclear whether genes that regulate the early development of the nervous system and its consequent circuitry influence homeostasis and longevity during adulthood. At the same time, although many genes affecting aging are conserved, both the nervous system and the aging process are highly variable within populations and among taxa. Accordingly, the role of natural genetic variation in shaping the neurobiology of aging is also presently unknown.

The aim of this Research Topic is therefore to highlight the genetic, developmental, and physiological aspects of the signaling networks that mediate the neuronal inputs and outputs that are required to maintain organismal homeostasis. The elucidation of the effects of these neuronal activities on homeostasis may thus provide much-needed insight into mechanisms that affect aging and longevity.

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The role of the nervous system in aging and longevity

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The connections between the nervous system, aging and longevity are manifold and profound. On the one hand, the nervous system plays an important role in processing complex information from the environment, which has a major influence on an animal's aging and longevity. Accordingly, environmental signals are received and integrated by this organ system, leading to diverse physiological outputs that can have pervasive effects on homeostasis and lifespan. Thus, an animal's nervous system not only controls its homeostatic responses but can also alter its lifespan and aging process. On the other hand, similar to the feedback regulation that characterizes homeostatic mechanisms, aging also impacts the functional state of the nervous system, as exemplified, for instance, by the prevalence of age-associated neurodegenerative diseases.

The lead review (Alcedo et al., 2013) in this collection of articles introduces different aspects of neuronal inputs and outputs of signaling pathways that affect homeostasis, and consequently longevity and aging. A number of these inputs, which can be detected by sensory neurons acting at the interface between an animal's external and internal environments, have been found to either shorten or lengthen lifespan. Jeong et al. (2012) describe more explicitly how gustatory, olfactory and thermosensory cues affect invertebrate lifespan and the possible implications on mammalian aging. In mammals, these sensory cues likely modulate hypothalamic function and the neuroendocrine systems required for maintaining homeostasis [reviewed in Alcedo et al. (2010, 2013); Jeong et al. (2012)]. Consistent with this notion, Bartfai and Conti (2012) discuss how nutrient signals act on heat-sensing hypothalamic neurons to regulate energy expenditure by maintaining mammalian core body temperature, which has been previously shown to affect lifespan (Conti et al., 2006).

In addition to nutritional and temperature cues, the nervous system can sense other environmental cues and stressors (Alcedo et al., 2013). Iranon and Miller (2012) focus on one such stressor—i.e., low oxygen availability, which compromises many important physiological processes; in their paper, the authors review the mechanisms animals use to maintain oxygen homeostasis in response to hypoxia. In contrast, Kagias et al. (2012) elaborate on different types of stressors and the neuronal responses that these elicit. Besides extrinsic environmental factors, they also discuss how the processes of development and aging generate intrinsic stress (Kagias et al., 2012).

Neuronal inputs are integrated by neural circuits, which can then lead to longevity-modulating outputs. Because of the central role that calcium signaling plays in processing neural information, Nikoletopoulou and Tavernarakis (2012) highlight the importance of maintaining calcium homeostasis. They discuss how loss of calcium homeostasis increases the risk for neurodegenerative diseases and how aging itself can impair this homeostasis (Nikoletopoulou and Tavernarakis, 2012). Calcium homeostasis requires the coordinated function of different organelles, including that of the mitochondria (Nikoletopoulou and Tavernarakis, 2012). Troulinaki and Bano (2012) further underscores the involvement of mitochondria in known longevity pathways and their dual role in aging and neurodegeneration. They review how the decline of mitochondrial activity significantly contributes to age-related impairment of neural circuits (Troulinaki and Bano, 2012).

Consistent with this idea, several reviews discuss evidence suggesting that impaired, aging neurons can modulate the functional outputs of the nervous system, such as protein homeostasis (David, 2012), learning, memory (Stein and Murphy, 2012), and emotional state (McKinney et al., 2012). The review of David (2012) focuses on the role of protein aggregation and protein-quality control in the aging brain. Of note, several reviews discuss how neuronal signaling upon mitochondrial dysfunction, in addition to other stimuli, plays a role in coordinating the mitochondrial unfolded protein response, which in turn affects protein misfolding and polyglutamine aggregation in non-neuronal tissues (David, 2012; Kagias et al., 2012; Alcedo et al., 2013).

McKinney et al. (2012) and Stein and Murphy (2012) present other mechanisms through which aging neurons affect the rate of organismal senescence. The impact of neuronal aging on cognitive and psychological states can be observed through consistent and specific age-dependent gene expression changes in the brain. The review by McKinney et al. (2012) also supports the important notion that naturally occurring individual variation in the rates of gene expression changes during brain aging can determine the onset of senescence and of developing age-related brain disorders. Last but not least, the paper by Huffman (2012) reviews the intimate links between the regulation of development and aging by discussing how patterns of neocortical gene expression and neocortical sensory-motor axonal connections develop and change throughout the lifespan of the animal and how they affect aging.

Collectively, the reviews in this Special Topic provide ample evidence from recent literature that show how aging is modulated by a complex interplay between the environment, genes, signaling networks and tissues. In particular, they highlight the key role in aging and longevity played by the nervous system, which is *the* central integrator of information from both the external

environment and the inner "milieu" intrinsic to the organism. In discussing the state-of-the-art, these papers also illustrate key areas for future work. For example, our current understanding of the sensory perception of environmental cues and the signals that integrate and process these cues in affecting lifespan and aging is still very limited. Moreover, major and unresolved questions remain about the mechanistic relationship between the neuronal regulation of lifespan and the senescence of neuronal and cognitive function. Finally, we still lack essential information on the evolutionary conservation of the neuronal inputs and outputs of longevity and aging. Such information might prove to be extremely helpful in generating therapeutic/pharmacological interventions in the future. As is clearly demonstrated by the review articles in this Special Topic, the neurobiology of aging is a rapidly developing field; at the same time, numerous difficult problems remain to be solved in future work, making this vibrant field a major frontier in aging research.

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Neuronal inputs and outputs of aging and longevity

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An animal's survival strongly depends on its ability to maintain homeostasis in response to the changing quality of its external and internal environment. This is achieved through intracellular and intercellular communication within and among different tissues. One of the organ systems that plays a major role in this communication and the maintenance of homeostasis is the nervous system. Here we highlight different aspects of the neuronal inputs and outputs of pathways that affect aging and longevity. Accordingly, we discuss how sensory inputs influence homeostasis and lifespan through the modulation of different types of neuronal signals, which reflects the complexity of the environmental cues that affect physiology. We also describe feedback, compensatory, and feed-forward mechanisms in these longevity-modulating pathways that are necessary for homeostasis. Finally, we consider the temporal requirements for these neuronal processes and the potential role of natural genetic variation in shaping the neurobiology of aging.

Keywords: aging, longevity, homeostasis, brain, nervous system, neuroendocrine system

INTRODUCTION

The study of aging is the study of an open system, where tissues and organs within the whole animal regularly exchange information not only with each other but also with their external environment during the course of the animal's lifespan. These exchanges in information allow the animal to maintain a stable internal environment, known as homeostasis, which is necessary for survival amid the constant flux in the animal's external environment. An important node within this flow of information is the nervous system, which serves as an interface between the animal's external and internal environments. Not surprisingly, neuronal signaling activities and their regulation have a major influence on the animal's survival and aging process. Here we address the role of the nervous system in maintaining homeostasis and its consequent impact on longevity and aging.

SIGNALING NETWORKS: INTRACELLULAR, INTERCELLULAR, AND INTERORGAN COMMUNICATION IN HOMEOSTATIC MAINTENANCE – THE INFLUENCE ON LIFESPAN

The nervous system is a network of specialized cells that relay information between different organ systems and the environment. Sensory neurons perceive environmental cues, whose information are transmitted to non-neuronal tissues either directly or indirectly via neural circuits that consist of interneurons and/or other types of neurons, like motor neurons. These intercellular and interorgan communications involve different types of signaling molecules that range from small molecule neurotransmitters to neuropeptides and hormones (**Figure 1**; reviewed in Alcedo et al., 2010). Indeed, consistent with the findings that the nervous system affects longevity, the processing of environmental information by sensory neurons and the corresponding neural circuitries can modulate hormonal secretions

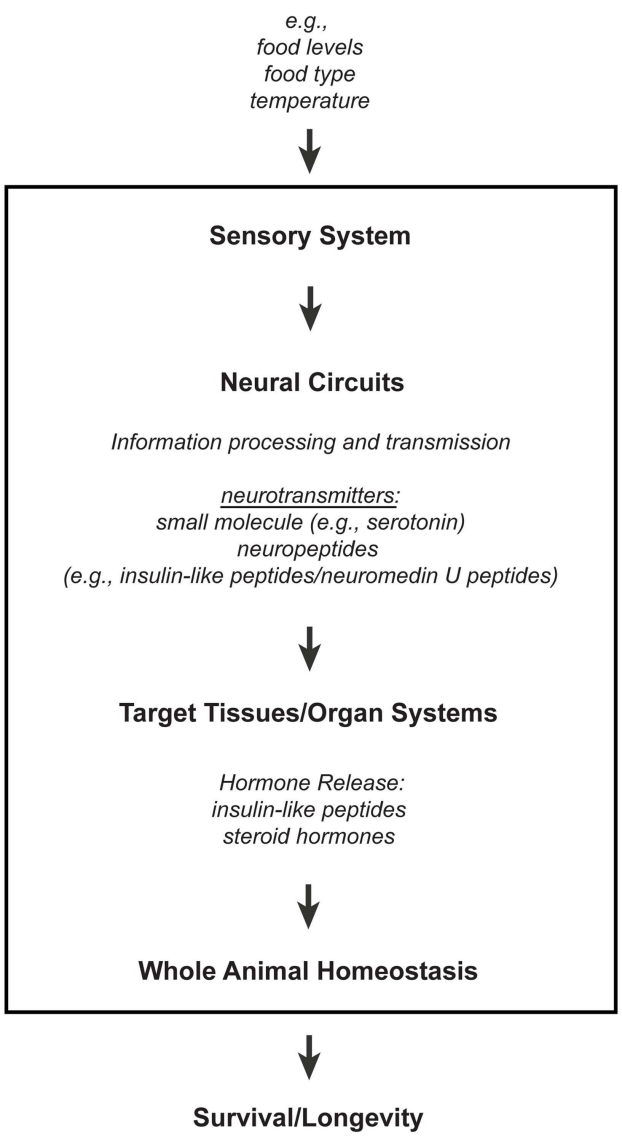

FIGURE 1 | A model for how the nervous system processes environmental information through neuronal and non-neuronal circuits to maintain homeostasis for optimal survival. Information processing of environmental inputs at the neuronal level will involve the function of (1) small molecule neurotransmitters and neuropeptides, such as insulin-like peptides, (2) stress-sensing pathways, and (3) mitochondria-associated signals. These neuronal signaling outputs will in turn target other tissues to regulate the production of secondary signals, like hormones, and thus promote homeostasis and longevity. Insulin-like peptides can function either as short-range peptide neurotransmitters (Chen et al., 2013) or as peptide hormones.

that maintain homeostasis (**Figure 1**; reviewed in Alcedo et al., 2010).

SENSORY INFLUENCE ON HOMEOSTASIS AND LIFESPAN

Sensory perception can alter a number of physiological processes, from circadian clocks (Wurtman et al., 1963, 1964; la Fleur et al., 2001; Challet et al., 2003; Ha et al., 2006), developmental plasticity (Bargmann and Horvitz, 1991; Schackwitz et al., 1996) and metabolism (Zafra et al., 2006; Greer et al., 2008) to reproduction (Yoon et al., 2005), and stress responses (Prahlad et al., 2008). Similarly, sensory neurons have been found to affect lifespan in the nematode worm *C. elegans* (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004; Bishop and Guarente, 2007; Lee and Kenyon, 2009) and in the fruit fly *Drosophila* (Libert et al., 2007; Poon et al., 2010). This influence on lifespan involves positive or negative inputs from gustatory, olfactory, and thermosensory neurons that can modulate the activities of different peptide or steroid hormones (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004; Libert et al., 2007; Lee and Kenyon, 2009), which would in turn presumably affect different homeostatic mechanisms (reviewed in Fielenbach and Antebi, 2008; Kenyon, 2010). The above studies demonstrating the sensory influence on *C. elegans* and *Drosophila* lifespan have been reviewed in greater detail by Jeong et al. (2012), as part of this Research Topic.

The nature of some of these neurons suggests that some of the cues that affect lifespan are food-derived, which agrees with the observation that some olfactory inputs are involved in the lifespan effects of restricting food intake levels (Libert et al., 2007), a phenomenon that is commonly known as calorie restriction (Klass, 1977; Weindruch and Walford, 1988). However, the longevity-promoting effects of food-level restriction are linked to changes in feeding rates, delayed development, and decreased reproduction (Klass, 1977; Weindruch and Walford, 1988). In contrast, the sensory influence on lifespan does not always correlate with the sensory effects on feeding behaviors, development, and reproduction (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004; Poon et al., 2010), which suggests that the sensory system will affect lifespan through more than one mechanism. This would be expected since different types of sensory neurons can perceive a wide variety of environmental cues, ranging from temperature (Lee and Kenyon, 2009; Xiao et al., 2013) or the inherent complexity of food sources (Libert et al., 2007; Maier et al., 2010; Poon et al., 2010) to other types of cues, many of which can potentially alter organismal homeostasis and affect lifespan.

Recently, the sensory system has been shown to promote another form of dietary influence on lifespan – dependence on food-type/composition, which is distinct from the lifespan effects of food-level restriction (Maier et al., 2010). This is consistent with the previous observation that only a subset of gustatory and olfactory neurons affects lifespan in a given environment (Alcedo and Kenyon, 2004), i.e., the presence of a specific set of lifespan-influencing cues in some food sources will only be detected by a specific set of sensory neurons. Indeed, this is supported by the recent identification of a monocarboxylate-like transporter (MCT-1) that mediates the lifespan effects of only certain sensory neurons, suggesting that MCT-1 will transport some, but not all, small metabolites (Gaglia et al., 2012).

The sensory influence on lifespan via food-type recognition has also been shown to involve the activities of specific neuropeptide signaling pathways under certain environmental conditions (Maier et al., 2010). For example, a neuropeptide neuromedin U

pathway processes food-type information that alters *C. elegans* lifespan, independent of food intake levels (Maier et al., 2010). Considering that many species have a large repertoire of neuropeptide ligands and receptors, many of which are expressed in the nervous system (Bargmann, 1998; Strand, 1999), these neuropeptide signaling pathways could presumably process distinct sets of sensory information into physiological responses that would optimize survival.

MODULATION OF LIFESPAN AND AGING BY NEURONAL INSULIN/IGF SIGNALING

The sensory influence on lifespan can be mediated by insulin/insulin-like peptides (ILPs) and their corresponding signaling pathway(s), IIS (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004), which are also known to play a central role in regulating various aspects of growth, development, metabolism, and reproduction. Indeed, among the molecular pathways known to affect longevity, IIS is probably the best-known, and perhaps the most important, mainly due to its major, evolutionarily conserved effects on lifespan in various model organisms, from invertebrates to mammals (reviewed in Tatar et al., 2003; Taguchi and White, 2008; Partridge et al., 2011). Here we provide a brief overview of recent studies suggesting that, among the many tissues affected by this endocrine pathway, IIS action in the central nervous system (CNS) is of special importance for modulating aging and longevity (reviewed in Broughton and Partridge, 2009).

IIS in the CNS has essentially two roles in aging. On the one hand, it can have local, neuroprotective effects in the CNS itself, for example, by promoting neuronal survival under neurodegenerative conditions (Chrysis et al., 2001; Schubert et al., 2004; Plum et al., 2005; Bateman and McNeill, 2006). On the other hand, in response to environmental cues, some of which could be food-derived, CNS-acting factors could regulate the production and release of ILPs, which in turn systemically act to influence whole-organismal aging. Here we focus on such CNS-mediated, lifespan-promoting effects of reduced IIS in worms, flies, and mice (reviewed in Tatar et al., 2003; Fielenbach and Antebi, 2008; Alcedo et al., 2010).

The worm *C. elegans* has 40 genes that are predicted to encode ILPs, many of which are expressed in sensory neurons and interneurons and can function as ligands for the insulin receptor ortholog DAF-2 (Pierce et al., 2001; Li et al., 2003; Cornils et al., 2011). Consistent with the notion that sensory neurons produce and release ILPs that regulate lifespan by influencing IIS in remote tissues, mutations that cause defects in ciliated sensory neurons or targeted ablation of gustatory and olfactory neurons extend lifespan in a manner that is fully or partially dependent on DAF-16/FOXO, a forkhead transcription factor downstream of IIS that becomes activated when IIS is reduced (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004; Shen et al., 2010). The central role of the CNS in the IIS modulation of longevity is further underscored by the fact that the extended lifespan due to mutations in *daf-2* and *age-1/PI-3K*, a central kinase downstream of DAF-2, can be largely or fully rescued, when wild-type *daf-2* or *age-1* is expressed in the neurons of the corresponding mutants (Wolkow et al., 2000; Iser et al., 2007). In contrast, neuronal activity of DAF-16/FOXO seems to be less important for lifespan extension in animals with impaired IIS (Libina et al., 2003; Iser et al., 2007; also, see below). However, expression of the microRNA *mir-71* in the nervous system mediates the lifespan extension in germline-ablated worms in a fashion that depends upon intestinal DAF-16 activity, revealing a complex signaling interaction between the CNS, the intestine, and the gonad in IIS-mediated lifespan regulation (Boulias and Horvitz, 2012).

Work in the fruit fly *Drosophila melanogaster* reveals remarkable parallels to these observations in worms. In the adult fly, three out of seven distinct ILPs are produced in specialized median neurosecretory cells (also called insulin-producing cells, IPCs) in the pars intercerebralis of the CNS (Rulifson et al., 2002; Grönke et al., 2010), and ablation of the IPCs significantly extends lifespan (Wessells et al., 2004; Broughton et al., 2005; Haselton et al., 2010), presumably due to reduced levels of ILP2, ILP3, and ILP5 (Broughton et al., 2008; Grönke et al., 2010). Consistent with these observations, several factors that regulate the production and/or release of ILPs affect IIS and lifespan. These factors include the metabotropic GABA receptors or uncoupling proteins (UCPs) expressed in the IPCs (Fridell et al., 2009; Humphrey et al., 2009; Enell et al., 2010) and short neuropeptide F (sNPF) expressed in the CNS (Lee et al., 2008, 2009). In addition, downregulation of p53 in the IPCs extends lifespan by reducing ILP levels and inhibiting PI-3K activity in peripheral tissues (Bauer et al., 2007). Similarly, the stress-responsive Jun kinase (JNK) in the IPCs promotes longevity by downregulating ILP2 through activation of FOXO (Wang et al., 2005). In contrast, and similar to the above-mentioned findings in *C. elegans*, activation of FOXO in the CNS, either pan-neuronally, in the neurolemma or in glial cells, is not sufficient to extend lifespan, whereas its downregulation in head fat body tissues promotes longevity (Hwangbo et al., 2004).

In mammals, the CNS also seems to play an important role in regulating the production and release of insulin-like hormones, although the bulk of insulin or IGF-1 is produced outside the brain. For example, mice with certain mutations affecting the so-called hypothalamic-pituitary-somatotropic growth hormone (GH-IIS) axis, known to regulate the release of insulin/insulin-like hormones, are long-lived, presumably due to downregulation of IIS (reviewed in Tatar et al., 2003; Holzenberger et al., 2004; Berryman et al., 2008). More direct evidence for a role of IIS in affecting mammalian lifespan via the nervous system comes from studies with transgenic or mutant mice with impaired IIS. Mice with a brain-specific deletion of the *insulin receptor substrate-2* (*Irs2*) locus are 14% longer lived than control mice, despite being hyperinsulinemic, obese, and insulin-resistant (Taguchi et al., 2007). Similarly, partial genetic inactivation of the *IGF-1 receptor* (*IGF-1R*) gene in the embryonic mouse brain inhibits GH and IGF-1 signaling after birth, which leads to growth retardation, small adult size, metabolic changes, and prolonged mean lifespan (Kappeler et al., 2008).

While much future work remains to be done for a detailed understanding of the underlying regulatory mechanisms, the available studies in worms, flies, and mice to date clearly show that neuroendocrine processes in the CNS are critically important for modulating the lifespan effects of IIS.

THE EFFECTS OF NEURONAL STRESS-SENSING PATHWAYS ON LIFESPAN AND AGING

The nervous system not only perceives a variety of environmental stressors but also integrates these information, which are then converted into appropriate physiological and behavioral adaptive responses. Below we discuss two such examples and their possible consequent effects on lifespan.

Exposure to acute stress, like heat, heavy metals, or toxins, can lead to proteotoxicity, as a result of protein misfolding within the animal (reviewed in Åkerfelt et al., 2010). To survive such insults, the animal activates its heat shock response, which is mediated by the heat shock transcription factor 1 (HSF-1; (Hsu et al., 2003; Morley and Morimoto, 2004; Cohen et al., 2006). For example, Kourtis et al. (2012) have shown that HSF-1 is required to protect the animal against cytotoxicity that is induced by thermal or other stresses through activation of the small heat shock protein HSP-16.1. This mechanism, which also protects against neurodegeneration, has been found to be conserved across species (Kourtis et al., 2012). Since thermosensory neurons and their associated neuronal circuitry can regulate the *C. elegans* heat shock responses non-autonomously (Prahlad et al., 2008; Prahlad and Morimoto, 2011), it is possible that the sensory regulation of the HSF-1/HSP-16.1 response is similarly conserved.

However, HSF-1 activity promotes longevity not only in the presence, but also in the absence, of acute stress (Hsu et al., 2003; Morley and Morimoto, 2004). Intriguingly, protein misfolding, whether it is mediated (Morley et al., 2002; van Ham et al., 2010) or not (David et al., 2010) by polyglutamine repeats, increases with age. This suggests that protein aggregation is inherent with age and is not restricted to a subset of proteins that have been implicated in diseases like neurodegeneration (David et al., 2010). Hence, given the role of HSF-1 in promoting protein disaggregation (Cohen et al., 2006), it is not surprising that HSF-1 activity in multiple tissues affects lifespan even in the absence of acute stress (Hsu et al., 2003; Morley and Morimoto, 2004).

Animals also employ different sensors for different types of gases that are required and/or affect important physiological processes. Some examples are the mechanisms through which animals perceive oxygen levels within their environment. For example, environmental oxygen is sensed by specific soluble guanylyl cyclases (sGCs) in specific sensory neurons in *C. elegans* and *Drosophila* (Cheung et al., 2005; Chang et al., 2006; Rogers et al., 2006; Vermehren-Schmaedick et al., 2010). These sGCs regulate the aerotactic behaviors of the animals: *C. elegans* prefers 7–11% ambient oxygen and is repelled by hypoxic (<5% O_2) and hyperoxic (>14% O_2) environments (Cheung et al., 2005; Chang et al., 2006; Rogers et al., 2006); whereas *Drosophila* larvae prefer a more restricted range of O_2 concentration (∼21%) (Vermehren-Schmaedick et al., 2010). Besides the sGC-expressing neurons, the avoidance of hyperoxia, i.e., in *C. elegans*, also depends on the activities of neurons that sense pain and neurons that integrate information about food availability and population density (Chang et al., 2006; Rogers et al., 2006). Thus, these different sensory neurons together allow the animals to generate rapid behavioral responses to ambient O_2, so that they can migrate to environments with the optimal O_2 levels necessary for their survival. At present, none of the sGCs are known to affect lifespan, unlike the receptor guanylyl cyclases for which a few have been reported to inhibit longevity (Murphy et al., 2003; Alcedo and Kenyon, 2004).

There are also many other cells that respond to O_2, albeit more slowly, through the hypoxia-inducible transcription factor HIF-1, which modifies the activities of the above O_2-sensing neurons and existing neural circuitries (Chang and Bargmann, 2008; Pocock and Hobert, 2010). Hypoxic activation of HIF-1 shifts the animal's preferences to lower oxygen concentrations and eliminates the dependence on some neurons, e.g., those that integrate information about food and population density, in promoting O_2-dependent responses (Chang and Bargmann, 2008). Interestingly, this HIF-1 effect requires that it acts coordinately in neuronal and gonadal cells (Chang and Bargmann, 2008), whose outputs are known to affect lifespan (Apfeld and Kenyon, 1999; Hsin and Kenyon, 1999; Wolkow et al., 2000; Broughton et al., 2005; Flatt et al., 2008).

Indeed, the HIF-1 pathway has been recently found to influence *C. elegans* lifespan and that these lifespan effects depend on environmental context (Chen et al., 2009; Mehta et al., 2009; Zhang et al., 2009; Lee et al., 2010; Leiser et al., 2011). Particularly, loss of *hif-1* can extend *C. elegans* lifespan at higher temperatures (25°C; Chen et al., 2009; Leiser et al., 2011) or shorten lifespan at lower temperatures (20°C; Mehta et al., 2009; Lee et al., 2010). Since O_2 perception can be modulated by food-derived information (Chang et al., 2006; Rogers et al., 2006; Chang and Bargmann, 2008; Pocock and Hobert, 2010), these temperature-dependent effects of HIF-1 may also reflect differences in the animal's bacterial food sources grown at 25 versus 20°C. Consistent with this idea, an interaction between HIF-1 and the food-dependent TOR pathway has been observed in affecting lifespan (Chen et al., 2009). Likewise, because O_2-sensing is also subject to population density (Chang et al., 2006; Rogers et al., 2006), the *hif-1* lifespan effects observed by Zhang et al. (2009) might reflect the higher density of animals used in their assays. Thus, HIF-1 function nicely illustrates how environmental context and its perception can modulate the effects of a signaling pathway on lifespan.

THE ROLE OF MITOCHONDRIA IN BRAIN AGING AND LONGEVITY

Mitochondria are among the most important cellular organelles that contribute to the aging process, mainly through respiratory chain dysfunction, changes in redox status, or by generating reactive oxygen species (ROS; Humphries et al., 2006; Mattson, 2006). It is therefore not surprising that the nervous system exhibits a highly active mitochondrial metabolism, especially because of the high energetic demands associated with processes such as ion homeostasis, neurotransmission, or the firing of action potentials.

Indeed in mammals, structural impairments in mitochondrial DNA and an age-dependent reduction in brain mitochondrial function are correlated with the age-dependent decrease in cognitive function and neuromuscular coordination (reviewed in Bishop et al., 2010; Escames et al., 2010; Chakrabarti et al., 2011; Yin et al., 2012). Similarly, mitochondrial dysfunction has been implicated in neurodegenerative diseases (reviewed in Eckert et al., 2011; Reddy and Reddy, 2011; Swerdlow, 2011; Troulinaki and Bano, 2012; Yin et al., 2012), although it remains unclear whether the functional changes seen in the healthily aging brain are distinct

from the pathological processes associated with neurodegenerative diseases. The empirical evidence at hand today thus suggests that neuronal mitochondria play an important role in maintaining organismal homeostasis and in influencing aging.

Several observations support the importance of proper neuronal mitochondrial function for lifespan and healthy aging. As mentioned previously, expression of human mitochondrial UCPs, which can uncouple mitochondrial respiration from ATP synthesis, in the neurons of adult flies extends lifespan (Fridell et al., 2005, 2009; Humphrey et al., 2009). This effect is likely to occur through reduced secretion of ILPs (Fridell et al., 2009; Humphrey et al., 2009), since the human UCP2 is known to regulate insulin secretion (Zhang et al., 2001). Interestingly, while moderate levels of neuronal UCP expression lengthen lifespan (Fridell et al., 2005; Humphrey et al., 2009), high levels have the opposite effect (Humphrey et al., 2009; **Figure 2**). This is reminiscent of previous studies that show a mild reduction of mitochondrial function can extend lifespan, whereas a strong functional impairment shortens lifespan (Rea et al., 2007). Therefore, hypothetically, mild mitochondrial dysfunction may cause (1) a change in levels of ROS production, e.g., a decrease that ensures preservation of DNA and protein structures or a mild increase that leads to compensatory mechanisms, or (2) a change in the types of ROS produced, which would then stimulate the expression of longevity-promoting genes. Together these data suggest that the increased lifespan associated with mild impairment of neuronal mitochondrial function (Dillin et al., 2002a; Rea and Johnson, 2003; Morrow et al., 2004; Fridell et al., 2005, 2009; Conti et al., 2006; Rea et al., 2007; Copeland et al., 2009; Humphrey et al., 2009; Lee et al., 2010; **Figure 2**) represents a compensatory mechanism that enables the maintenance of homeostasis.

A reduction of the function of the mitochondrial respiratory chain in the nervous system has also been shown to induce a mitochondria-specific unfolded protein response (UPR^{mt}) in intestinal cells and to extend lifespan (Durieux et al., 2011; **Figure 2**). Interestingly, a similar impairment of mitochondrial function in muscle cells can also induce UPR^{mt}, but this does not cause lifespan extension (Durieux et al., 2011), which could suggest that UPR^{mt} by itself is not sufficient for promoting longevity. On the other hand, the induction of UPR^{mt} has been found to be necessary for the long-life phenotype due to reduced mitochondrial respiration (Durieux et al., 2011). Thus, these findings suggest that mitochondrial dysfunction in neurons extends lifespan by producing an unknown signal that acts together with the UPR^{mt}-inducing signal. While the nature of this additional signal remains unknown, it is tempting to speculate about the possible

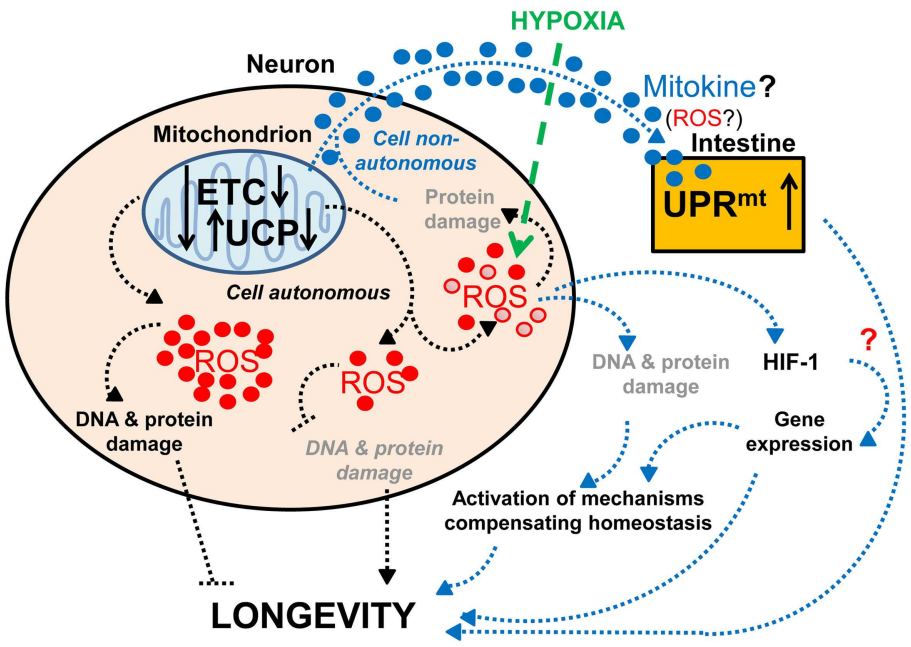

FIGURE 2 | Effects of neuronal mitochondrial UCP and the electron transport chain on longevity. Lifespan is modulated by altered mitochondrial function in neurons: a lower level of UCP and electron transport chain (ETC) expression lengthens lifespan, whereas a higher level of UCP and ETC expression has the opposite effect on lifespan (Fridell et al., 2005; Rea et al., 2007; Copeland et al., 2009; Humphrey et al., 2009; Durieux et al., 2011). The lifespan increase observed with mild mitochondrial dysfunction may hypothetically be due to (1) a decrease in ROS production and DNA and protein damage (denoted in gray and italics) or (2) a mild increase in ROS production and DNA and protein damage (denoted in gray), which can activate compensatory mechanisms. Alternatively, mitochondria-dependent lifespan increases might also be due to other compensatory mechanisms induced by a change in the types of ROS produced (red • versus red ○). Neuronal mitochondrial dysfunction can also induce a cell non-autonomous UPR^{mt} in intestinal cells and lead to lifespan extension, via a proposed mitokine, like ROS (Durieux et al., 2011). However, intestinal UPR^{mt} response is necessary but not sufficient to promote longevity (Durieux et al., 2011). Since HIF-1 activates survival genes in response to hypoxia and a mild inhibition of mitochondrial ETC, which involves an increase in ROS levels (Lee et al., 2010), it is tempting to speculate about the possible role of HIF-1 in this process (denoted by a red "?").

role of the HIF-1 pathway in this process. Indeed, HIF-1 not only modifies neuronal activities (Chang and Bargmann, 2008; Pocock and Hobert, 2010), but also promotes longevity in response to mild inhibition of mitochondrial respiration through increased ROS levels (Lee et al., 2010). Although the longevity-promoting effects of increased ROS (Lee et al., 2010) contradict a previous hypothesis that ROS would shorten lifespan through increased oxidative damage (Humphries et al., 2006; Mattson, 2006), this observation is consistent with the more recent hypothesis of mitohormesis, where higher ROS subsequently leads to increased stress resistance (Schulz et al., 2007). Alternatively, it is conceivable that certain types of ROS act as signaling molecules to activate survival pathways (Bishop et al., 2010; Lee et al., 2010; Durieux et al., 2011).

As the major source of ROS, the mitochondria are intimately involved in crosstalk among different pathways. Not surprisingly, mitochondrial activity is also regulated by major pathways that affect longevity, including the IIS, TOR, and JNK signaling pathways (reviewed in Troulinaki and Bano, 2012, as part of this Research Topic). Indeed, the ROS-mediated induction of JNK activity (Wang et al., 2005), which leads to translocation of JNK from the cytoplasm to the mitochondria, has been proposed to be of fundamental importance in the transduction of cytosolic signals to the mitochondria in the aging mammalian brain Schroeter et al., 2003; Eminel et al., 2004; Zhou et al., 2008, 2009).

Reactive oxygen species signaling itself also modulates mitochondrial homeostasis, which involves constant remodeling of this organelle, i.e., through mitochondrial fusion, fission, and autophagy (reviewed in Lemasters, 2005; Lee et al., 2012; Palikaras and Tavernarakis, 2012; Liesa and Shirihai, 2013). Such remodeling, which is tightly regulated, appears to be an adaptive response to the cell's energy expenditure and demands (reviewed in Liesa and Shirihai, 2013). However, mitochondrial fusion and fission have also been proposed to distribute damaged organelle components across the cell's mitochondrial network, whereas mitochondrial autophagy, known as mitophagy, removes highly damaged mitochondria (reviewed in Lemasters, 2005; Lee et al., 2012; Palikaras and Tavernarakis, 2012). Thus, an increase in ROS levels can shift the balance between fusion and fission to mitophagy (reviewed in Lemasters, 2005; Lee et al., 2012; Palikaras and Tavernarakis, 2012). Interestingly, mitophagy requires genes that have been implicated in the neurodegenerative Parkinson's disease, i.e., the serine/threonine kinase PINK1 and the E3 ubiquitin ligase Parkin, where PINK1 senses the damaged mitochondria and recruits Parkin to induce mitophagy (Narendra et al., 2008, 2010). Thus, dysregulation of mitochondrial remodeling, including mitophagy, through excess ROS, likely contributes to the onset and progression of several age-associated neurodegenerative diseases (reviewed in Batlevi and La Spada, 2011; Palikaras and Tavernarakis, 2012).

FEEDBACK, COMPENSATORY, AND FEED-FORWARD MECHANISMS IN LONGEVITY-MODULATING PATHWAYS

The studies discussed above point to the existence of major feedback mechanisms within the nervous system. Feedback loops are critically important in regulating physiology and metabolism, particularly with respect to homeostasis, and are often controlled by hormones (reviewed in Baker and Thummel, 2007; Leopold and Perrimon, 2007; Fielenbach and Antebi, 2008; Rajan and Perrimon, 2011; Hill et al., 2012). Notably, many such endocrine feedback mechanisms are thought to modulate aging and lifespan (Tatar et al., 2003; Murphy et al., 2007; Fielenbach and Antebi, 2008; Broughton and Partridge, 2009; Karpac and Jasper, 2009; Karpac et al., 2009; Tazearslan et al., 2009; Landis and Murphy, 2010), and the nervous system has been implicated in several of them (Hwangbo et al., 2004; Broughton et al., 2008; Flatt et al., 2008; Grönke et al., 2010; Alic et al., 2011; Boulias and Horvitz, 2012). Here, we focus on a few examples of feedback mechanisms that involve IIS and the nervous system.

A first example concerns the communication between adipose tissue and the brain via IIS. Hwangbo et al. (2004) found that in *D. melanogaster* overexpression of FOXO in the head fat body (equivalent of mammalian liver and adipose) extends lifespan and – remarkably – reduces the levels of ILP2 produced in the IPCs of the CNS, suggesting that lifespan extension is caused by FOXO-mediated negative feedback regulation of neural ILP production. This is consistent with the observation that ablation of IPCs extends lifespan (Wessells et al., 2004; Broughton et al., 2005), probably due to lowered levels of the ILP2, ILP3, and ILP5 ligands (Broughton et al., 2008; Grönke et al., 2010). Moreover, these findings are particularly interesting in view of the fact that a humoral factor produced by the fat body has been found to remotely control insulin secretion from the IPCs (Geminard et al., 2009; Tatar, 2009), yet whether this factor itself modulates lifespan remains unknown.

Another example is the existence of endocrine communication between the gonad and the brain. Similar to previous findings in *C. elegans* (Hsin and Kenyon, 1999; Arantes-Oliveira et al., 2002), Flatt et al. (2008) found that ablation of germline stem cells (GSCs) extends *Drosophila* lifespan. However, despite evidence of impaired IIS in peripheral tissues, fly GSC ablation also upregulates the production of ILP2, ILP3, and ILP5 in the brain IPCs (Flatt et al., 2008). Since neurally produced ILPs are known to bind to the insulin-like receptor (InR) on GSCs to regulate GSC proliferation in the gonad (LaFever and Drummond-Barbosa, 2005; Hsu et al., 2008), it is tempting to speculate that GSCs in the gonad exert negative feedback on ILP production in the brain. Although the nature of the signal that relays this communication remains unknown, a promising candidate may be IMP-L2, an insulin-binding protein. IMP-L2, which is expressed in the germline niche, among other tissues (Terry et al., 2006), limits the availability of free ILPs by sequestering them away from the InR, thereby antagonizing systemic IIS (Honegger et al., 2008). Interestingly, this protein is upregulated in germline-less, long-lived flies exhibiting ILP overproduction (Flatt et al., 2008). Moreover, similar to the phenotypes seen in germline-less flies, the Partridge group has shown that direct upregulation of IMP-L2 itself extends lifespan and increases ILP2, ILP3, and ILP5 levels, whereas genetic deletion of the *ilp2*, *ilp3*, and *ilp5* loci decreases IMP-L2 (Grönke et al., 2010; Alic et al., 2011). Together these observations support the hypothesis that IMP-L2 is part of a gonad-brain signaling circuit that regulates neural ILP levels (**Figure 3**).

While the detailed consequences for physiology, and in particular for aging and longevity, are in most cases still unknown,

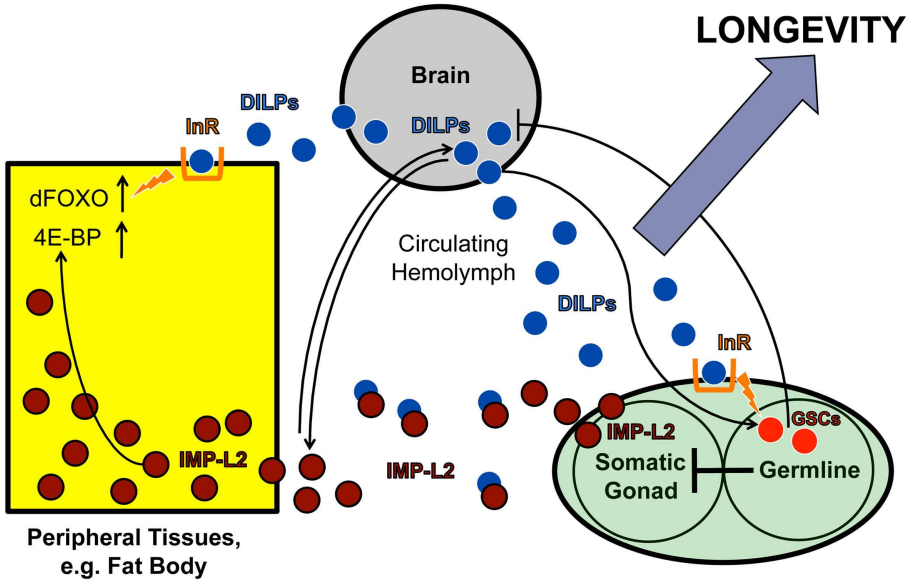

FIGURE 3 | IMP-L2-mediated endocrine feedback loop between brain and ovary. Model of the endocrine feedback loop between brain and ovary mediated by the ILP-binding protein IMP-L2, based on findings in LaFever and Drummond-Barbosa (2005), Flatt et al. (2008), and Alic et al. (2011). ILPs produced in the brain bind to the ovarian InR and stimulate GSC proliferation. GSC proliferation likely downregulates ILP production in the IPCs since GSC ablation causes ILP transcription to increase, suggesting the existence of a negative feedback loop between the brain and ovarian tissues. This putative feedback loop might be mediated, at least in part, by the ILP-binding protein, IMP-L2, which is known to inhibit aspects of insulin signaling. Remarkably, GSC ablation results in a strong upregulation of IMP-L2. Consistent with this observation, GSC ablation and IMP-L2 overexpression cause very similar phenotypes: in both cases, flies exhibit increased lifespan, upregulation of *ilp2, ilp3,* and *ilp5,* and increased expression of DAF-16/FOXO targets (such as 4E-BP), although other aspects of DAF-16/FOXO activity (e.g., subcellular localization and phosphorylation status) remain unaltered. Together this suggests that the long-lifespan phenotype of GSC-ablated flies is mediated by IMP-L2, which in turn modulates insulin signaling. See text for further details.

feedback mechanisms also occur at the level of transcriptional regulation. For example, some of the seven different *Drosophila* ILPs demonstrate feedback regulation of each other (**Figure 4**): IPC-*expressed ilp3* is required for the normal expression of *ilp2* and *ilp5* in the IPCs, whereas knockdown of *ilp2* leads to upregulation of *ilp3* and *ilp5* expression in the IPCs (Broughton et al., 2008; Grönke et al., 2010). Similar feedback loops also exist for other components of IIS: *Drosophila* FOXO (dFOXO), which is activated when InR signaling is downregulated, activates the transcription of InR (Puig et al., 2003; Puig and Tjian, 2005).

Intriguingly, besides feedback loops, the genetic study of aging is also beginning to uncover other types of regulatory motifs, e.g., compensatory and feed-forward regulatory mechanisms. For instance, upregulation of the fat body-specific *ilp6* seems to compensate for the loss of the brain-specific *ilp2*, *ilp3*, and *ilp5* (**Figure 4**; Grönke et al., 2010). Moreover, like in *Drosophila*, *C. elegans* exhibits feed-forward regulation between ILPs (Murphy et al., 2007). Increased DAF-16/FOXO activity in a specific tissue is shown to increase the activity of DAF-16/FOXO in other tissues through feed-forward regulation that requires the inhibition of the ILP *ins-7* expression in the *C. elegans* intestine (**Figure 4**; Murphy et al., 2003, 2007). Thus, these studies beautifully exemplify the complexity of existing feedback, compensatory, and feed-forward mechanisms that may be relevant for modulating aging and longevity.

TEMPORAL REQUIREMENTS OF LONGEVITY-INFLUENCING GENES

The experimental data available today suggest that adult-expressed neuronal genes may have important effects on aging and longevity (e.g., reviewed in Broughton and Partridge, 2009). However, to what extent genes that regulate the development of the nervous system and its circuitry also influence homeostasis and longevity remains presently unclear. Interestingly, several data support the notion that early-life environmental influences might have "carry-over" effects into adulthood and might thus impact lifespan (Gavrilov and Gavrilova, 2011; Saino et al., 2012). For example, putative biomarkers of aging that affect gene activity and chromosome structure at an early age have been shown to predict life expectancy (Baeriswyl et al., 2009; Pincus and Slack, 2010; Heidinger et al., 2012). Similarly, newly emerging data from *C. elegans* show that age-related behaviors are associated with distinct transcriptomes and that the statistical analysis of these aggregate gene expression profiles can predict age and health states (Golden et al., 2008). Thus, such data tempt one to speculate that genes involved in developmental canalization (or "robustness") might also have long-term effects on physiological homeostasis and somatic maintenance later in life. This canalization has been predicted to be a generic feature of developmental gene networks (Siegal and Bergman, 2002; Flatt, 2005).

A particularly plausible mechanism underlying these "carry-over" effects on adult lifespan is pleiotropic gene action, whereby

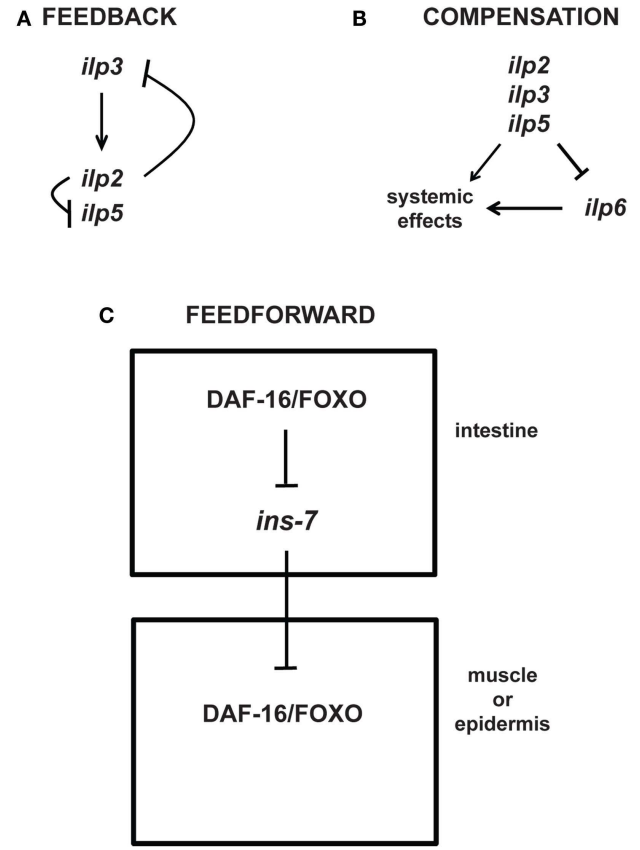

FIGURE 4 | Feedback, compensatory, and feed-forward mechanisms in the longevity-modulating insulin signaling pathway. **(A)** Neuronally produced *Drosophila* insulin-like peptides exhibit feedback regulation among each other (Broughton et al., 2008; Grönke et al., 2010). **(B)** The systemic activities of the *Drosophila* neuronal *ilp2*, *ilp3*, and *ilp5* can be compensated by the systemic activity of the *ilp6* produced from the head fat body (Grönke et al., 2010). **(C)** *C. elegans* ILP signaling between tissues (i.e., intestine to muscle or epidermis) involves feed-forward regulation via transcriptional inhibition of the ILP *ins-7* (Murphy et al., 2007).

one gene's effect during development differs from its effect in adulthood, i.e., the same gene variant might have pleiotropic roles in affecting development versus lifespan (e.g., see Dillin et al., 2002b). On the other hand, a gene could also have different lifespan effects that depend on its temporal activity, as has been observed with the overexpression of different *p53* constructs in *Drosophila*: this can lead to different lifespan effects in females and males depending on whether expression was driven during development versus adulthood (Waskar et al., 2009). Indeed, several key lifespan modulators, like the mitochondrial electron transport chain, microRNAs, HSF-1, and FOXO, can have "carry-over" effects on adult lifespan when manipulated (e.g., overexpressed or silenced) during early larval development and/or early adulthood (Dillin et al., 2002a,b; Giannakou et al., 2007; Rea et al., 2007; Durieux et al., 2011; Pincus et al., 2011; Volovik et al., 2012). Other examples are the age-dependent expression changes in neocortical genes, which not only play a role during development but also in altered neocortical function that is observed during age-related cognitive decline and brain dysfunction (reviewed in Huffman, 2012, as part of this Research Topic).

The distinct functional roles of pleiotropic genes during development versus aging are also demonstrated by the uncoupling of their gene functions between these two processes (Chen et al., 2007; Shen et al., 2009; Thyagarajan et al., 2010). In some cases, strong loss-of-function (or null) mutations have been found to affect embryonic development in *C. elegans*, whereas weaker mutant alleles of the same gene have been shown to affect adult lifespan (Kenyon et al., 1993; Kimura et al., 1997; Gems et al., 1998; Boehm and Slack, 2005), suggesting that essential developmental genes can have deleterious effects late in life. To neutralize these late-acting deleterious effects, Liu et al. (2012) have shown that miRNA signaling is involved in specifically silencing a set of these developmental genes in adulthood, thereby restricting the pleiotropic "carry-over" effects of such genes. This is exemplified by the miRNA *miR-34*-mediated silencing of the steroid pathway gene *E74A* in *Drosophila* adults to maintain brain integrity and viability (Liu et al., 2012).

Another obvious mechanism that might play a role in "carry-over" effects on lifespan and aging are epigenetic modifications. Experiments in rodents, for instance, have shown that experiences during sensitive periods of brain development influence DNA methylation patterns, which in turn could alter gene transcription throughout life and promote specific phenotypic outcomes (Roth and Sweatt, 2011). In a similar vein, the "heterochromatin loss model of aging" posits that heterochromatin domains that are set up early in embryogenesis are gradually lost with age, which results in aberrant and age-associated gene expression patterns (Villeponteau, 1997). In support of this hypothesis, genetic manipulation of HP1 levels and JAK/STAT signaling suggests that heterochromatin formation contributes to the prevention of premature aging (Larson et al., 2012). These are intriguing preliminary observations and it will be interesting to learn more about the role of epigenetic changes in aging and lifespan in future work.

EVOLUTIONARY IMPLICATIONS OF LONGEVITY-MODULATING NEURONAL MECHANISMS

Although the classical evolutionary theory of aging posits that aging should be affected by different mechanisms in different species (Williams, 1957; Reznick, 2005), recent studies suggest that several pathways have conserved effects on longevity (reviewed in Partridge and Gems, 2002; Tatar et al., 2003; Kenyon, 2005; Partridge et al., 2005; Smith et al., 2008; Flatt and Schmidt, 2009; Fontana et al., 2010; Nakagawa et al., 2012; Wuttke et al., 2012). Whereas lifespan can vary by several orders of magnitude across different species (Finch, 1990; Stearns, 1992; Nabholz et al., 2008; Li and de Magalhães, 2011), the molecular underpinnings of longevity have so far been mainly studied in a few short-lived and genetically tractable model systems, suggesting that our current understanding of the mechanisms of aging might be biased (Deweerdt, 2012). Moreover, while many of the conserved, pleiotropic signaling pathways implicated in aging have neuronal roles, not all of these functions might directly impinge on aging. Therefore, the extent to which the neuronal mechanisms of longevity are evolutionarily conserved remains largely unclear.

A recent study directly comparing gene expression profiles during aging in mouse, rhesus macaque and human brains indicates that only a small subset of the age-dependent expression changes might be conserved (Loerch et al., 2008). These few genes include the neuroprotective gene apolipoprotein D (APOD), which is robustly upregulated with age in all three species and whose two *Drosophila* homologs are known to affect lifespan (Ruiz et al., 2011). Another example is the calcium/calmodulin-dependent protein kinase IV (CAMK4), which has been shown to regulate synaptic plasticity (Ho et al., 2000) and is downregulated with age in all three species (Loerch et al., 2008). In contrast, most genes did not show a consistent age-dependent pattern across species, leading the authors to conclude that humans and rhesus macaques have greatly diverged from mice as demonstrated by a dramatic increase in age-dependent repression of human and macaque neuronal genes (Loerch et al., 2008). While these results indicate that the neuronal mechanisms of aging and longevity might not be highly conserved among different taxa, a study by Fonseca et al. (2005) provides a remarkable counter-example. Across a range of terrestrial, freshwater, marine, tropical, and temperate arthropods, whose lifespans vary by three orders of magnitude, the neuronal deposition of lipofuscin, a lipid-protein aggregate, is highly correlated with lifespan. This suggests that age-dependent damage accumulation in the brain might be the primary driver of senescence (Fonseca et al., 2005).

Similarly, at the microevolutionary or intraspecies level, it is still unclear whether natural variation in lifespan is based on allelic variation within the same genes and pathways that have already been previously found to affect longevity in laboratory studies of mutant or transgenic model organisms (Flatt, 2004; Paaby and Schmidt, 2008; Flatt and Schmidt, 2009). On the one hand, some studies have failed to confirm the lifespan effects of natural variants of candidate longevity genes (Geiger-Thornsberry and Mackay, 2004). On the other hand, there is increasing evidence that genetic variation in candidate longevity genes might indeed contribute to variation in lifespan, as well as life history traits, in natural populations (Schmidt et al., 2000; Paaby and Schmidt, 2008; Suh et al., 2008; Paaby et al., 2010; Rose et al., 2010, 2011; Pijpe et al., 2011; Luisi et al., 2012).

A particularly striking example of such a variant is the gene FOXO3A, a human ortholog of *Drosophila* FOXO and *C. elegans* DAF-16. Several independent studies of natural polymorphisms in FOXO3A in Japanese, German, French, Italian, and Han Chinese populations have found that specific variants in this gene are associated with exceptional longevity among human centenarians (Willcox et al., 2008; Anselmi et al., 2009; Flachsbart et al., 2009; Li et al., 2009; Pawlikowska et al., 2009; Soerensen et al., 2010; Zeng et al., 2010). Although one cannot rule out a certain level of ascertainment bias, these results suggest that FOXO not only plays a functional role in regulating lifespan in laboratory model organisms, but that naturally occurring alleles can also have measurable effects on lifespan. Similar associations between natural polymorphisms and human longevity have been identified for IGF-1R (Suh et al., 2008). Likewise, evidence from *Drosophila* indicates that natural alleles in the InR locus do affect life history traits that are closely linked to longevity (Paaby et al., 2010).

Finally, a similar pattern appears to be emerging with regard to natural variants of genes involved in the neuronal regulation of lifespan: correlations have been found between longevity and genes that function in (1) neuronal development (Rybina and Pasyukova, 2010; Walter et al., 2011), (2) in neural circuitry (De Benedictis et al., 1998; De Luca et al., 2001, 2003; Carbone et al., 2006), or (3) in the uncoupling process in neuronal tissues (Rose et al., 2011).

CONCLUSIONS AND PERSPECTIVES

Here we have provided a review of the recent knowledge about the neuronal inputs and outputs that affect aging and longevity, mainly by focusing on the latest work in genetically tractable model organisms, such as flies, worms, and mice. Even though many details remain to be discovered, it is amply clear today that aging and longevity are profoundly influenced by neuronal activities. Indeed, given that the nervous system (especially, the neuroendocrine system) is intimately involved in regulating an animal's physiology, e.g., its homeostasis and survival, in response to environmental changes, such a role for this organ system in the aging process is not surprising, both from a physiological and evolutionary perspective. Yet numerous difficult puzzles remain to be solved in future work. For example, with regard to IIS, we know that downregulation of this pathway can have positive effects on lifespan; however, at the same time such downregulation can severely impair neuronal survival and CNS function in old age (also, see discussion in Broughton and Partridge, 2009). Perhaps these distinct effects of IIS on animal physiology could depend on the tissue- or temporal-specific activities of the pathway. Hence, these pleiotropic effects of IIS highlight our need for a much better understanding of how, why, and when "brain aging" and "organismal aging" are exactly coupled or decoupled. More generally, understanding the developmental "carry-over" effects on adult lifespan will require us to gain further insight into the tissue-, age-, and stage-specificity of the neuronal effects on aging and longevity. Similarly, our current knowledge of the intricate interactions involved in the neuronal regulation of aging and longevity is still extremely rudimentary. For instance, not much is known about the interactions between different "longevity" pathways in the brain, or how different tissues (such as the gonad or adipose tissue) cross-talk with the CNS in the modulation of whole-organism lifespan. Thus, despite the fact that recent years have witnessed a lot of progress in this area, there are clearly very exciting times and novel discoveries ahead in the elucidation of the neuronal aspects of aging and longevity.

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Regulation of lifespan by chemosensory and thermosensory systems: findings in invertebrates and their implications in mammalian aging

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Many environmental factors that dynamically change in nature influence various aspects of animal physiology. Animals are equipped with sensory neuronal systems that help them properly sense and respond to environmental factors. Several studies have shown that chemosensory and thermosensory neurons affect the lifespan of invertebrate model animals, including *Caenorhabditis elegans* and *Drosophila melanogaster*. Although the mechanisms by which these sensory systems modulate lifespan are incompletely understood, hormonal signaling pathways have been implicated in sensory system-mediated lifespan regulation. In this review, we describe findings regarding how sensory nervous system components elicit physiological changes to regulate lifespan in invertebrate models, and discuss their implications in mammalian aging.

Keywords: aging, sensory neuron, chemosensation, thermosensation, *Caenorhabditis elegans*, *Drosophila melanogaster*, endocrine signaling, hibernation

INTRODUCTION

Organisms are subject to continuously changing environments and numerous stimuli that originate from various sources. These stimuli are perceived and processed by different mechanisms to allow the organism to respond appropriately. In most animals, these ambient cues are perceived by a system that consists of many sensory neurons and protects animals from harmful stimuli, such as burns and noxious poisons, by allowing them to avoid these damaging conditions. Therefore, the sensory system is of vital importance for many organisms to thrive in their niches.

The perception of external stimuli and subsequent signal transmission for proper responses are the primary function of sensory neurons. These neurons were assigned another important function after remarkable discoveries in invertebrate model animals that lifespan is actively regulated by sensory neuronal systems. Inhibiting chemosensation modulates lifespan in *Caenorhabditis elegans* and *Drosophila melanogaster*, and defects in thermosensation shorten *C. elegans* lifespan at high temperature (**Figure 1**). Here, we describe how sensory neuronal systems regulate aging processes in these invertebrate model animals and speculate the implications of these findings with regard to mammalian aging.

THE ROLE OF THE CHEMOSENSORY SYSTEM IN AGING

CHEMOSENSORY SYSTEMS OF *C. elegans* AND *D. melanogaster*

Chemosensation is initiated by the detection of chemical cues by receptors in chemosensory neurons that form neural circuits with other neurons (Bargmann, 2006). Chemosensory neural circuits include motor neurons; therefore, the organism's sensory system can elicit behavioral responses to environmental stimuli. Model invertebrates with relatively simple nervous systems have been utilized to genetically dissect functions and mechanisms of chemosensory systems. One of them is the nematode *C. elegans*, a simple multi-cellular animal that lives in decomposing organic material. Approximately 60 of the 302 *C. elegans* neurons are ciliated sensory neurons, including chemosensory neurons, some of which are in the amphid organ in the head (Bargmann, 2006) (**Figure 2**). Chemosensory signals are transduced by many effector proteins in the neurons, including G protein-coupled receptors (GPCRs) that are activated by binding with their ligands (**Figure 2**). GPCRs activate G protein signaling to influence the level of cyclic GMP (cGMP), which functions as a second messenger for the chemosensory signal transduction. cGMP binds to and opens cyclic nucleotide-gated channels to regulate cation flux that is required for chemosensation (Bargmann, 2006).

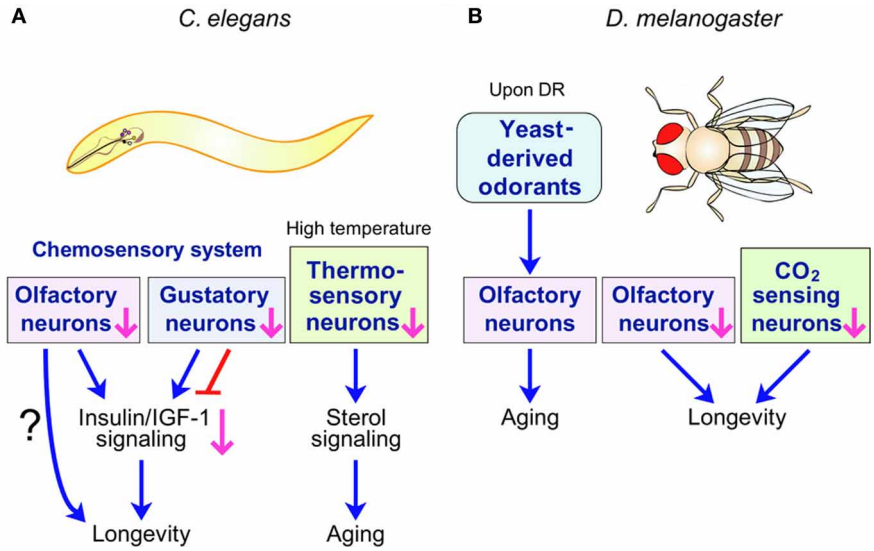

FIGURE 1 | *C. elegans* and *D. melanogaster* sensory systems influence lifespan. (A) *C. elegans* chemosensory and thermosensory systems regulate lifespan through hormonal signaling. Specific chemosensory (olfactory or gustatory) neurons promote or limit longevity. Insulin/IGF-1 signaling can mediate this longevity response downstream of chemosensory neurons. Perturbation of thermosensory system accelerates aging at high temperatures by influencing the sterol hormonal signaling pathway. **(B)** Chemosensory systems regulate *D. melanogaster* lifespan. Olfaction of nutrient-derived odorants promotes the aging of long-lived *D. melanogaster* upon dietary restriction (DR). Perturbation of the *D. melanogaster* olfactory system and inhibition of the CO_2-sensing system both prolong lifespan. However, the signaling pathways regulated by *D. melanogaster* chemosensory systems to influence lifespan are unknown.

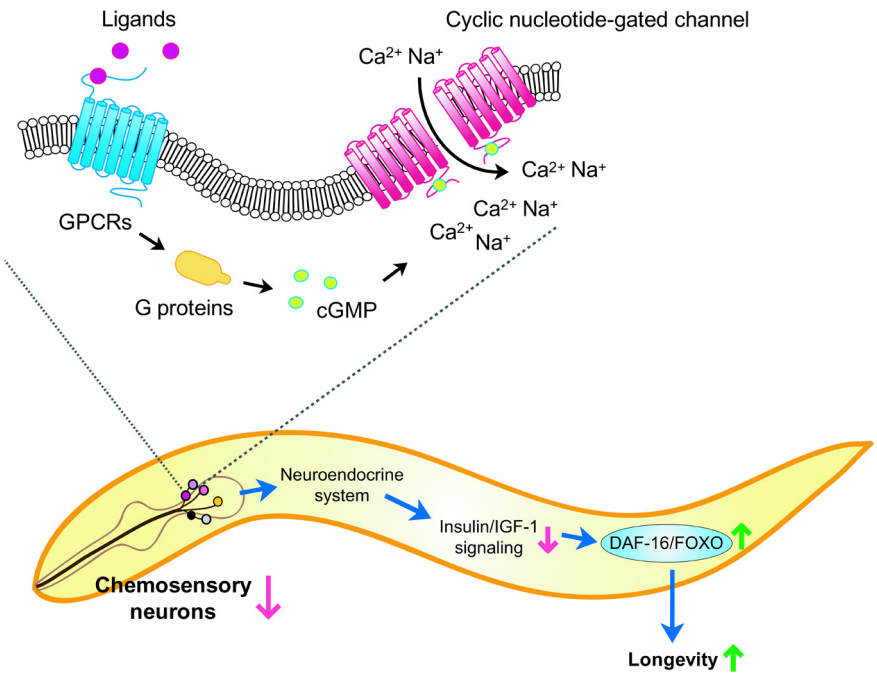

FIGURE 2 | Model of lifespan control by chemosensation and insulin/IGF-1 signaling in *C. elegans*. A subset of ciliated neurons in the head region (for example, in the amphid) perceive chemosensory cues and trigger signal transduction cascades. Upon binding to ligands, chemosensory G protein-coupled receptors (GPCRs) activate G proteins, which lead to cGMP production. This in turn opens cyclic nucleotide-gated channels and increases Ca^{2+} and Na^{+} influx. Inhibiting chemosensory neuronal structure or function in specific neurons may alter neuroendocrine signaling and reduces insulin/IGF-1 signaling, which activates the DAF-16/FOXO transcription factor and promotes longevity.

Another invaluable invertebrate model organism that has been used to understand the chemosensory neural system is the fruit fly *D. melanogaster*. The fly genome expresses 62 odorant receptors in approximately 2500 olfactory receptor neurons (ORNs), which are covered by sensilla. These structures cover the third segment of antenna and maxillary palp, which are specialized olfactory appendages in the head that sense environmental odors (Vosshall and Stocker, 2007). Olfactory neurons innervate glomeruli in the antennal lobe and target to local interneurons that connect with cholinergic projection neurons to form neural circuits for signal transmission. In contrast to the olfactory neurons, gustatory neurons are gathered in taste bristles on the body of *D. melanogaster*, including the proboscis, legs, and wings. This wide distribution of gustatory organs may allow *D. melanogaster* to seek preferred foods and find appropriate egg-laying locations (Vosshall and Stocker, 2007).

CHEMOSENSORY PERTURBATION INFLUENCES THE LIFESPAN OF *C. elegans* AND *D. melanogaster*

Studies have shown that inhibiting chemosensory systems leads to lifespan change in *C. elegans* and *D. melanogaster*. The first evidence regarding the effect of sensory systems on aging was revealed by Apfeld and Kenyon, who found that mutations that impair sensory neural structure and/or their function extend *C. elegans* lifespan (Apfeld and Kenyon, 1999). Various mutant worms with malformed sensory cilia, including *che-2*, *daf-10*, *daf-19*, and *osm-5* mutants, live up to 50% longer than wild-type worms. Lifespan is also increased by direct laser ablation of amphid sheath cells, which support the structure of amphid neurons (Apfeld and Kenyon, 1999). Alcedo and Kenyon used this technique to directly determine the roles of chemosensory neurons in *C. elegans* lifespan regulation (Alcedo and Kenyon, 2004). Laser ablation of either gustatory ASI and ASG neurons or olfactory AWA and AWC neurons prolongs lifespan. Gustatory and olfactory neurons appear to influence lifespan independently of each other because ablation of olfactory AWA and AWC neurons further lengthens the lifespan of gustatory ASI-ablated *C. elegans*. They also demonstrated that some normal chemosensory neurons limit *C. elegans* lifespan, while others appear to promote long lifespan. Laser ablation of either ASJ or ASK gustatory neurons does not influence lifespan in control worms and decreases the longevity resulting from ASI ablation, suggesting that ASJ and ASK neurons contribute to longevity in ASI-ablated animals (Alcedo and Kenyon, 2004). Overall, these pioneering studies established the role of *C. elegans* chemosensory neurons in the lifespan regulation at the organismal level.

Inhibiting components of chemosensory signal transduction extends lifespan. Mutations in *str-2*, which encodes a putative chemosensory GPCR, promote long lifespan (Alcedo and Kenyon, 2004). In addition, mutations in *kin-29*, a serine/threonine kinase that regulates the expression of a set of chemoreceptor genes, confer lifespan extension similar to that of other chemosensory mutants (Lanjuin and Sengupta, 2002). Multiple G proteins located downstream of chemosensory receptors in GPCR signaling cascades also influence longevity (Alcedo and Kenyon, 2004; Lans and Jansen, 2007; Hahm et al., 2009). Either loss-of-function mutations or overexpression of subsets of G proteins increases lifespan. Mutants in genes encoding Gα subunits including *gpa-1*, *gpa-5*, *gpa-9*, and *odr-3* live longer than wild type (Alcedo and Kenyon, 2004; Lans and Jansen, 2007; Hahm et al., 2009). Seemingly paradoxically, overexpression of *gpa-2*, *gpa-9*, or *gpa-11*, and constitutively active *gpa-3* mutations also lengthen lifespan (Lans and Jansen, 2007; Hahm et al., 2009). It seems likely that both decrease and increase in the activity of G proteins cause defects in chemosensation, which lead to life extension. Mutations in the cyclic nucleotide-gated channel subunit encoded by *tax-4* also extend lifespan at low temperatures (Apfeld and Kenyon, 1999; Lee and Kenyon, 2009). Together, these studies indicate that chemosensory signal transduction cascades regulate longevity in *C. elegans*.

Lifespan extension is observed in *C. elegans* mutants that are defective in neurosecretory processes. Mutations in *unc-31*, the *C. elegans* homolog of Ca^{2+}-dependent activator protein for secretion (CAPS), or *unc-64*, the syntaxin required for synaptic transmission and neurosecretion, prolong lifespan (Ailion et al., 1999). In addition, mutations in *ocr-2*, which encodes a transient receptor potential vanilloid (TRPV) channel expressed in neurons, including chemosensory neurons, increase adult lifespan through the same genetic pathway as *unc-31* (Lee and Ashrafi, 2008). Interestingly, mutants that have defects in neurosecretion or sensory cilia display increased resistance to chronic starvation, as well as long lifespan. Increased survival of *unc-31* mutants during starvation is partially rescued by *unc-31* expression in ADL and ASH amphid chemosensory neurons, suggesting that perturbing neurosecretion in these chemosensory neurons underlies increased survival (Lee and Ashrafi, 2008). Together, these studies indicate that lifespan regulation by the chemosensory system is linked to neurosecretory system control.

Pharmacological perturbation of chemosensory neurons also increases lifespan. It has been shown that anticonvulsants used for treating seizure disorders in humans promote the longevity of invertebrate model animals; ethosuximide and trimethadione confer long lifespan in *C. elegans* (Evason et al., 2005; Collins et al., 2008) and lamotrigine extends lifespan in *D. melanogaster* (Avanesian et al., 2010). The Kornfeld group showed that treatment with ethosuximide lengthens *C. elegans* lifespan and prevents age-related physiological decline, such as decrease in feeding rates (Evason et al., 2005). Subsequently, Collins et al. screened for mutants resistant to high-dose ethosuximide-induced larval lethality and isolated those with defects in sensory cilium structure (Collins et al., 2008). They also showed that ethosuximide treatment abrogates chemotaxis in wild-type *C. elegans*, suggesting that the lifespan-increasing effects of ethosuximide act through inhibiting chemosensory function. The Buck group then performed a high-throughput chemical screen to identify anti-aging chemicals and discovered that mianserin and methiothepin prolong the lifespan of *C. elegans* (Petrascheck et al., 2007). These compounds are known to influence serotonin-mediated neural signaling and are used to treat depression in humans. Mianserin and methiothepin require serotonin receptor *ser-4* and a probable octopamine receptor *ser-3* to promote the longevity in worms (Petrascheck et al., 2007). Similar to the effects on the human serotonergic system, pre-incubation of mianserin or

methiothepin antagonizes the actions of *C. elegans* SER-4 and SER-3 receptors in cultured mammalian cells, suggesting that these chemicals disrupt serotonin and octopamine neurotransmitter signaling to elicit lifespan-lengthening effects (Petrascheck et al., 2007).

The observations that chemosensory system impairment promotes longevity raise an important question of whether environmental chemical cues directly influence aging through sensory neurons. Worms fed with sub-strains of *E. coli* with different lipopolysaccharide (LPS) structures on the external cellular membrane have different lifespans (Soukas et al., 2009; Maier et al., 2010). Maier et al. raised the possibility that chemosensory neurons modulate the effects of consumed *E. coli* on lifespan probably through sensing the LPS structure (Maier et al., 2010). They further found that *nmur-1*, which encodes a homolog of neuromedin U receptor, mediates food type-dependent lifespan changes in *C. elegans* (Maier et al., 2010).

The role of the chemosensory system in lifespan regulation appears to be conserved in fruit flies. Pletcher's group demonstrated that the lifespan of flies is extended by mutations in the *Or83b* gene (Libert et al., 2007), which encodes an atypical co-receptor required for recruiting odorant receptors to ciliated dendrites of olfactory neurons (Larsson et al., 2004). In addition, nutrient-derived odorants directly accelerate aging in flies under dietary restriction, suggesting that dietary restriction increases lifespan at least partially through reducing the level of transmission of olfactory information to target cells and/or tissues (Libert et al., 2007). In addition to odorant sensation, a recent report suggests that carbon dioxide (CO_2) sensation also regulates the aging of *D. melanogaster* (Poon et al., 2010). Mutations in the *Gr63a* gene, which encodes a CO_2 olfactory receptor, or genetic ablation of CO_2-sensing ab1C neurons increases the lifespan of *D. melanogaster* (Poon et al., 2010). Together, these findings strongly support the hypothesis that chemosensory systems use environmental cues to regulate lifespan in *D. melanogaster* and *C. elegans*.

SIGNALING PATHWAYS THAT MEDIATE THE LIFESPAN REGULATION BY CHEMOSENSORY SYSTEMS

How does chemosensation influence lifespan? Although detailed mechanisms remain to be elucidated, recent studies suggest that chemosensory systems appear to affect lifespan at least partially by altering insulin/IGF-1 signaling in *C. elegans* (**Figure 2**). Activation of the insulin/IGF-1 receptor leads to the phosphorylation of downstream kinase cascades, including AGE-1/phosphoinositide-3 kinase (PI3K), phosphoinositide-dependent kinase 1 (PDK-1), AKT/protein kinase B (PKB), and serum- and glucocorticoid-induced kinase 1 (SGK-1) (Kenyon, 2010). This results in the subsequent phosphorylation of DAF-16/FOXO transcription factor, prevents its nuclear localization, and therefore inhibits its activation. When insulin/IGF-1 signaling is reduced, activated DAF-16/FOXO translocates to the nucleus and regulates the expression of downstream targets, including genes required for stress resistance, protein homeostasis, and lifespan extension (Kenyon, 2010).

Several lines of evidence support the idea that the *C. elegans* chemosensory system can influence lifespan through the insulin/IGF-1 signaling pathway. Mutations causing defects in sensory cilia, direct ablation of ASI chemosensory neurons, or defects in neurosecretion by *unc-64* or *unc-31* mutations do not further lengthen the lifespan of long-lived *daf-2*/insulin/IGF-1 receptor mutants, suggesting that chemosensory inhibition and reduced insulin/IGF-1 signaling act through the same genetic pathway (Ailion et al., 1999; Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004). In addition, inhibiting chemosensation in *C. elegans* appears to promote longevity by activating DAF-16/FOXO. Chemosensory defects caused by mutations in *daf-10*, *odr-3*, or *gpa-1* promote nuclear localization of DAF-16/FOXO (Lin et al., 2001; Lans and Jansen, 2007). Consistently, the expression level of *sod-3*, one of the direct targets of DAF-16/FOXO, is increased by chemosensory *daf-11* loss-of-function or *gpa-3* gain-of-function mutations (Hahm et al., 2009). Furthermore, longevity conferred by inhibiting chemosensory function or neurosecretion is largely dependent on DAF-16/FOXO (Ailion et al., 1999; Apfeld and Kenyon, 1999; Lanjuin and Sengupta, 2002; Alcedo and Kenyon, 2004; Lans and Jansen, 2007; Lee and Ashrafi, 2008). Together, these studies suggest that chemosensory perception influences the insulin/IGF-1 signaling pathway to regulate lifespan.

How do chemosensory defects in *C. elegans* modulate the insulin/IGF-1 signaling? A study by Ashrafi's laboratory showed that chemosensory neurons regulate insulin-like peptide (ILP) secretion (Lee and Ashrafi, 2008). They generated transgenic worms expressing red fluorescent protein (mCherry)-tagged *daf-28*, which encodes an ILP, under the control of an ADL sensory neuron-specific promoter. They found that *unc-31* or *ocr-2* mutations disrupt mCherry-DAF-28 release from ADL sensory neurons (Lee and Ashrafi, 2008), raising the possibility that loss of chemosensory function reduces the levels of ILPs, which may lead to longevity.

In addition to insulin/IGF-1 signaling, there seem to be other signaling pathways that influence lifespan downstream of chemosensory transduction, because the longevity of several chemosensory-defective animals is partially independent of DAF-16/FOXO. For example, mutations in *daf-10*, *osm-3*, *osm-5*, or *gpa-1* slightly but significantly increase *daf-16*/FOXO mutant lifespan (Apfeld and Kenyon, 1999; Lans and Jansen, 2007). Moreover, mutations in G protein genes such as *odr-3* and *gpa-11* further enhance the long lifespan of *daf-2*/insulin/IGF-1 mutants (Lans and Jansen, 2007). The identification of these insulin/IGF-1-independent components will be a crucial next step towards better understanding of how chemosensory systems influence lifespan.

CHEMOSENSATION AND MAMMALIAN AGING

Although there is no evidence for a chemosensory neural system influencing the lifespan of mammals to date, it has been established that the mammalian nervous system controls endocrine signaling and influences lifespan. The regulation of aging by the insulin/IGF-1 signaling pathway is evolutionarily well conserved from yeast to mammals (Kenyon, 2010). Interestingly, mammalian insulin/IGF-1 signaling is modulated by a neural system as in *C. elegans*. Insulin signaling in the brain influences not only metabolism and reproduction but also lifespan in mice (Bruning, 2000; Plum et al., 2005; Taguchi et al., 2007; Kappeler et al., 2008;

Scherer et al., 2011). Insulin levels in humans are increased by the sensation of foods (Sjostrom et al., 1980), suggesting that chemosensation itself can influence insulin signaling in mammals (**Figure 3**). It will be interesting to determine whether insulin signaling regulated by the mammalian chemosensory system influences aging processes.

THE ROLE OF THERMOSENSORY SYSTEM IN AGING

THERMOSENSORY NEURAL SYSTEM

Sensing and responding to ambient temperature are vital processes for organisms to maintain their body functions. Environmental temperature fluctuations are tolerated by the homeostatic adjustment of body temperature by neuronal circuitry in warm-blooded animals (endotherms). In the case of cold-blooded animals (ectotherms), body temperature cannot be adjusted and is dependent on ambient temperature; therefore, locomotion towards the optimum temperature is the most suitable strategy for survival (Garrity et al., 2010). Thus, environmental temperature can have drastic effects on ectotherm physiology, especially in small organisms. In principle, thermoregulatory behaviors are triggered by thermosensory receptors located on the surface or inside of the body (Garrity et al., 2010).

Along with stimulants such as mechanical forces and chemical cues, temperature is perceived by sensory neurons in the peripheral nervous system, which originate from dorsal root ganglia (DRG) and trigeminal ganglia (TG) in mammals. These neurons have two nerve endings with a branched axonal structure. One nerve ending innervates peripheral tissues and contains a variety of receptors to perceive stimuli, including temperature, and the other connects to the spinal cord to transmit these stimuli (Dhaka et al., 2006). Temperature-sensitive neurons localized in the preoptic area (POA) and anterior hypothalamus are the major regulators of mammalian body temperature homeostasis (Morrison and Nakamura, 2011). The POA is thought to limit core body temperature fluctuations regardless of extreme environmental temperature changes (Clapham, 2011). Slight temperature changes perceived by the POA or lateral hypothalamus are almost immediately balanced by body temperature regulation (Clapham, 2011). At the molecular level, the transient receptor potential (TRP) family of cation channels is thought to be the major mammalian thermosensor (Morrison and Nakamura, 2011). TRPM8 and TRPA1 are cold-sensitive channels, whereas four TRPV channels (TRPV1-4) are activated by warm temperatures (Morrison et al., 2008; Morrison and Nakamura, 2011).

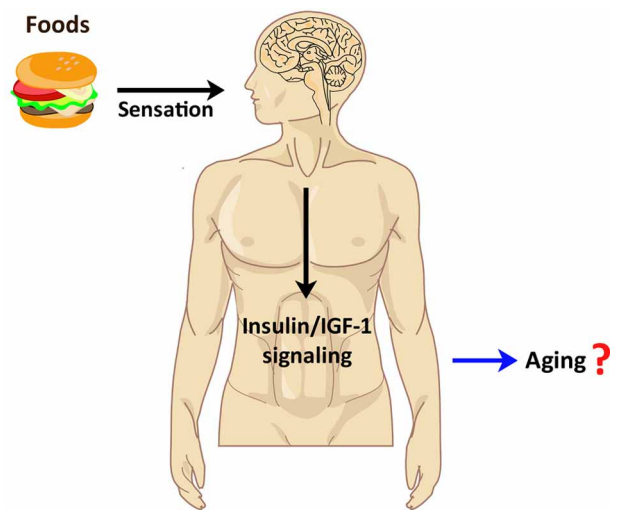

FIGURE 3 | Implication of chemosensory regulation in human aging. Food perception by sensory systems in humans appears to increase human insulin/IGF-1 signaling. Because the insulin/IGF-1 signaling pathway is known to regulate mammalian lifespan, perhaps human chemosensation also influences aging via the insulin/IGF-1 pathway.

C. elegans and *D. melanogaster* have served as useful model animals to characterize thermosensory perception and thermoregulatory pathways. The *C. elegans* thermosensory system allows movement towards temperatures previously associated with the existence of food and movement away from those temperatures associated with food deprivation. It has been shown that AFD, AWC, and ASI neurons respond to temperature changes, and play key roles in these temperature-associated thermotactic behaviors (Mori and Ohshima, 1995; Biron et al., 2008; Kuhara et al., 2008; Beverly et al., 2011). Among them AFD neurons are the major thermosensory neurons, which form neural circuits with AIY, AIZ, and RIA interneurons (Mori and Ohshima, 1995; Mori et al., 2007). Temperature shifts are perceived by AFD thermosensory neurons upon exceeding the threshold temperature, which is likely stored as a memory acquired during previous growth conditions. At the molecular level, AFD neurons are activated by cGMP-dependent cation channels composed of TAX-2 and TAX-4 subunits, the functions of which rely on cGMP production by AFD thermosensory neuron-specific guanylyl cyclases GCY-8, GCY-18, and GCY-23 (Inada et al., 2006). Increased ambient temperature appears to result in the activation of these GCYs, which promotes the elevation of intracellular cGMP levels (Wasserman et al., 2011). This leads to TAX-2/TAX-4 cation channel opening, which causes cation flux into AFD neurons (Satterlee et al., 2004).

Unlike worms, the temperature preference of fruit flies is mostly constant within a small range, rather than experience dependent. *Drosophila* first instar larvae exhibit negative thermotaxis above 30°C and positive thermotaxis below 20°C towards their preferred temperatures, which range between 24–28°C (Garrity et al., 2010). Although it is known that the temperature preference of adult flies is similar to that of larvae, their thermotactic behaviors and the involvement of thermosensation are not well understood due to the free movement of adult flies (Garrity et al., 2010). The sensation of cool or warm temperatures requires three types of thermosensory TRP channels: dTRPA1, Pyrexia (dTRPA2), and Painless (Garrity et al., 2010). dTRPA1 is activated at warm temperatures with a threshold of 27°C in a heterologous system (Viswanath et al., 2003) and is important for larval thermotaxis (Rosenzweig, 2005). Pyrexia is activated by temperatures over 40°C *in vitro*, and *pyrexia* mutants display defects in thermotolerance (Lee et al., 2005). Painless is activated at temperatures higher than 42°C *in vitro* (Sokabe et al., 2008) and helps flies avoid noxious heat (McKemy, 2007).

REGULATION OF LONGEVITY IN *C. elegans* BY THE THERMOSENSORY SYSTEM

Despite relatively well-defined thermosensory system structures and functions for the perception of ambient temperature, the effects of these systems on the regulation of animal physiology, including aging, remain poorly understood. Lee and Kenyon demonstrated that AFD thermosensory neurons regulate worm lifespan through a sterol endocrine signaling pathway at high temperatures (Lee and Kenyon, 2009) (**Figure 4**). Impairment of AFD neurons by either laser ablation or mutations causes a significant lifespan decrease at high temperature (25°C) but have no effect at low temperatures (20 and 15°C). Perturbation of AFD neurons reduces the expression of *daf-9*, which encodes a cytochrome P450 (CYP) that is responsible for producing the sterol hormones known as dafachronic acids. This subsequently influences the activity of DAF-12, a nuclear hormone receptor (NHR) whose activity is regulated by dafachronic acids. They proposed a model in which AFD neurons stimulate *daf-9*/*CYP* expression, which in turn regulates DAF-12/NHR activity and leads to altered lifespan at high temperature. Another noteworthy study suggested that AFD thermosensory neurons regulate the transient heat shock response of whole worms upon perception of acute high temperature (Prahlad et al., 2008). When mutant worms that have defects in AFD thermosensory neurons are exposed to transient heat shock, the expression of heat-shock responsive chaperone genes in neurons and other tissues are reduced. In addition, these animals are less tolerant to heat shock than wild-type worms. A subsequent study showed that regulation of chaperone gene expression by AFD neurons also influences protein aggregation in a neurodegenerative disease model (Prahlad and Morimoto, 2011). Therefore, signaling from thermosensory neurons to other tissues appears to mediate the proper heat shock response of whole worms. The induction of heat-shock responsive genes by AFD neurons is solely dependent on the heat shock factor-1 (HSF-1), which is a leucine-zipper transcription factor crucial for various biological functions, including heat-shock response, lifespan regulation, and organismal development (Neef et al., 2011). The interplay between the thermosensory neural system and HSF-1 was further demonstrated by Sugi et al. who found that HSF-1 is required for the thermotactic behavior of worms towards a preferred cultivation temperature (Sugi et al., 2011). Non-neuronal expression of *hsf-1* is sufficient to rescue thermotactic defects and does so by regulating AFD neurons through estrogen signaling. Thus, regulation of HSF-1 activity in non-neuronal cells, as well as AFD neurons in *C. elegans* appears to act as thermosensors. Interestingly, both AFD thermosensory neurons and the HSF-1 transcription factor are required for maintaining normal worm lifespan at high temperatures (Lee and Kenyon, 2009). It is tempting to speculate that AFD neuronal signaling and HSF-1 activity regulation

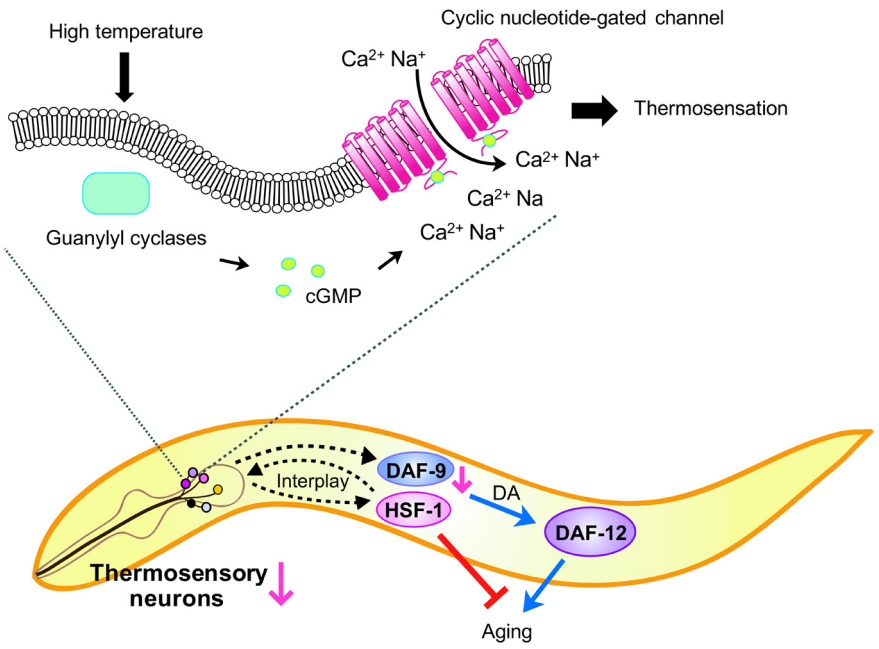

FIGURE 4 | Model of *C. elegans* lifespan control by thermosensory AFD neurons. Increased ambient temperature is perceived by AFD thermosensory neurons. Although the exact mechanism for thermal cue perception is not understood, upon sensing temperature elevation, G protein signaling promotes cyclic GMP production and facilitates the influx of Ca^{2+} and Na^{+} cations by opening cyclic nucleotide-gated channels (CNGs). The signal perceived by the thermosensory neurons is transmitted to downstream effectors, such as DAF-12/NHR and heat shock factor 1 (HSF-1), via intercellular (cell-nonautonomous) signaling. Impairment of AFD thermosensory neurons reduces DAF-9/CYP levels, which in turn decreases the level of sterol hormones, dafachronic acids (DA). This leads to altered DAF-12/NHR activity, which accelerates aging at high temperature. In addition, AFD neurons and HSF-1 regulate each other in a cell-nonautonomous manner to affect physiological processes of whole worms. Defects in thermosensory neurons decrease the activity of HSF-1 in non-neuronal tissues, while impairment of *hsf-1* in non-neuronal tissues decreases thermosensory neural function. It has also been shown that inhibiting HSF-1 speeds up aging.

in the non-neuronal cells of ectothermic animals, such as *C. elegans*, operate together to properly tune physiological processes, including aging (**Figure 4**).

IMPLICATIONS IN MAMMALIAN AGING

Although there is no known relationship between the thermosensory system and mammalian aging, there are hypotheses that mammalian thermosensory and thermoregulatory systems may influence aging, as well as other biological processes. Hibernation is defined as the controlled and reversible reduction and/or inhibition of body temperature, metabolic rate, oxygen consumption, and other physiological activities (Geiser, 2004). An early study on the relationship between thermosensation and hibernation showed that reduced body temperature and arousal from deep hibernation are sensed and regulated by the POA of the hypothalamus in hibernating mammals (Heller and Hammel, 1972). The role of hibernation in mammalian longevity has long been debated, and direct data have been scarce to date. Most bats hibernate during the winter and are known to have long lifespan compared to mammals of similar size and metabolic rate; bats can live as long as 30 years, whereas the maximum lifespans of mice and rats are 4 and 5 years, respectively (Brunet-Rossinni and Austad, 2004). Wilkinson and South found that hibernating bats live approximately 6 years longer than non-hibernating bats on average (Wilkinson and South, 2002). Lyman et al. suggested that hibernation also prolongs the lifespan of the Turkish hamster *Mesocricetus brandti* (Lyman et al., 1981). Animals that hibernate for a longer time (19–33% of their lives) live ∼50% longer than animals that hibernate for a shorter time (0–11% of their lives) at cold temperatures. It has been proposed that hibernators keep their metabolic rates at very low levels, which may lead to an extended lifespan, while animals that are exposed to cold and do not take the advantage of hibernation may suffer from cold stress (Lyman et al., 1981).

What are the potential molecular mechanisms behind the beneficial effects of hibernation on health and longevity? Recent research using the Djungarian hamster *Phodopus sungorus* suggests that changes in relative telomere length (RTL) may underlie the potential benefits of hibernation (Turbill et al., 2011). Djungarian hamsters use daily torpor, which can be considered as temporary hibernation, upon exposure to winter conditions for over 180 days. Animals that are kept at cold temperature (9°C) and use daily torpor have increased RTL compared to animals kept at warm temperature (20°C). Therefore, the use of daily torpor may delay aging during harsh environmental conditions by increasing telomere length in hamsters. Other studies suggest that endocrine signaling plays a role in hibernation and perhaps the longevity associated with it. The level of neuropeptide somatostatin, a negative regulator of growth hormone and thyroid-stimulating hormone (TSH) (Tichomirowa et al., 2005), increases before hibernation in the golden-mantled squirrel *Spermaphilus lateralis* (Muchlinski et al., 1983). Interestingly, growth hormone knock-out mice have long lifespan (Coschigano et al., 2000), significantly reduced levels of thyroid hormone, and decreased body core temperature compared to normal mice (Hauck et al., 2001). In addition, Ames dwarf mice, which are deficient in growth hormone, TSH, and prolactin, are long lived (Brown-Borg et al., 1996). A recent study suggests that growth hormone receptor deficiency in humans is associated with reduced incidences of age-related diseases, including cancer and diabetes (Guevara-Aguirre et al., 2011). Collectively, one can speculate that before the onset of hibernation, somatostatin inhibits growth hormone and TSH secretion, leading to lifespan extension (**Figure 5**).

One of the common features of mammalian hibernation is decreased body temperature. Is there a cause-and-effect relationship between body temperature and aging? One striking report showed that reducing core body temperature significantly prolongs lifespan (Conti et al., 2006). The method was based on an assumption that temperature increase in the lateral hypothalamus, which is in close proximity to thermosensory neuron-rich POA, would lead to a counter-balanced response to decrease

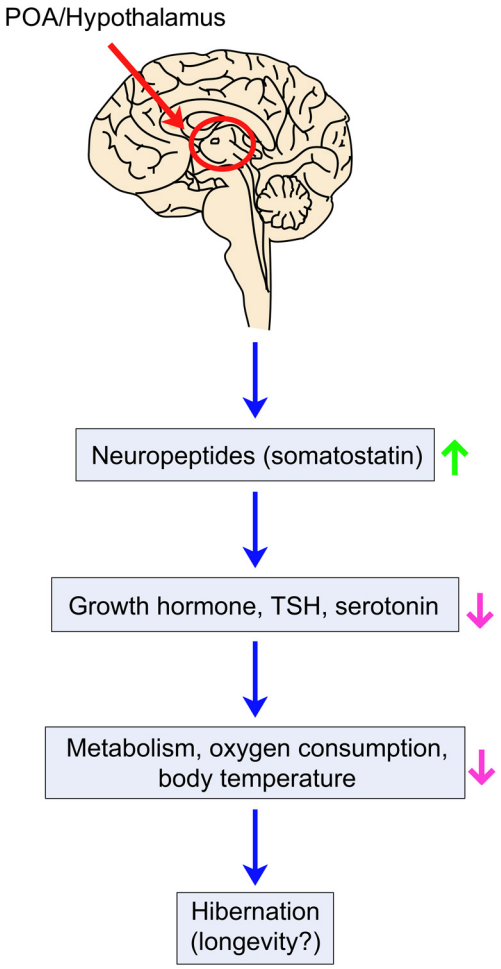

FIGURE 5 | Hypothetical model of longevity and hibernation modulation by thermoregulation in mammals. Ambient temperature decrease is sensed by cold-activated receptors and transmitted to the POA of the hypothalamus. Neuropeptides such as somatostatin may be secreted to inhibit growth, cellular proliferation, body core temperature, oxygen consumption, and metabolic activities by decreasing the levels of growth hormone, thyroid-stimulating hormone (TSH) and/or serotonin. These changes appear to contribute to the hibernation process and may influence longevity.

core body temperature. Overexpression of uncoupling protein 2 (UCP2), which generates heat by uncoupling the mitochondrial proton gradient and oxidative phosphorylation, in hypocretin neurons in mice elevates the level of heat perceived by thermosensory neurons located in the POA and lateral hypothalamus. This leads to a decrease in core body temperature and a subsequent reduction of energy requirement. These long-lived transgenic mice show diminished levels of free radicals and oxidative stress. Their prolonged lifespan may be due to similar mechanisms in dietary restriction-induced longevity, as the reduced core body temperature leads to more efficient energy consumption and lowered toxic metabolic byproduct production, which are similarly observed upon dietary restriction (Conti et al., 2006). It will be interesting to test whether thermosensory neurons affect lifespan extension induced by decreased body temperature.

CONCLUDING REMARKS

In this review, we discussed studies regarding the effects of sensory perception on aging. Although over a decade of research have established a role for sensory systems in the lifespan regulation of *C. elegans* and *D. melanogaster*, many important questions remain unanswered. It will be interesting to test whether insulin/IGF-1 signaling mediates the lifespan effects of chemosensory neurons and whether thermosensory neurons affect the aging processes in *D. melanogaster*. Also, it will be crucial to identify genes and signaling pathways, other than the insulin/IGF-1 pathway, which are involved in lifespan modulation by chemosensation. Perhaps the most important question in the field is whether mammalian sensory systems influence aging. Because many findings on aging regulation in invertebrate model organisms have been shown to be conserved in mammals, including humans, it would not be surprising to find homologous mechanisms in mammals. If so, it will be a fascinating starting point for future research, including anti-aging drug screens, because mammalian sensory systems are relatively well understood at the molecular level; therefore, components in sensory systems, such as receptors, can be modulated to promote healthy aging.

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Molecules affecting hypothalamic control of core body temperature in response to calorie intake

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Core body temperature (CBT) and calorie intake are main components of energy homeostasis and two important regulators of health, longevity, and aging. In homeotherms, CBT can be influenced by calorie intake as food deprivation or calorie restriction (CR) lowers CBT whereas feeding has hyperthermic effects. The finding that in mice CBT prolonged lifespan independently of CR, suggested that the mechanisms modulating CBT may represent important regulators of aging. Here we summarize the current knowledge on the signaling molecules and their receptors that participate in the regulation of CBT responses to calorie intake. These include hypothalamic neuropeptides regulating feeding but also energy expenditure via modulation of thermogenesis. We also report studies indicating that nutrient signals can contribute to regulation of CBT by direct action on hypothalamic preoptic warm-sensitive neurons that in turn regulate adaptive thermogenesis and hence CBT. Finally, we show the role played by two orphans G protein-coupled receptor: GPR50 and GPR83, that were recently demonstrated to regulate temperature-dependent energy expenditure.

Keywords: core body temperature, calorie restriction, hypothalamus, neuropeptides, GPCR, homeostasis, warm-sensitive neurons

INTRODUCTION

Experimental work on calorie restriction (CR), core body temperature (CBT), and the insulin-like growth factor 1 (IGF1)/Insulin pathway point at energy homeostasis as an important regulator of health, longevity, and aging. The two main components of energy homeostasis are nutrient and temperature homeostasis. Each contributes to energy intake and energy expenditure, respectively, and in homeotherms, they are regulated primarily in the hypothalamus. Although nutrient and temperature homeostasis are typically investigated independently, there is a distinct relationship between them. Calorie intake can affect CBT, with feeding producing acute hyperthermic effects, whereas food deprivation as well as the controlled reduction of nutrient intake in CR, can induce longer lasting hypothermia (Rampone and Shirasu, 1964; Walford and Spindler, 1997; Smirnov and Kiyatkin, 2008). CBT response to calorie intake reduction is regarded as an adaptive mechanism, decreasing energy expenditure when nutrient availability is limited. This mechanism may have evolved to prolong survival until food became available, and at least under controlled experimental conditions of CR, it contributes to increased lifespan. With some strain and diet specific differences, CR reduced CBT across species including mice, rats, primates, and humans (Duffy et al., 1989; Lane et al., 1996; Rikke et al., 2003; Soare et al., 2011). Work on transgenic mice with lowered CBT showed that even a modest (∼0.5°C) but prolonged CBT reduction increased median lifespan of up to 20%. This was observed in animals on *ad libitum* diet and with a calorie intake similar to that of wild type (wt) littermates, demonstrating that the effects of CBT on longevity were independent from those of CR (Conti et al., 2006). These findings also suggested that the reduction of CBT occurring during CR may contribute to the effects of CR on longevity. Thus, the molecules and the pathways regulating CBT responses to calorie intake may be important regulators of aging.

Adaptive thermogenesis is controlled via the sympathetic nervous system (SNS), which influences heat production in the brown adipose tissue (BAT). BAT is a specialized tissue responsible for producing heat for adaptive thermogenesis by dissipating the mitochondrial proton gradient via the uncoupling protein 1 (UCP1). In rodents and human infants, BAT has been shown to be the major source of induced heat (Cannon and Nedergaard, 2004). In addition to BAT, the SNS can also influence CBT by affecting heat production in the skeletal muscles and the liver, as well as by restricting heat dissipation via regulation of peripheral vasoconstriction.

This review will focus on signaling molecules demonstrated in mouse or in rat to be produced by and/or to act on two hypothalamic regions pivotal in the regulation of temperature or nutrient homeostasis, and that are in polysynaptic contact

Abbreviations: α-MSH, α-melanocyte-stimulating hormone; AgRP, agouti-related protein; ARC, arcuate nucleus; BAT, brown adipose tissue; CART, cocaine- and amphetamine-regulated transcript; CBT, core body temperature; CR, calorie restriction; DMH, dorsomedial hypothalamus nucleus; DMNX, dorsal motor nucleus of the vagus; Glut2, glucose transporter type 2; GOAT, ghrelin *O*-acyltransferase; GPR83, G protein-coupled receptor; IGF-1, insulin-like growth factor 1; IR, insulin receptor; LH, lateral hypothalamus; MCH, melanin-concentrating hormone; MC4R, melanocortin 4 receptor; MRR, melatonin-related receptor; NE, norepinephrine; NPY, neuropeptide Y; NTS, nucleus of the solitary tract; POA, preoptic area; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus; rRPa, raphe pallidus; SNS, sympathetic nervous system; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormones; UCP1, uncoupling protein 1; WSN, warm-sensitive neurons.

with the BAT (Elmquist et al., 2005). One such region includes the paraventricular (PVN), the arcuate (ARC), and the lateral hypothalamic (LH) nuclei (**Figure 1**); another is the preoptic area (POA; **Figure 2**).

The PVN, ARC, and LH express neuropeptides and their receptors, which together regulate feeding in addition to influencing CBT. These peptides include the neuropeptide Y (NPY), the cocaine- and amphetamine-regulated transcript (CART), the agouti-related protein (AgRP), the α-melanocyte-stimulating hormone (α-MSH), the melanin-concentrating hormone (MCH) and the thyrotropin-releasing hormone (TRH). These hypothalamic regions and neuropeptides are only reviewed here for their role in temperature homeostasis, and we refer to comprehensive reviews for their role in feeding (Elmquist et al., 2005; Morton et al., 2006; Gao and Horvath, 2007; Sanchez-Lasheras et al., 2010).

The POA contains temperature sensitive neurons that are pivotal in the sensing and the regulation of CBT (Hammel et al., 1960; Nakayama et al., 1963; Boulant, 2000). Among them are the warm-sensitive neurons (WSN), which are GABAergic neurons that exert a tonic inhibition on the dorsomedial hypothalamus (DMH) and the raphe pallidus (rRPa), both of which can activate spinal sympathetic and somatic motor circuits to drive adaptive thermogenesis in BAT (Morrison and Nakamura, 2011). WSN are typically investigated for their role in regulating fever or response to peripheral (skin) and local changes in temperature. However, electrophysiological studies and more recent molecular characterization have demonstrated that these specialized cells also respond to nutrient signals including glucose, insulin, and adiponectin (Silva and Boulant, 1984; Sanchez-Alavez et al., 2010; Eberwine and Bartfai, 2011; Klein et al., 2011). Evidence that these and other peripheral nutrient signals may contribute to CBT regulation via their action in the PVN, the ARC and the LH will also be summarized. Finally, we will discuss the role of the two orphan G protein-coupled receptors (GPCRs) GPR50 and GPR83 that were recently proposed to

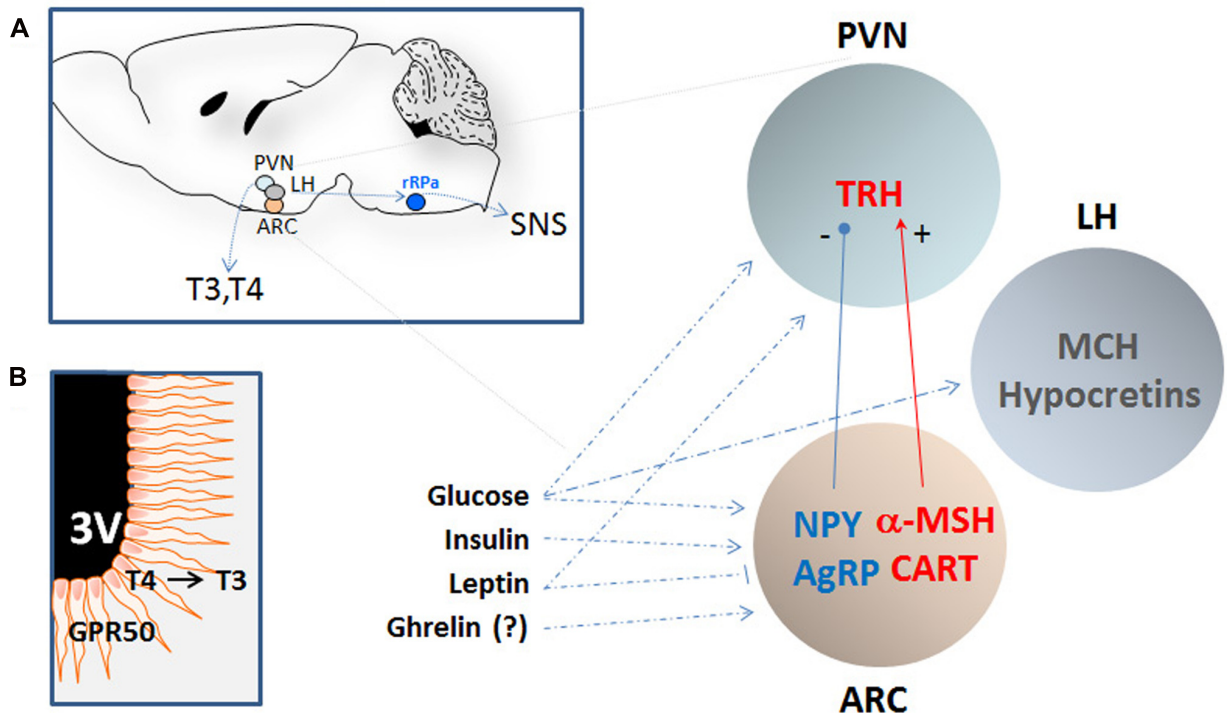

FIGURE 1 | Schematic representation of the hypothalamic nuclei and the neuropeptides that regulates calorie intake and energy expenditure by affecting core body temperature. (A) The approximate location of the periventricular nucleus (PVN), the arcuate nucleus (ARC), and the lateral hypothalamus (LH) are shown in a sagittal section of the mouse brain (left). These regions are in polysynaptic contact with the BAT (not shown) via the raphe pallidus (RPa) and can regulate CBT by affecting the sympathetic nervous system (SNS). In addition, CBT can also be affected by thyrotropin-releasing hormones (TRH) that determines the levels of circulating thyroid hormones T3 and T4. The PVN and the ARC contain neurons that can respond to nutritional state by modulating the level of specific orexigenic or anorexigenic neuropeptides. The orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) are elevated during calorie restriction or food deprivation and are strong stimulator of appetite and reduce CBT by acting on the SNS as well as by reducing the level of TRH in the PVN. In contrast, the anorexigenic peptide α-melanocyte-stimulating hormone (α-MSH), and possibly the cocaine-and amphetamine-regulated transcript (CART), stimulates thermogenesis. The LH is also an area important in the regulation of energy homeostasis and the melanin-concentrating hormone (MCH) and the hypocretins (Hcrt) can contribute to CBT elevation at least indirectly by increasing the locomotor activity associated with food seeking behavior and possibly via BAT activation. Neurons in these nuclei are sensitive to changes in the level of glucose, leptin, insulin, and possibly ghrelin all positively correlated to CBT. **(B)** Schematic representation of tanycytes cells lining the third ventricle (3V) and projecting into the hypothalamus. These cells can contribute to the regulation of CBT in response to nutrient signals in at least two ways: (1) by producing of T3 from T4 thus increasing its local concentration and in doing so inhibiting the synthesis of TRH even when circulating T3 levels are low; (2) by expressing GPR50, an orphan G-coupled protein receptor proposed to serve as a regulator of adaptive thermogenesis in response to nutrient signals. See text for details and references.

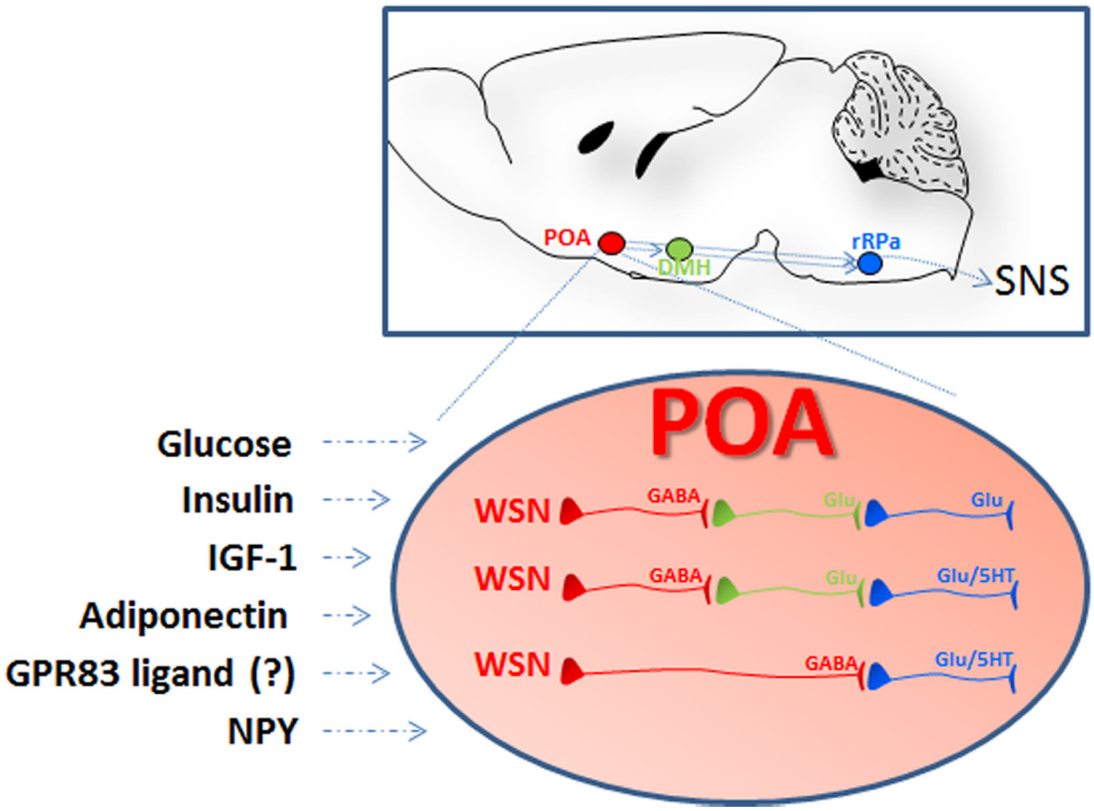

FIGURE 2 | Schematic representation of the localization and the organization of the nuclei and the cells participating to central thermoregulation. Lesions studies indicate that the hypothalamic preoptic area (POA) exerts the function of a bona fide thermostat allowing sensing and proper thermoregulatory responses to local as well as peripheral temperature changes (afferent pathways are not shown). In addition to respond to changes in temperature the POA can also sense nutrient signals: POA injection of insulin, IGF-1, and adiponectin were followed by hyperthermia via BAT activation while treatment with NPY or downregulation of the G-protein coupled receptor 83 (GPR83) induced hypothermia. The receptors for some of these ligands, as well as GPR83, were demonstrated in the POA warm-sensitive neurons (WSN). These specialized GABAergic cells exert a tonic inhibition on the raphe pallidus (aRP) either directly or through neurons in the dorsomedial hypothalamus (DMH) and control thermogenesis by activation of brown adipose tissue (BAT), muscular shivering, or the regulation of vasodilation (scheme adapted from Morrison and Nakamura, 2011). WSN are one component of the POA thermoregulatory neurocircuitry that comprises also temperature insensitive and cold-sensitive neurons that might also participate in the POA responses to nutrients (not shown in this scheme).

mediate effects of yet unidentified endogenous signals on energy expenditure via CBT regulation.

HYPOTHALAMIC OREXIGENIC AND ANOREXIGENIC PEPTIDES

NEUROPEPTIDE Y

Neuropeptide Y is a 36 aa, C terminally amidated neuropeptide. NPY acts at five different GPCR type of NPY-receptors (Y1–Y5), and is found in the autonomic nervous system and the brain, where its expression is highest in the ARC (**Figure 1**). NPY is a strong stimulator of feeding: its expression and synaptic level in the ARC is associated with hunger and is elevated during food deprivation or CR. Importantly, central administration of NPY not only increased food intake but also caused hypothermia (−1 to −3°C), reducing metabolic rate (Stanley et al., 1986). Such effect is at least in part due to decreased SNS-mediated thermogenesis, resulting from the NPY-mediated presynaptic (auto) inhibition of noradrenaline release from neurons that contain both norepinephrine (NE) and NPY. This also leads to a lower level of thermogenesis in BAT. In addition, Y1 and Y5 postsynaptic receptors on brown adipocytes also counteract the effect of NE at beta 3 adrenoreceptors (Billington et al., 1991; Egawa et al., 1991; Walker and Romsos, 1993; Bouali et al., 1995; Currie and Coscina, 1995; Pedrazzini et al., 1998; Williams et al., 2001). The Y5 agonists increased feeding, reduced oxygen consumption and energy expenditure in rats, probably by acting on ARC and BAT. Furthermore, the Y5 subtype selective antagonist increased CBT and the transcription of UCP1 in the BAT of mice (Hwa et al., 1999; Mashiko et al., 2007). Working with cold-acclimated Siberian hamsters, (Pelz and Dark (2007) and Dark and Pelz (2008)) found that activation of Y1 induced a prolonged reduction in CBT similar to that observed during natural torpor. Finally, inhibition of Y1 (albeit not of Y5) in hamster or its downregulation (knock-down) with antisense oligodeoxynucleotides in rats produced a transient hyperthermia (Lopez-Valpuesta et al., 1996; Pelz and Dark, 2007; Dark and Pelz, 2008).

Since the hypothermic action of NPY was observed not only after its administration into the ARC or the PVN, but

also the POA, it was proposed that NPY influenced the activity of thermoregulatory neurons (Currie and Coscina, 1995; Jolicoeur et al., 1995; Dark and Pelz, 2008). Molecular profiling showed that POA WSN express Y2 as well as the GPR83, an orphan receptor sharing homology to Y2 and found by one group to interact with NPY *in vitro* (Sah et al., 2007; Eberwine and Bartfai, 2011; Dubins et al., 2012). Interestingly, downregulation of GPR83 expression in the POA by shRNA was recently shown to reduce CBT (discussed below; Dubins et al., 2012).

AGOUTI-RELATED PROTEIN AND α-MELANOCYTE-STIMULATING HORMONE

The neuropeptides AgRP and α-MSH are the endogenous antagonist and agonist, respectively, of the melanocortin receptors and the main ligands of the central melanocortin system (**Figure 1**). In the hypothalamus they are produced in the ARC, where AgRP co-localizes with NPY, and where the precursor of α-MSH, the pro-opiomelanocortin (POMC), is co-expressed with CART (discussed below). AgRP and α-MSH stimulate and inhibit appetite, respectively, to modulate nutrient intake, but they can also contribute to the regulation of energy expenditure and can influence CBT (reviewed in Cone, 1999; Robinson et al., 2000; Schwartz et al., 2000; Spiegelman and Flier, 2001; Fan et al., 2005). This action is mediated by the melanocortin 4 receptor (MC4R) subtype. Mice null for MC4R, or treated with MC4R antagonists, including AgRP, have reduced thermogenesis and fail to upregulate UCP1 in the intercapsular BAT when fed high fat diet or when exposed to cold (Ste Marie et al., 2000; Butler et al., 2001; Voss-Andreae et al., 2007). Conversely, administration of MC3/4R agonist into the ventricle or in the RPa increased temperature via BAT activation (Yasuda et al., 2004).

Double labeling with retrograde tracing experiments demonstrated a connection between BAT and MC4R neurons in the RPa, the PVN, and the DMH. MC4R mRNA was also demonstrated in LH, VMH, as well as in the POA, suggesting that the α-MSH and AgRP regulate BAT thermogenesis by acting on several different neuronal circuits (Kishi et al., 2003; Liu et al., 2003).

The melanocortin system can also influence the circulating levels of thyroid hormones (TRH and T3/T4), which were increased or reduced by intracerebroventricular (i.c.v.) injection of α-MSH or AgRP, respectively (Fekete et al., 2000a, 2002a; Kim et al., 2000). This is achieved by direct innervation of both α-MSH and AgRP fibers in the ARC to the TRH neurons in the PVN (Legradi and Lechan, 1999; Fekete et al., 2004; see below for the thermoregulatory action of TRH and thyroid hormones).

Interestingly, the melanocortin system was also recently demonstrated to be modulated by the NAD^+-dependent deacetylase Sirt1, the mammalian homolog of Sir2 which was implicated in mediating some of the lifespan prolonging effects of CR (Guarente and Picard, 2005; Coppari, 2012). Sirt1 is expressed in the hypothalamus and its pharmacological inhibition decreased AgRP neuronal activity, producing a reduced inhibition of POMC neurons that was MCR4-dependent. Similar results were observed in animals with ablation of Sirt1 in AgRP neurons: these mice displayed decreased sensitivity to ghrelin, food intake, and body weight (Dietrich et al., 2010).

COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT

The CART is a neuropeptide co-expressed with POMC in neurons in the ARC (**Figure 1**). CART primarily affects energy homeostasis through its anorexigenic action, and can possibly contribute to energy expenditure by influencing CBT. In the rat, i.c.v. injection of CART was followed by hypothermic effects that were reduced by exendin-9-39, an antagonist of the glucagon like peptide 1 receptor also demonstrated to mediate the hypophagic of CART (Skibicka et al., 2009). However, a different study showed that CART injected in the PVN induced the expression of UCP1 in the BAT, suggesting that CART may be capable of stimulating adaptive thermogenesis by mitochondrial uncoupling (Wang et al., 2000).

MELANIN-CONCENTRATING HORMONE AND HYPOCRETINS

The LH is another region involved in the regulation of feeding and energy expenditure, and lesions to this nucleus caused hypophagia as well as hyperthermia (Teitelbaum and Epstein, 1962; Stevenson and Montemurro, 1963). Two distinct families of signaling molecules are recognized as main modulators of nutrient homeostasis and energy expenditure in the LH: the MCH and hypocretins (also known as Orexins; **Figure 1**). MCH is a powerful stimulator of feeding and its ablation results in a lean phenotype (Shimada et al., 1998). Hypocretins are neuropeptides involved in the regulation of sleep, wakefulness, and reward, and they are also able to increase appetite and CBT (de Lecea et al., 1998; Sakurai et al., 1998; Yoshimichi et al., 2001; Adamantidis and de Lecea, 2009; Bonnavion and de Lecea, 2010).

Retrograde labeling studies demonstrated a link between MCH neurons and hypocretin neurons and BAT innervation and activity (Oldfield et al., 2002; Cerri and Morrison, 2005). The level of UCP1 transcripts in brown adipocytes was increased in mice null for MCH, and this mutation normalized CBT in leptin-deficient animals (Segal-Lieberman et al., 2003). Consistently, UCP1 mRNA levels in BAT were reduced by MCH infusion (Ito et al., 2003). However, MCH and hypocretins have profound effects on locomotion, rendering the contribution of muscular activity to energy expenditure difficult to evaluate. In our experience, for instance, a deletion of up to 90% of hypocretin neurons reduced locomotion without affecting CBT (Conti et al., 2006).

THYROTROPIN-RELEASING HORMONE

The TRH is recognized as an important regulator of energy metabolism (reviewed in Lechan and Fekete, 2006). TRH exerts this action mainly via the modulation of the hypothalamic–pituitary–thyroid (HPT) axis, regulating the level of the thyroid hormones thyroxine (T4) and triiodothyroxine (T3). TRH neurons in the PVN regulate the release of the pituitary thyroid-stimulating hormones (TSH) into the circulation that, in turn, act on the thyroid gland to release T3 and T4 (**Figure 1**). Importantly, thyroid hormones inhibit TRH secretion, providing a negative regulatory feedback onto the axis. Thyroid hormones have long been recognized as mediators of thyroid thermogenesis, a phenomenon mainly investigated as a peripheral event evoked via direct thyroid action on muscle cells, involving altered muscle cell Ca^{2+} homeostasis, and possibly UCP3 (reviewed in Silva, 2006). More recently, it was proposed that the mechanisms of T3-induced

thermogenesis are central and involve the sympathetic activation of BAT, requiring the activation of the lipogenic pathway in the ventromedial hypothalamus (Cannon and Nedergaard, 2010; Lopez et al., 2010).

Thyrotropin-releasing hormone, as well as T3, are also important regulators of feeding, and experimental work indicated that both hormones can regulate thermogenesis in response to calorie intake. Fasting induced a fall in T3 and T4 levels and a reduction of TRH expression in the PVN, an effect at least in part due to decreased level of anorexigenic peptides (Spencer et al., 1983; Connors et al., 1985; Blake et al., 1991, 1992; Ahima et al., 1996; Legradi et al., 1998). These actions are mediated in two manners: by leptin acting directly on TRH neurons in the PVN, or by leptin acting indirectly by exerting opposite actions on α-MSH/CART and NPY/AgRP neurons localized in the ARC and projecting to the PVN (Ahima et al., 1996, 2000; Ahima, 2000; Nillni et al., 2000; Harris et al., 2001; Bjorbaek and Hollenberg, 2002; Perello et al., 2010). Fasting-induced reduction of TRH can be restored by i.c.v. injection of α-MSH or CART, which activate TRH neurons and stimulate hormone release (Fekete et al., 2000a,b; Kim et al., 2000; Nillni et al., 2000). Both AgRP and NPY can inhibit TRH neurons, reducing TRH transcript and circulating thyroid hormone levels. The NPY action is also mediated via Y1 and Y5 receptors (Fekete et al., 2001, 2002a,b; Costa-e-Sousa et al., 2011; Vella et al., 2011). Recently, it was reported that MC4R and NPY are both required for hepatic metabolism of T4 during fasting (Vella et al., 2011).

A distinct hypothalamic mechanism for the downregulation of the HPT axis is represented by local increase of T3 via fast-induced elevation of type 2 iodothyronine deiodinase in tanycytes, a group of ependymal cells that are located at the base of the third ventricle and extend into the hypothalamus (**Figure 1B**). During fasting, the type 2 deiodinase, D2 can convert T4 into the more potent T3 whose feedback inhibits the HPT axis, lowering the level of circulating thyroid hormones (Diano et al., 1998). Interestingly, these cells produce high level of GPR50, an orphan receptor also expressed in several hypothalamic nuclei and in pituitary neurons. GPR50 was recently shown to be a strong regulator of energy expenditure and thermogenesis in the context of the state of torpor (Bechtold et al., 2012; and see below).

Finally, TRH may also influence thermogenesis by direct action on POA temperature sensitive neurons and without affecting the HPT axis. Central injection of TRH was, in fact, capable of decreasing the activity of a fraction of WSN and increasing that of cold-sensitive neurons in the POA (Hori et al., 1988). This finding is consistent with the central hyperthermic effect of TRH, although its possible role in influencing CBT in response to nutrient intake remains to be investigated.

PERIPHERAL NUTRIENT SIGNALS

GLUCOSE

A central role of glucose in influencing CBT was first revealed by experiments in which i.c.v. injection of the glucose analog 2-DG was followed by reduced sympathetic activation of BAT and hypothermia (Freinkel et al., 1972; Egawa et al., 1989). Glucose-sensing neurons are found in most hypothalamic nuclei (Ritter and Dinh, 1994; Dunn-Meynell et al., 1998; Silver and Erecinska, 1998; Briski and Sylvester, 2001; Fioramonti et al., 2004; Wang et al., 2004; Yang et al., 2004) as well as at the brain stem level in the area postrema (AP), the nucleus of the solitary tract (NTS), the dorsal motor nucleus of the vagus (DMNX), and the basolateral medulla (BLM) (Adachi et al., 1984; Mizuno and Oomura, 1984; Ritter and Dinh, 1994; Yettefti et al., 1995; Dunn-Meynell et al., 1998; Dallaporta et al., 1999; Briski and Sylvester, 2001). It is possible to distinguish two categories of neurons depending on whether the elevation of extracellular glucose level has excitatory or inhibitory action on their activity (Anand et al., 1964; Adachi et al., 1984; Routh, 2002; Yang et al., 2004). Using mice with inactivation of the glucose transporter type 2 (Glut2), Mounien et al. (2010) demonstrated that the effects of glucose on thermogenesis are at least in part mediated via decreased leptin sensitivity of NPY and POMC expressing neurons in the ARC. These actions on the ARC may not be direct, but mediated by glucose-sensing neurons located in the LH, the dorsal vagal complex, and the basal medulla.

Interestingly, Glut2 neurons were also found in the DMH, an area that receives projections from POA WSN, suggesting the possibility that glucose may also influence adaptive thermogenesis via this neuronal circuitry. Finally, electrophysiological studies revealed that POA neurons, including a fraction of warm and cold-sensitive neurons, are sensitive to glucose (Silva and Boulant, 1984; **Figure 2**).

LEPTIN

Leptin is a small protein produced by adipose tissue that acts peripherally as well as centrally to regulate appetite and energy expenditure (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). Mice null for leptin receptor or for the transcription factor STAT3, which is involved in leptin receptor signaling, are obese and have reduced CBT and oxygen consumption (Gao et al., 2004). Leptin-deficient mice spontaneously enter into torpor when deprived of food, a response that is prevented by leptin administration (Gavrilova et al., 1999). Conversely, in wt mice leptin reduced food intake, elevated CBT, and increased the sympathetic activation of BAT (Pelleymounter et al., 1995; Haynes et al., 1997).

The effects of leptin on energy expenditure and thermogenesis have mostly been investigated for leptin's ability to regulate TRH either by direct action on PVN neurons, or by indirect action via inhibition of NPY/AgRP and stimulation of POMC/CART neurons in the ARC (Ahima et al., 1996, 2000; Ahima, 2000; Nillni et al., 2000; Schwartz et al., 2000; Harris et al., 2001; Bjorbaek and Hollenberg, 2002; Perello et al., 2010; see also Thyrotropin-Releasing Hormone).

A distinct mechanism of action for leptin-induced thermogenesis was also proposed to occur via stimulation of the release of the endogenous pyrogen interleukin-1β and prostaglandins (Luheshi et al., 1999) acting on POA and MPO neurons.

GHRELIN

The gastrointestinal peptide ghrelin is a hunger-stimulating hormone produced mainly by specialized cells in the fundus of the stomach and the pancreas. Ghrelin promotes an increase in food intake and a reduction in energy expenditure, resulting in a positive energy balance and an increase in body weight (Tschop et al., 2000; Lawrence et al., 2002; Theander-Carrillo et al., 2006).

Definitive proof for a role of ghrelin in regulating CBT is still lacking since findings remain few and contrasting. Central i.c.v. injection of ghrelin was reported to not only to be able to provoke a transient reduction of CBT associated with decreased spontaneous activity, but also to promote a small but significant reduction of BAT temperature, which indicates that ghrelin may be capable of reducing energy expenditure by affecting temperature homeostasis (Lawrence et al., 2002; Yasuda et al., 2003; Tang-Christensen et al., 2004). A single case of severe hypothermia in humans subject to prolonged treatment with ghrelin was also reported (Wiedmer et al., 2011). When the same group further investigated the hypothermic effect of ghrelin in rodents, they found evidence that ghrelin could bind to axon terminals in the POA, but they did not see any effects on CBT when the peptide was injected i.c.v. or subcutaneously.

Findings that CBT reduction may not be one of the mechanisms by which ghrelin regulates energy expenditure also came from experiments using mice null for ghrelin *O*-acyltransferase (GOAT), the enzyme that catalyzes the octanoylation of ghrelin, that is a post-translational modification necessary for the biological activity of this peptide. CBT profiles in GOAT null mice were similar to that of their wt littermates in different nutritional states, including fasting, or when exposed to different ambient temperatures (Heppner et al., 2012).

Instead, two distinct studies suggest that ghrelin may have a role in fasting-induced torpor. One found that the torpor induced by food deprivation was more severe if animals were treated with ghrelin peripherally. These effects were lost in animals with chemical ablation of the ARC, or in mice null for NPY, but not in mice blocked in α-MSH pathway (Gluck et al., 2006). Another study found that mice null for pre-pro-ghrelin had increased sensitivity to fasting and lowered ambient temperature, resulting in a precipitous drop of CBT, impaired sleep pattern, and decreased survival (Szentirmai et al., 2009). However, such a phenotype was not observed in mice lacking ghrelin receptor, suggesting that additional ghrelin receptor subtypes may exist. In addition, some of the differences in these studies may be due to the distinct ambient temperature at which experiments were carried out, with the hypothermic effects of ghrelin reported only at 17–18°C, but not at 25°C, a value closer to thermo-neutrality.

INSULIN/IGF-1

The pancreatic hormone insulin is the main regulator of peripheral glucose homeostasis and has been also investigated for its role as regulator of energy homeostasis in the central nervous system (Woods et al., 1979; Baskin et al., 1987; for recent reviews, see Plum et al., 2006; Belgardt and Bruning, 2010). Indeed, the insulin receptor (IR) is expressed in several brain regions, including the hypothalamus where it is abundant in the ARC (Havrankova et al., 1978; Werther et al., 1987; Marks et al., 1990). Pharmacological studies with central insulin injection, as well as elegant transgenic models of selective IR-ablation, showed that insulin can act centrally to cause reduced food intake, increased weight loss, and helped to regulate peripheral glucose homeostasis (Woods et al., 1979; McGowan et al., 1992a; Chavez et al., 1995; Bruning et al., 2000; Obici et al., 2002; Brown et al., 2006; Koch et al., 2008).

A role of insulin in regulating thermogenesis in response to feeding was proposed when it was observed that pharmacological inhibition of its secretion effectively attenuated diet-induced thermogenesis (Rothwell and Stock, 1981, 1986, 1988). Since either peripheral or central administration of insulin activated the SNS, the involvement of BAT in this response was promptly hypothesized (McCormack, 1982; Rothwell and Stock, 1986; Muntzel et al., 1995).

Injection of insulin into the hypothalamus had hyperthermic effects, increasing CBT and energy expenditure (Menendez and Atrens, 1991; McGowan et al., 1992a,b). This was proposed to occur via the insulin-mediated inhibition of the NPY/AgRP neurons expressing IR (Porte et al., 2002, 2005; Fekete et al., 2006; Mayer and Belsham, 2009).

The presence of IRs in the POA raised the possibility that insulin may influence thermogenesis by also acting on neurons in this region (Unger et al., 1989; Cardona-Gomez et al., 2000; Plum et al., 2005; van Baak, 2008). Central i.c.v. injection of insulin reduced the unit activity of POA neurons sensitive to peripheral changes in scrotum temperature, indicating that this hormone may modulate thermoregulatory responses by affecting these specialized cells (Wang and Lin, 1985). Recently, IR was demonstrated on at least a fraction of POA WSN, and electrophysiological studies on hypothalamic slices demonstrated that insulin acted directly on intrinsically WSN, inducing hyperpolarization and reducing their firing rate (Sanchez-Alavez et al., 2010). Retrograde transport and double labeling studies also demonstrated that the IR-positive WSN are GABAergic and project to the RPa (thus a likely synaptic connection to BAT was established). Finally, POA injection of insulin induced a specific, PI3K-involving and dose-dependent elevation of CBT mediated by stimulation of BAT (**Figure 2**).

A similar finding was reported for the IGF-1 (Sanchez-Alavez et al., 2011). Its receptor can be expressed on WSN and POA, and an injection of IGF-1 elicited a dose-dependent increase of CBT and activated BAT. Although the effects of IGF-1 on WSN activity remain to be demonstrated, the CBT effects of central IGF-1 were reduced in mice lacking neuronal IR. Since IGF-1 can also activate IR, the IR homodimers or the IGF-1R/IR heterodimers may contribute to the thermogenic action of IGF-1 (Sanchez-Alavez et al., 2011).

ADIPONECTIN

Adiponectin is a protein hormone secreted by adipose tissue. It has insulin-sensitizing effects, and is an important regulator of metabolism in peripheral tissues, enhancing fatty acid oxidation and glucose uptake in muscle, and reducing hepatic glucose production (Berg et al., 2001, 2002; Fruebis et al., 2001; Yamauchi et al., 2001; Tomas et al., 2002; Shklyaev et al., 2003; Qi et al., 2004). The adiponectin receptors AdipoR1 and AdipoR2 are expressed in different brain regions such as the hypothalamus, where adiponectin is beginning to be investigated for its possible central effects (Yamauchi et al., 2003; Fry et al., 2006; Kos et al., 2007; Kubota et al., 2007; Coope et al., 2008; Guillod-Maximin et al., 2009; Psilopanagioti et al., 2009; Hoyda and Ferguson, 2010; Thundyil et al., 2011).

So far, only a limited number of studies have measured the effects of adiponectin on CBT and energy expenditure and these

have reported contrasting findings. For instance, one group found i.c.v. injection of adiponectin recapitulated its peripheral effects and increased energy expenditure via BAT-induced thermogenesis (Qi et al., 2004). Another showed increased BAT UCP1 and rectal temperature following peripheral, but not central injection with adiponectin (Masaki et al., 2003). A third group instead reported that intravenous injection of adiponectin reduced BAT UCP1 mRNA and energy expenditure while exerting central orexigenic effects via direct action on the ARC (Kubota et al., 2007). The effects of central injections of adiponectin on calorie intake are also contrasting, reporting either pro-anorexigenic or pro-orexigenic effects, as well as no effects at all (Masaki et al., 2003; Kubota et al., 2007; Coope et al., 2008).

Since both AdipoR1 or AdipoR2 were recently found in a fraction of POA WSN we tested the effects of adiponectin on thermogenesis in mice null for either one of the adiponectin receptors (Eberwine and Bartfai, 2011; Klein et al., 2011). When injected locally into the POA of wt mice, adiponectin had thermogenic effects elevating CBT and fatty acid oxidation (measured as decreased respiratory exchange ratio). These effects were nearly abolished in mice lacking AdipoR1, and were only diminished in animals null for AdipoR2. It is possible that some of the contrasting findings may be due to differences in the experimental conditions used, or to the putative opposite roles that AdipoR1 and AdipoR2 were found to have on energy metabolism (Bjursell et al., 2007). Another confounding factor may be that the oligomer form of adiponectin used as the adiponectin monomer can oligomerize to form 3-mers that can further aggregate into 6-, 12-, or 18-mers (Pajvani et al., 2003). Kubota et al. (2007) reported that in mice, only 3- and 6-mers can enter the CSF from the circulation.

TWO INTERESTING ORPHAN GPCRs WITH HYPOTHALAMIC EXPRESSION

The GPCRs are the favorite drug target class of the pharmaceutical industry and many of the most used and safest drugs are ligands to this class of receptors, including beta blockers, the antihistamines, and the D2 receptor antagonist antipsychotics to mention a few. The relative ease by which ligands to GPCRs are developed is the reason for excitement in the discovery of orphan GPCRs with physiologically and pharmacologically interesting and robust effects. Thus we examine into the effects mediated by GPR83 and GPR50, because it is likely that the validation of their role in integration of nutrient and energy homeostasis will lead to the development of useful drugs that affect feeding body weight and life span.

GPR83

Profiling of WSN revealed that these cells express several orphan GPCRs (Eberwine and Bartfai, 2011). Among these is GPR83 (also known as GIR, GPR72, or JP05), originally identified as a stress–response element from a murine thymoma cDNA library treated with glucocorticoids and forskolin (Harrigan et al., 1989, 1991; Baughman et al., 1991), and subsequently shown to be highly expressed in several brain regions including the hypothalamus, the cortex, the thalamus, the hippocampus, and the amygdala (Pesini et al., 1998; Brezillon et al., 2001; Wang et al., 2001; Adams et al., 2003; Sah et al., 2005). GPR83 shares some homology with a variety of known peptide receptors, including the neuropeptide Y2 receptor. One study reported that NPY C-terminus fragments can bind and activate rat GPR83 with moderate affinity suggesting that GPR83 might participate in the regulation of nutrient intake (Sah et al., 2007).

Local downregulation of GPR83 in the hypothalamic POA, by injection of lentiviral vectors expressing a pool of shRNAs directed against all known isoforms of mouse GPR83 recently demonstrated its role in temperature homeostasis (Dubins et al., 2012). Reduction of POA GPR83 in the range of 30–50% caused a modest (0.15°C) but significant reduction of CBT, starting at day 4 post-treatment, that lasted at least until recording was stopped at day 18. CBT reduction was observed only in the dark period of the day, when the mice are active, and was not significant during the light-inactive phase. The downregulation of the expression GPR83 did not alter calorie intake, and animals treated with silencing GPR83 shRNA ate similarly to those treated with the non-silencing counterpart. However, the silencing shRNA treated group showed an increase in body weight gain that became significant 3 weeks after treatment and was associated with reduced hypothalamic receptor expression. This phenotype was similar to that observed in the long-lived transgenic mice, with reduced CBT achieved by producing heat through uncoupling neuronal mitochondria in the vicinity of WSN cells in the POA (Conti et al., 2006).

GPR50

GPR50 is a GPCR recently demonstrated to play an important role in adaptive thermogenesis in response to calorie intake (Bechtold et al., 2012). It was originally cloned from human pituitary gland and termed melatonin-related receptor (MRR) for its homology with the melatonin receptors (Reppert et al., 1996; Dufourny et al., 2008, 2012). GPR50 does not bind to melatonin, and although it may dimerize with melatonin receptors (possibly influencing melatonin action) to date it remains an orphan receptor (Levoye et al., 2006). Expression of GPR50 is high in the hypothalamus, where it localized in the medial POA, the LH neurons of the dorsomedial nucleus, and in tanycytes (Reppert et al., 1996; Drew et al., 1998, 2001; Hamouda et al., 2007; Sidibe et al., 2010; Batailler et al., 2011; Bechtold et al., 2012).

When fed *ad libitum*, mice null for GPR50 (*Gpr50*$^{-/-}$) showed a modest (∼0.5°C) reduction of CBT, that like in the Hcrt-UCP2 mice and the GPR83 shRNA mice, respectively, was observed only during the dark-active part of the day. In response to 24 h food deprivation, CBT of *Gpr50*$^{-/-}$ mice dropped up to 10°C. O_2 consumption and CO_2 production were also reduced, and mice entered a torpor-like state. The exact mechanisms by which GPR50 may affect thermogenesis remain to be elucidated. The experimental evidence collected thus far suggest that GPR50 can affect thermal responses to energy signals by directly reducing the responses to leptin and melanocortin during fasting in the ARC, and indirectly by suppressing TRH in the PVN, possibly normally inhibiting entry into a hypometabolic state (Bechtold et al., 2012).

SUMMARY

Lowered CBT increased lifespan and its value in homeotherms can be affected by calorie intake. Here we reviewed the current

knowledge on the molecules and signals that mediate CBT responses to calorie intake as these may influence longevity and aging.

At least two hypothalamic regions are involved in mediating these responses. One is the region containing the ARC, the PVN, and the LH nuclei, which synthesize neuropeptides to regulate feeding, but are also able to affect CBT. The second is the POA, recognized for integrating and regulating peripheral as well as central temperature information, and containing temperature sensitive neurons that can also respond to nutrient signals. Both regions can activate the SNS and are in polysynaptic contact with BAT, a tissue specialized in heat production, and that in small animals, such as mice and rats, is the main contributor to thermogenesis. Within the ARC and the LH, the anabolic neuropeptides NPY, AgRP, and MCH are elevated during food deprivation, stimulating appetite and concomitantly reducing thermogenesis. In contrast, the catabolic neuropeptide α-MSH has opposite effects. In addition, AgRP and α-MSH were also proposed to regulate thermogenesis by inhibiting or stimulating the release of TRH from the PVN, thus influencing the level of circulating thyroid hormone.

Nutrient signals can influence thermoregulation by affecting the level of the aforementioned neuropeptides or by inhibiting the activity of WSN POA neurons. In particular, glucose, insulin, and leptin can act on neurons in the PVN and the ARC, stimulating the release of TRH and α-MSH, and reducing that of NPY and AgRP, promoting CBT increase. The POA was demonstrated to be responsive to glucose, insulin, IGF-1, NPY, and adiponectin, and some of these signals were demonstrated to modulate WSN activity. Two orphan GPCRs, GPR83 and GPR50, were also recently proposed to be instrumental in influencing CBT in response to calorie intake. GPR83 is elevated in the hypothalamus during CR, is expressed on WSN, and its local downregulation in the POA reduced CBT. GPR50, primarily expressed in tanycytes lining the third ventricle, appears to be an important regulator of the hypothermic response to fasting.

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Interactions between oxygen homeostasis, food availability, and hydrogen sulfide signaling

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The ability to sense and respond to stressful conditions is essential to maintain organismal homeostasis. It has long been recognized that stress response factors that improve survival in changing conditions can also influence longevity. In this review, we discuss different strategies used by animals in response to decreased O_2 (hypoxia) to maintain O_2 homeostasis, and consider interactions between hypoxia responses, nutritional status, and H_2S signaling. O_2 is an essential environmental nutrient for almost all metazoans as it plays a fundamental role in development and cellular metabolism. However, the physiological response(s) to hypoxia depend greatly on the amount of O_2 available. Animals must sense declining O_2 availability to coordinate fundamental metabolic and signaling pathways. It is not surprising that factors involved in the response to hypoxia are also involved in responding to other key environmental signals, particularly food availability. Recent studies in mammals have also shown that the small gaseous signaling molecule hydrogen sulfide (H_2S) protects against cellular damage and death in hypoxia. These results suggest that H_2S signaling also integrates with hypoxia response(s). Many of the signaling pathways that mediate the effects of hypoxia, food deprivation, and H_2S signaling have also been implicated in the control of lifespan. Understanding how these pathways are coordinated therefore has the potential to reveal new cellular and organismal homeostatic mechanisms that contribute to longevity assurance in animals.

Keywords: hypoxia, anoxia, oxygen, hydrogen sulfide, suspended animation, diapause, dietary restriction, homeostasis

All organisms must maintain homeostasis to survive. Walter Cannon defined the modern concept of homeostasis as "the coordinated physiological reactions which maintain most of the steady states in the body. . .." (Cannon, 1929). At the cellular level, maintaining homeostasis requires the coordination of metabolic reactions and cellular processes with environmental conditions. Homeostatic mechanisms are also centrally important for regulating longevity assurance. One consequence of the physiological decline associated with aging is degradation of the ability to maintain homeostasis, which narrows the range of conditions that can be tolerated. At least partly as a result of this defect in homeostasis, the likelihood of death from injury, infection, and disease increases. Oxygen (O_2) is an essential environmental resource for all metazoans, with only one known exception (Danovaro et al., 2010). The ability to sense and respond to changes in O_2 likely arose early in evolution (O'Farrell, 2001). Nevertheless, even short exposure to decreased O_2 availability (hypoxia) leads to irreversible cellular damage and death in most metazoans. Interestingly, responses to hypoxia have molecular and physiological similarities to the effects of food deprivation. Moreover, there is accumulating evidence that hydrogen sulfide (H_2S) improves outcome after ischemia, suggesting that H_2S signaling can modulate effects of hypoxia in animals. In this article, we review physiological responses to hypoxia and consider similarities and interactions with adaptation to food deprivation and H_2S signaling (**Figure 1**).

There is great diversity in sensitivity to hypoxia between different animals and even between cell types in the same animal. For example, hibernating mammals have decreased respiration, with up to 30 min between breaths, and can survive in hypoxic conditions that are damaging to related euthermic non-hibernators (Drew et al., 2004). In global cerebral ischemia, CA1 pyramidal neurons in the hippocampus begin to die before other neurons when blood flow is disrupted (Lipton, 1999). This variation suggests there are mechanisms that promote homeostasis in hypoxia, but that they are only employed in specific physiological contexts. It is important also to consider the precise hypoxic conditions experienced by the cells and organism. The physiological consequences of hypoxia depend greatly on the duration and severity of the hypoxic insult. Hypoxia, where O_2 levels are "less than normal" or low enough to disrupt normal function, includes a wide range of conditions (**Figure 2**). The ambient concentration of O_2 at sea level (1 atm atmospheric pressure) is 210,000 ppm (21%) O_2. At high altitude, though the concentration of O_2 remains the same, the lower atmospheric pressure results in decreased effective ambient O_2 tension. O_2 is poorly soluble in aqueous solutions and diffuses slowly. Therefore, steep O_2 gradients can exist in poorly mixed water environments and waterlogged soil. It can take >3 h for a 100 mm tissue culture dish to equilibrate with ambient O_2 levels (Chapman et al., 1970). In large animals, O_2 is delivered to cells by a complex circulatory system. The concentration of

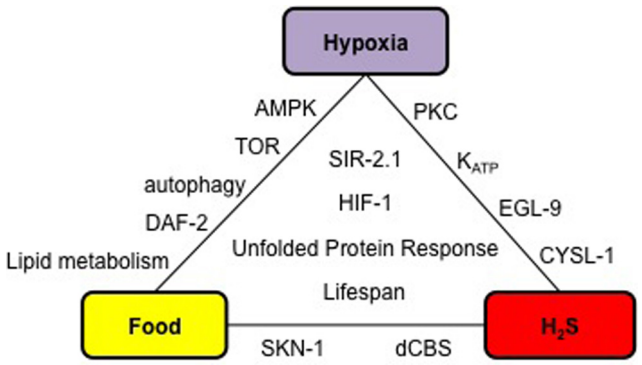

FIGURE 1 | Physiological and molecular relationships between hypoxia, H_2S signaling, and food. Factors listed inside the triangle are common to all three conditions, and those on the edges are shared by two conditions. Details and references are included in the main text.

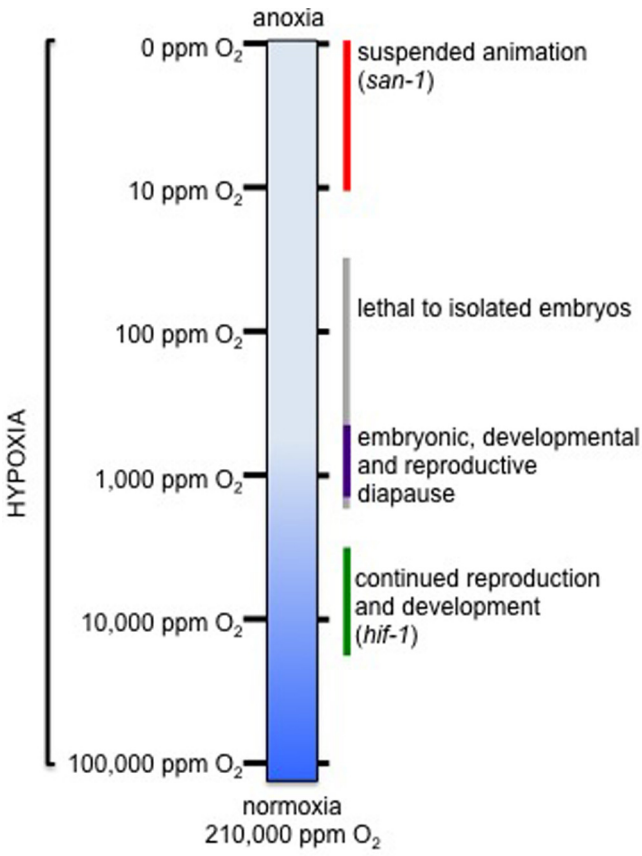

FIGURE 2 | Hypoxia responses at difference concentrations of O_2. The bar represents decreasing O_2 levels, with normoxia at the bottom and anoxia at the top. For the purposes of this review, normoxia is considered to be room air, which is 210,000 ppm (21%) O_2. Hypoxia includes all concentrations of O_2 that are less than this. On the right, the physiological response of *C. elegans* to different O_2 concentrations is noted, as described in the main text.

O_2 at the tissue level is lower than ambient, varies between tissue types, and depends both on O_2 delivery and tissue metabolic activity (Montgomery, 1957; Dyson and Singer, 2011). Fluctuations in ambient O_2 supply or tissue metabolic demand stimulate compensatory responses to increase blood flow and O_2 delivery, including vasodilation, increased respiratory rate, and production of red blood cells. This makes it difficult to experimentally control the hypoxic exposure of cells in an intact animal in order to investigate different cellular responses to hypoxia. It is important also to consider that it is experimentally difficult or impossible to separate damage that occurs in hypoxia or ischemia from effects that occur as a result of reoxygenation. In contrast, *C. elegans* does not have a circulatory system, relying instead on diffusion for O_2 delivery to cells. This allows for precise experimental control of both genotype and cellular environment (Shen and Powell-Coffman, 2003; Fawcett et al., 2012). Because it is an attractive model for hypoxia research we have built a framework of hypoxia responses as a function of O_2 tension using *C. elegans*, drawing connections with other systems when possible. There have been several excellent reviews recently about signaling pathways that coordinate cellular responses to hypoxia (Gorr et al., 2006; Powell-Coffman, 2010; Hand et al., 2011; Padilla and Ladage, 2012). In this review we compare how strategies to respond to hypoxia vary with O_2 concentration, and focus on how response mechanisms could integrate with other signaling pathways to influence organism physiology and lifespan.

ADAPTATIONS TO ANOXIA

In the laboratory, *C. elegans*, *Drosophila melanogaster*, and *Danio rerio* all survive without O_2 (anoxia; operationally defined as <10 ppm O_2) by entering into a state of suspended animation (Foe and Alberts, 1985; DiGregorio et al., 2001; Padilla and Roth, 2001; Padilla et al., 2002). In suspended animation, all microscopically observable activity reversibly arrests, including embryonic cell divisions, post-embryonic development, movement, and reproduction. Upon reoxygenation, developmental processes resume and animals grow to healthy, fertile adults. Suspended animation can be successfully maintained for several days in *C. elegans*, weeks in *Drosophila* embryos, and years in the brine shrimp *Artemia franciscana* (Foe and Alberts, 1985; Clegg, 1997; Padilla et al., 2002). Mechanisms that underlie the ability to survive severe hypometabolic and quiescent states may be widely conserved. Metabolism is dramatically reduced in dogs that survive for several hours after total exsanguination with cold saline flush, for example (Behringer et al., 2003).

One common feature of suspended animation is the reversible arrest of cell divisions. The point at which cell cycle arrest occurs differs between organisms. *C. elegans* embryonic blastomeres arrest in interphase, prophase, and metaphase, but the transition to anaphase will not occur in anoxia (Padilla et al., 2002; Nystul et al., 2003; Hajeri et al., 2005). The spindle assembly checkpoint is activated by anoxia, and stopping the cell cycle is important to prevent lethal chromosome segregation defects. Embryos that have been depleted of *san-1*, a component of the spindle assembly checkpoint, by RNAi die when exposed to anoxia and exhibit chromosome segregation defects (Nystul et al., 2003). In cells that arrest in interphase or prophase, the chromatin condenses and

chromosomes align near the nuclear envelope, whereas metaphase blastomeres display reduced spindle and astral microtubule density. The prophase arrest is characterized by inactivation of *cdk-1*, and requires the *npp-16* nucleoporin (Hajeri et al., 2005). These results indicate that there are at least two distinct cell cycle checkpoints activated to arrest embryonic cell divisions in anoxia-induced suspended animation in *C. elegans*. The spindle assembly checkpoint is not required for suspended animation in adults, possibly because somatic cells are all post-mitotic. However, germline stem cell divisions arrest in adults in suspended animation without any apparent decrease in full reproductive potential (Padilla et al., 2002; our unpublished observation). Thus, there may be other mechanisms that contribute to anoxia-induced suspension of cell division post-embryonically. The mechanisms by which anoxia signaling integrates with the spindle checkpoint are not well understood, though the effect is conserved. *Drosophila* embryos exposed to anoxia also arrest during interphase, prophase, and metaphase, and the arrest is characterized by chromatin localization near the nuclear membrane (Foe and Alberts, 1985;Douglas et al., 2001). Similarly, *Danio rerio* embryos suspend cell division in anoxia, though arrest is exclusively during interphase (Padilla and Roth, 2001).

In anoxia metabolic networks must be substantially rearranged, with important phenotypic consequences. O_2 is essential for both mitochondrial respiration and fatty acid oxidation. A major consequence of O_2 deprivation is that cellular energy metabolism is disrupted. The survival of both embryos and adult *C. elegans* in anoxia is correlated with available glycogen stores, which serve as a source for glycolytic energy production (Frazier and Roth, 2009; LaRue and Padilla, 2011). Glycogen decreases progressively as embryos are exposed to anoxia (Frazier and Roth, 2009). Mutations in genes that have little in common, other than decreased glycogen content, all show an anoxia-sensitive phenotype during embryogenesis (Frazier and Roth, 2009). Similarly, hyperosmotic shock, an environmental perturbation that increases glycerol production at the expense of glycogen, reduces the viability of embryos in anoxia (Frazier and Roth, 2009). In contrast, in adults hypomorphic loss-of-function mutations in the insulin/IGF receptor homolog *daf-2* increase glycogen content and survival in anoxia (Scott et al., 2002; Mendenhall et al., 2006; Frazier and Roth, 2009; LaRue and Padilla, 2011). Diet-induced increases in glycogen are also associated with increased survival in anoxia in *Drosophila* (Vigne et al., 2009). Depletion of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (*gpd-2/3*) by RNAi decreases survival of adult *daf-2* mutant animals in anoxia (Mendenhall et al., 2006). The significance of this result is not clear, insofar as *gpd-2/3(RNAi)* does not reduce survival of wild-type animals in anoxia (Mendenhall et al., 2006). One possibility is that the difference between wild-type and *daf-2* mutant animals reflects a difference in metabolic state. Both gene expression, oxygen consumption measurements, and physiological studies suggest that the *daf-2* mutant animals have a metabolic architecture that is very different from wild-type (Van Voorhies and Ward, 1999; Lee et al., 2003; Murphy et al., 2003; Houthoofd et al., 2005). Moreover, RNAi directed against other glycolytic enzymes does not alter survival in anoxia (Mendenhall et al., 2006). This may suggest that simply decreasing glycolysis does not explain the effect on anoxia survival. However, it is difficult to assess whether the RNAi treatment sufficiently decreased the activity of the glycolytic enzymes in these experiments, and no direct measurements of effects on glycogen were reported.

In anoxia, fatty acid oxidation is not possible. Instead, increased fatty acid synthesis may be important for anabolic activity and to regenerate reducing equivalents for continued glycolytic activity. Fatty acid synthesis is a hallmark of hypoxic tumor cells (Romero-Garcia et al., 2011), and in *C. elegans* the SREBP homolog *sbp-1* is required for fatty acid accumulation after anoxia (Taghibiglou et al., 2009). This result suggests that changes in lipid metabolism are essential parts of the response to hypoxia. However, it is also possible that lipid signaling plays an important role during O_2 deprivation. Consistent with this view, mutations that are predicted to disrupt ceramide synthesis modulate survival in anoxia. Survival was decreased by loss-of-function of *hyl-2*, whereas similar mutations in the related *hyl-1* increase survival in anoxia (Menuz et al., 2009). In mammalian models, altered ceramide signaling has been associated with hypoxia-induced changes in tumors and may contribute to cell death in neurological disorders including cerebral ischemia (Jana et al., 2009; Yin et al., 2010). *hyl-1* and *hyl-2* are functional homologs, of LAG1 (longevity assurance gene 1), which was reported to increase replicative lifespan in *Saccharomyces cerevisiae* (D'Mello et al., 1994). However, RNAi knockdown of neither *hyl-1* nor *hyl-2* increase lifespan in *C. elegans* (Menuz et al., 2009). Lipid metabolism and signaling are increasingly recognized as playing an important role in the regulation of aging and lifespan (Lapierre and Hansen, 2012). Considering the important role that aberrant lipid signaling plays in the progression of cancer cells, elucidating the role that these processes play in adaptations to hypoxia is likely to be a productive direction for future research.

There is surprising overlap between genes and pathways that increase survival in anoxia and those that modulate lifespan, though the mechanistic basis of this correlation is not understood. In a screen for genes that increased survival in anoxia when depleted by RNAi, 11 of 198 hits (5.6%) had previously been identified to increase lifespan in *C. elegans* (Mabon et al., 2009). In contrast, the frequency of finding genes that increase lifespan from RNAi screens that use longevity as the primary phenotype ranged from 0.1 to 0.5% (Hamilton et al., 2005; Hansen et al., 2005). Thus, the genes identified by enhanced anoxia survival are enriched for longevity genes. In addition to a variety of metabolic genes identified in this screen, anoxia survival also requires autophagy, which may serve as an important source for catabolic energy production. Disruption of genes important for autophagy by RNAi or mutation reduce survival in anoxia (Samokhvalov et al., 2008). In mammalian systems, autophagy is regulated by hypoxia, particularly in cancer cells (Rouschop and Wouters, 2009; Eskelinen, 2011). Moreover, autophagy is important for increased lifespan by both *daf-2(lf)* loss-of-function mutations and dietary restriction (DR) in *C. elegans* (Meléndez et al., 2003; Hansen et al., 2008). Overexpression of autophagy gene LC3/Atg8 in the nervous system increases lifespan in *Drosophila* (Simonsen et al., 2008). The insulin/IGF1 signaling (IIS) pathway is another conserved pathway that is involved both in longevity assurance and the response to hypoxia. In. *C. elegans*, the IIS receptor homolog *daf-2* increases

lifespan as well as survival in anoxia (Kenyon et al., 1993; Scott et al., 2002; Mendenhall et al., 2006). Increased stress resistance is a well-known feature of *daf-2(lf)* mutant animals, suggesting that increased survival in anoxia is a consequence of a correlation between increased stress resistance and lifespan (Lithgow et al., 1995; Honda and Honda, 1999; Mendenhall et al., 2006; Scott et al., 2002). However, five of six *daf-2* regulated gene products depleted by RNAi increased resistance to anoxia but had no effect on lifespan (Mabon et al., 2009). Moreover, mutations that increase resistance to osmotic stress, including loss-of-function alleles of *dpy-10* and *osm-7*, decrease survival in anoxia (Wheeler and Thomas, 2006; Frazier and Roth, 2009). Thus, a general increase in stress resistance does not explain the relationship between lifespan and anoxia resistance.

Protein metabolism is another central aspect of cellular physiology affected by hypoxia. Protein synthesis and the chaperones that help to maintain cellular proteins in the correctly folded state are energetically expensive. The coordination of protein synthesis, quality control, and degradation, referred to as proteostasis, is essential to maintain cellular function (Hartl et al., 2011; Taylor and Dillin, 2011). Reduced protein translation is associated with increased lifespan in *C. elegans* (Hansen et al., 2007; Pan et al., 2007). Many genes that increase survival in anoxia when depleted by RNAi are involved in protein translation. Protein translation is inhibited in low O_2 (Hochachka et al., 1996; Teodoro and O'Farrell, 2003; Storey and Storey, 2004; Wouters et al., 2005; Liu et al., 2006), making it somewhat surprising that genetic manipulations that decrease translation would increase anoxia survival. It may be that indirect consequences of, or adaptations to, decreased translation confer the protective effect in anoxia. For instance, decreased energy utilization for protein translation could increase energy stores available in anoxia. Another possibility is that reduced translation rates improve proteostasis networks and improve the capacity to deal with unfolded protein stress in anoxia. In the endoplasmic reticulum, the ERO1 enzyme uses O_2 to catalyze oxidative protein folding (Tu and Weissman, 2002), which would be inhibited in anoxia. In *C. elegans*, the ER unfolded protein response (UPR) is activated in anoxia, and UPR genes *xbp-1* and *ire-1* are required for survival (Mao and Crowder, 2010). This suggests that anoxia increases the burden of misfolded proteins in the secretory path. Decreasing translation by knock-down of aminoacyl tRNA synthase genes reduces expression of UPR mediators, and increases survival in anoxia (Anderson et al., 2009). UPR activity is increased by decreased O_2 in pancreatic β-cells and liver (but not cardiomyocytes), suggesting that it plays a conserved role in the cellular response to hypoxia (Tagliavacca et al., 2012; Zheng et al., 2012). Understanding general mechanisms that integrate stress homeostasis pathways with the proteostasis network could reveal new strategies to manipulate proteostasis. This would have broad significance, particularly as defects in proteostasis have been associated with the aging process (Haigis and Yankner, 2010; Gidalevitz et al., 2011).

RESPONSES TO HYPOXIA WHEN SOME O_2 IS AVAILABLE

A common strategy to survive hypoxia is to avoid conditions with insufficient O_2. Indeed, animals have evolved sophisticated behavioral strategies to avoid hypoxic conditions. In a gradient of O_2 blue crabs, New Zealand snapper, and *C. elegans* will all avoid low O_2 and show preference for an optimal O_2 environment (Dusenbery, 1980; Bell et al., 2009; Gray et al., 2004; Cook and Herbert, 2012). Interestingly, other environmental conditions can modulate what is perceived as the optimal O_2 concentration. Hypoxia avoidance in *C. elegans* decreases as animals are starved (Dusenbery, 1980). Both alligators and cold-submerged frogs prefer lower ambient temperature in hypoxia (Branco et al., 1993; Tattersall and Boutilier, 1997). This may reflect a physiological interaction between temperature and O_2. Consistent with this idea, *C. elegans* survive much longer in anoxia at low temperature than at higher temperature (Padilla et al., 2002; Scott et al., 2002; Mendenhall et al., 2006). It is not clear if the mechanisms that regulate survival are identical in these conditions, though the insulin/IGF receptor ortholog *daf-2* can increase survival at both temperatures (Scott et al., 2002; Mendenhall et al., 2006). The interaction between temperature and hypoxia may also have clinical relevance, as therapeutic hypothermia can reduce neurodevelopmental disability in infants surviving hypoxic ischemic encephalopathy from perinatal asphyxiation, and is used in adults clinically to improve outcome after pelvic surgery, cardiac arrest, and brain ischemia (Selway, 2010; Finley, 2011; Sunde and Søreide, 2011; Yenari and Han, 2012).

In moderate hypoxia (5,000–20,000 ppm O_2) *C. elegans* embryos complete development and grow to gravid adults, albeit more slowly than in room air (Jiang et al., 2001; Nystul and Roth, 2004; Miller and Roth, 2009). This indicates that the response to these hypoxic conditions is physiologically distinct from anoxia, in which animals enter suspended animation. Consistent with this, embryos do not require *san-1*, the spindle assembly checkpoint protein essential for suspended animation (Nystul and Roth, 2004), to survive exposure to hypoxia. Instead, HIF-1, the single worm homolog of the hypoxia-inducible factor (HIF) is required for embryo survival in 5,000–20,000 ppm O_2 (Jiang et al., 2001; Nystul and Roth, 2004). HIF is a highly conserved bHLH-PAS domain transcription factor that helps maintain O_2 homeostasis by coordinating the transcriptional response to hypoxia in metazoans. There are many excellent reviews of HIF function and its role in development and disease (e.g., Semenza, 2009, 2010, 2011, 2012; Majmundar et al., 2010; Powell-Coffman, 2010). HIF was first identified biochemically as the factor that bound the erythropoietin promoter in hypoxia (Wang and Semenza, 1993). HIF is directly regulated by O_2 levels. HIF is hydroxylated at the conserved proline in the LxxLAP motif by a 2-oxoglutarate-dependent prolyl hydroxylase of the EGLN family, named after *egl-9* in *C. elegans* (Epstein et al., 2001). Hydroxylated HIF is then recognized by an E3-ubiquitin ligase, the Von Hippel–Lindau factor VHL-1, and degraded by the proteasome (Kaelin, 2008). In hypoxia the hydroxylation is inefficient and HIF accumulates, dimerizes with the aryl hydrocarbon nuclear translocator (ARNT; *aha-1*), and induces expression of target genes that facilitate adaptation to hypoxia. In mammals, HIF is essential for early developmental events, and both HIF1α and HIF2α mutant mice die early in embryogenesis (Iyer et al., 1998; Compernolle et al., 2002). HIF homologs are also important for tracheal branching in *Drosophila* and neuronal patterning in *C. elegans*, highlighting the conserved role for HIF in development (Keith and Simon, 2007; Centanin et al., 2008;

Pocock and Hobert, 2008). Constitutive stabilization of HIF has been implicated in tumor progression and mutations in VHL, a negative regulator of HIF, are associated with Von Hippel–Lindau syndrome, which is characterized by renal clear cell carcinoma (Kim and Kaelin, 2004; Shen and Kaelin, 2012). Importantly, HIF-1 is not required for embryos to survive suspended animation in *C. elegans*, demonstrating that these two physiological responses to low O_2 are genetically distinct. Although HIF has been the focus of most studies into transcriptional responses to hypoxia, there is also evidence that other factors are involved. HIF-independent transcriptional responses to hypoxia have been observed in *C. elegans* and mammals (Dong et al., 2001; Shen et al., 2005; Piret et al., 2006; Ndubuizu et al., 2010). The factors that mediate these effects are not well understood.

Despite the fact there are at least two separate adaptive responses to low O_2 – suspended animation in anoxia or continued development in moderate hypoxia – there are hypoxic conditions that are lethal during embryogenesis. Isolated embryos die when exposed to O_2 concentrations between 100 and 1,000 ppm O_2 (Nystul and Roth, 2004). In these conditions, continued developmental progression is associated with increased lethality. Embryos exposed to 1,000 ppm O_2 undergo more cell divisions and experience a higher rate of lethality than those exposed to 100 ppm O_2, for 24 h (Nystul and Roth, 2004). Although the cellular mechanisms that underlie these defects are not well understood, it has been demonstrated that inducing suspended animation in isolated embryos using carbon monoxide rescues embryo survival in hypoxia (Nystul and Roth, 2004). Anoxia-induced suspended animation also protects *C. elegans* embryos against otherwise lethal cold exposure (Chan et al., 2010). These results suggest that arresting cell division and development facilitates coordination between cellular events and prevents irrevocable errors. Although embryos cannot autonomously engage suspended animation in these hypoxic conditions, embryos exposed to 1,000 ppm O_2 *in utero* arrest development and survive (Miller and Roth, 2009). Embryo survival *in utero* requires *san-1*, suggesting that the embryos are in a state genetically related to anoxia-induced suspended animation (Miller and Roth, 2009). We refer to this as a hypoxia-induced diapause, because it is reminiscent of mammalian embryonic diapause, in which the adults remain active but arrest development of embryos *in utero* (Renfree and Shaw, 2000). This embryonic diapause is coordinated by as-yet uncharacterized maternal factors that alter the uterine environment to impinge on embryonic development. Many facets of suspended animation and the mechanisms by which suspended animation can be non-autonomously controlled in the presence of O_2 remain a mystery and are likely to be a fruitful area of future research.

Developmental context also influences the response to hypoxia, with greater flexibility after embryogenesis. Newly hatched larvae survive in hypoxic conditions that are lethal to embryos (1,000 ppm O_2), and survival is associated with a reversible arrest of postembryonic development (Miller and Roth, 2009). This suggests that there are mechanisms that can arrest cell division in 1,000 ppm O_2, but that embryos cannot enact this response. The arrest of post-embryonic cell divisions is genetically distinct from suspended animation, in that *san-1* is not required to arrest cell division of germline stem cells (Miller and Roth, 2009). One caveat to this interpretation is that it has not been demonstrated that *san-1* is required for successful suspension of germline stem cell divisions in adults exposed to anoxia, and it is possible that suspended animation in adults employs different strategies to arrest cell division. Further delineation of the mechanisms used to arrest cell division in these conditions is required to evaluate this possibility. In addition to this developmental arrest, adults exposed to 1,000 ppm O_2 enter a reproductive diapause (Miller and Roth, 2009). Gravid adults cease laying eggs, arrest the development and fertilization of oocytes, and halt embryonic development *in utero*. The arrest of progeny production ensures that embryos are not produced into conditions where they cannot survive. Moreover, energy shunted away from reproductive activity can be used instead for locomotion to search for a new environment. Therefore, by delaying progeny production animals can find a time and place more suited to successful reproduction. In this way, hypoxia-induced reproductive diapause is similar to diapause in insects and mammals that ensures progeny production is synchronized with seasonal and nutritional conditions that maximize fitness (Renfree and Shaw, 2000; Tatar et al., 2001; Allen, 2007; Guidetti et al., 2008; Tachibana and Watanabe, 2008).

HIF-1 is not required for hypoxia-induced diapause, as animals with a null allele of *hif-1* arrest post-embryonic development and reproduction in 1,000 ppm O_2 as efficiently wild-type animals (Miller and Roth, 2009). Unlike the situation in embryos, *hif-1(−)* mutant larvae and adults exposed to 5,000 ppm O_2 survive 24 h with >90% viability to adult upon reoxygenation (Nystul and Roth, 2004; Miller and Roth, 2009). Nevertheless, HIF-1 is necessary for the normal response to 5,000 ppm O_2. Whereas wild-type animals continue development in these conditions, *hif-1(−)* mutant animals precociously enter into hypoxia-induced developmental and reproductive diapause (Miller and Roth, 2009). This observation supports the idea that responses to hypoxia are specific to the concentration of O_2 that is available, and that HIF-1 does not play a major role in the response to 1,000 ppm O_2. In fact, even constitutive activation of HIF-1, by loss-of-function mutations in negative regulator *vhl-1* or *egl-9*, does not prevent diapause in 1,000 ppm O_2. This result further suggests that HIF-1 promotes continued developmental activity in both larvae and embryos, though it may have different targets in each developmental context. Although *hif-1* is expressed in most, if not all, cells (Jiang et al., 2001), expression only in neurons is sufficient to regulate hypoxia-induced diapause in 5,000 ppm O_2 (Miller and Roth, 2009). This suggests that there are neuroendocrine signaling pathways that coordinate development with the response to hypoxia. In contrast, early stage *hif-1* mutant embryos die in 5,000 ppm O_2, suggesting that HIF-1 acts autonomously during embryogenesis, when the nervous system is not fully developed, to protect against hypoxia (Jiang et al., 2001; Nystul and Roth, 2004). The neuronal circuits and neuroendocrine factors that coordinate the systemic response to hypoxia have not been delineated, though it has been shown that hypoxia-induced diapause does not require the same neurons that mediate hyperoxia avoidance behavior (Gray et al., 2004; Miller and Roth, 2009).

The AMP-activated protein kinase (AMPK)is also involved in regulating hypoxia-induced diapause in 5,000 ppm O_2. AMPK

is a conserved serine/threonine kinase that is important for cellular energy homeostasis. In response to disruptions of energy homeostasis, AMPK is activated and phosphorylates targets that increase energy production and decrease energy expenditures (Carling et al., 2011; Hardie, 2011; Mantovani and Roy, 2011; Mihaylova and Shaw, 2011). AMPK is a heterotrimeric protein that consists of a catalytic α subunit and the regulatory β and γ subunits (Hardie et al., 2003). The *C. elegans* genome encodes genes for two AMPK α subunits, *aak-1* and *aak-2*, two β subunits, *aakb-1* and *aakb-2*, and five γ subunits, *aakg-1–5* (Beale, 2008). In 1,000 ppm O_2 *aak-2(lf)* mutant animals are fully capable of entering into and surviving diapause. However, *aak-2(lf)* mutant animals precociously enter diapause in 5,000 ppm O_2 (Miller and Roth, 2009). Thus, like HIF-1, AAK-2 acts to oppose diapause in hypoxia and support continued developmental activity. AAK-2 is not required for embryonic or larval survival in either 1,000 or 5,000 ppm O_2 (Miller and Roth, 2009), though it is required for long-term survival in anoxia (LaRue and Padilla, 2011). The source of this discrepancy could be either the duration or severity of O_2 deprivation. Another possibility is that AMPK has different function in different hypoxic conditions. This could result if different AMPK complexes are active in each O_2 concentration. In addition to *aak-2*, *aakb-1/2* and *aakg-2* contribute to long-term survival in anoxia (LaRue and Padilla, 2011). It is not known which subunits other than *aak-2* are involved in coordinating hypoxia-induced diapause. Another possibility is that different AMPK substrates mediate these different physiological effects, depending on context.

The mechanisms by which AMPK integrate with cellular and developmental functions to regulate hypoxia-induced diapause have not been defined. Full activation of AMPK requires phosphorylation of the α subunit by an activating kinase. Genetic studies suggest there are at least three kinases upstream of AMPK, including LKB1 (*par-4*), Ca^{2+}/calmodulin-dependent protein kinase kinase (*ckk-1*), and the MAP kinase kinase kinase TAK1 (*tap-1*; Carling et al., 2008; www.wormbase.org WS231). AMPK is also stimulated by AMP (which increases when ATP levels fall), but in mammalian systems hypoxia activates AMPK independent of ATP levels (Laderoute et al., 2006; Liu et al., 2006; Papandreou et al., 2008). The importance of these upstream kinases in different hypoxia contexts has not been investigated. In mammalian cells, activation of AMPK by hypoxia is abrogated by depletion of CAMKKβ but not LKB1 (Mungai et al., 2011). The role of TAK1 in regulating AMPK homologs in animals is still a matter of investigation. Recent proteomic studies have revealed that AMPK directly phosphorylates many components of the cell cycle machinery (Banko et al., 2011). These studies suggest a preliminary model in which HIF-1 acts upstream or in parallel to AMPK, which regulates cell division in hypoxia. Working out the mechanistic details that govern this effect is likely to provide unique insight into how AMPK coordinates cellular activities in response to metabolic stress.

RELATIONSHIP BETWEEN HYPOXIA AND FOOD DEPRIVATION

Hypoxia and food deprivation are similar stresses in that they both affect central aspects of cellular metabolism. The absence of either food or O_2 disrupts energy-generating pathways, and there are similarities in physiological responses and molecular genetic pathways that are activated in these two situations. The integration of these pathways is highlighted by the interactions between hypoxia and nutrient availability. Rats that are subject to alternate-day feeding have reduced neuronal damage and improved behavioral outcomes after focal cerebral ischemia (Yu and Mattson, 1999). Similarly, mice that are fasted for only 3 days are resistant to surgically induced renal and hepatic ischemia/reperfusion (I/R) injury (Mitchell et al., 2010; Verweij et al., 2011). In contrast, both severe and moderate food restriction decrease survival after gut I/R from occlusion of the superior mesenteric artery (Ueno et al., 2005). Given the therapeutic potential, there is much interest in understanding the mechanistic basis of the interaction between fasting and hypoxia and I/R.

In both *C. elegans* and *Drosophila* exposure to hypoxia increases lifespan, though the relationship is not linear and different hypoxic conditions increase lifespan in these species (Honda et al., 1993; Mehta et al., 2009; Rascón and Harrison, 2010). Decreased food intake, DR, also increases lifespan in these and other species (Koubova and Guarente, 2003; Bishop and Guarente, 2007; Fontana et al., 2010), though there are some genetic backgrounds and species in which DR does not increase lifespan (Mockett et al., 2006; Swindell, 2012). Many genetic pathways that are involved in mediating the effects of DR on lifespan also have roles in the response to hypoxia, and vice versa. This suggests that responses to DR and hypoxia may physiologically interact as well. Indeed, dietary conditions that maximize lifespan are different for *Drosophila* in hypoxia and normoxia. Flies chronically adapted to 50,000 ppm O_2 live longer at lower yeast (protein) levels than normoxic cohorts (Vigne and Frelin, 2007). In *C. elegans* the IIS pathway downstream of *daf-2*, *aak-2*, and the target of rapamycin (TOR) kinase *let-363* have all been shown to be important for increased lifespan in DR (Greer and Brunet, 2009). As noted above, both *daf-2* and AMPK are important in mediating responses to decreased O_2. Although a role of TOR/*let-363* in *C. elegans* hypoxia response has not been demonstrated, TOR is negatively regulated by AMPK, and TOR mediates the translational arrest observed in mammalian cells exposed to hypoxia (Liu et al., 2006; Lee et al., 2008). This suggests the possibility that these factors mediate increased lifespan in response to both decreased food and hypoxia.

HIF-1 has recently been shown to modulate lifespan in *C. elegans*. Curiously, both *hif-1(−)* and *vhl-1(−)* mutant animals, which have constitutively stabilized HIF-1, exhibit increased lifespans (Chen et al., 2009; Mehta et al., 2009; Zhang et al., 2009). It may be that different environmental contexts underlie this effect. The *hif-1* mutant was subsequently shown to be long-lived at low temperature but not at high temperature (Leiser et al., 2011). HIF-1 is required for *C. elegans* to adapt to changes in temperature (Treinin et al., 2003), and HIF is stabilized in both crucian carp and mice exposed to high temperature (Katschinski et al., 2002; Rissanen et al., 2006). Thus, HIF may have an important role in responding to thermal stress as well as hypoxia. Notably, the *hif-1(−)* mutant animal does not have increased lifespan in DR. While *C. elegans* that overexpress HIF-1 due to a mutation in *egl-9* show modest increases in lifespan under DR, the effect is

blunted compared to wild-type animals (Chen et al., 2009). These results suggest that *hif-1* may be generally involved the response to decreased food and well as decreased O_2. Longevity mediated by both DR and mutation of *hif-1* require the ER stress signaling genes *ire-1* and *xbp-1*, which function to activate the UPR (Chen et al., 2009), suggesting an interaction between ER stress and nutrient sensing. In mice HIF is stabilized by glucose in POMC neurons in the hippocampus, and plays a role to regulate feeding and organismal energy balance (Zhang et al., 2011). Together, these observations suggest that HIF may coordinate a conserved integration of nutrient sensing with hypoxia. It will be important to further understand the mechanisms by which these response pathways to determine if this is a direct effect.

Developmental arrest is a common response to both food deprivation and hypoxia in *C. elegans*. Larvae that hatch in conditions without food do not initiate post-embryonic development and can persist for weeks in this state, referred to as the L1 diapause. Similarly, if food deprivation occurs in the last larval stage, L4, animals can enter into an adult reproductive diapause that is characterized by the arrest of oocyte production and fertilization (Angelo and Van Gilst, 2009). The arrest observed in hypoxia-induced diapause is superficially similar to L1 diapause, as both somatic and germline development arrest in animals on food in 1,000 ppm O_2. However, there are differences in the genetic factors required in each situation. Neither *daf-16*, the FOXO transcription factor downstream of the IIS pathway, nor the PTEN homolog *daf-18* is required for larvae to reversibly arrest development and survive for 24 h in 1,000 ppm O_2 (Miller and Roth, 2009). In contrast, loss-of-function mutations in *daf-16* or *daf-18* abrogate the ability to maintain developmental arrest and survive food deprivation (Baugh and Sternberg, 2006; Fukuyama et al., 2006). This discrepancy suggests that L1 arrest involves different mechanisms in each condition, though it is possible that the difference stems from longer duration of arrest in the starvation experiments. Another feature that distinguishes hypoxia-induced diapause from starvation is that hypoxia can arrest development at any point, whereas there seem to be specific points in development in which food withdrawal can cause developmental arrest (Angelo and Van Gilst, 2009; Miller and Roth, 2009; Seidel and Kimble, 2011). This difference may stem from the fact that O_2 must be continuously acquired from the environment whereas fats, proteins, and sugars can be stored for later use. After extended periods of starvation in the adult reproductive diapause the germline retracts until only a small population of stem cells remains (Angelo and Van Gilst, 2009). The nuclear hormone receptor *nhr-49* is required to appropriately enter into starvation-induced adult reproductive diapause. In contrast, the germline remains intact in hypoxia, and suspension of reproduction does not require *nhr-49* (Miller and Roth, 2009 and our unpublished observation). As in hypoxia, when gravid adults are removed from food they arrest egg-laying. In starved adults embryo development *in utero* continues until the progeny hatch and devour the adult from within, a process known as "bagging" or facultative vivipary (Chen and Caswell-Chen, 2004; Schafer, 2005). The arrest of embryo production in development in hypoxia prevents bagging, however. It has been reported that embryos also arrest in the uterus of adults in starvation-induced adult reproductive diapause (Angelo and Van Gilst, 2009), though this result has been recently questioned (Seidel and Kimble, 2011).

If food is scarce in development, *C. elegans* will enter an alternative larval stage called dauer, where development arrests until conditions improve. High temperature and crowding also influence the dauer decision. Developmental arrest in dauer is regulated by neuroendocrine signals as well as the IIS and TGFβ signaling pathways (Hu, 2007; Fielenbach and Antebi, 2008). The IIS pathway does not have an apparent role in hypoxia-induced diapause (Miller and Roth, 2009). However, some genes regulated by hypoxia are also regulated by entry into dauer, and at high temperature *hif-1(−)* mutant animals arrest as partial dauers (Shen et al., 2005). This suggests that there is cross-talk between the IIS pathway and *hif-1*. Similarly, AMPK is required for normal response to hypoxia and in dauer. In dauer, germ cell divisions do not arrest appropriately in *aak-2* mutant animals (Narbonne and Roy, 2006). Thus, in contrast to hypoxia, where AAK-2 promotes cell division and development, in dauer it is required to arrest of germline cell divisions. This observation further suggests that AMPK has different roles in regulating developmental progression in specific physiological contexts.

INTERACTIONS BETWEEN H_2S SIGNALING AND HYPOXIA

Emerging evidence suggests that H_2S signaling can modulate the physiological effects of hypoxia in mammals. H_2S is naturally produced in animal cells as a product of amino acid metabolism though the transsulfuration pathway (Dominy and Stipanuk, 2004; Stipanuk, 2004). Endogenously produced H_2S has many important roles in cellular signaling, neuromodulation, and regulation of vascular tone (Kimura, 2011; Vandiver and Snyder, 2012; Wang, 2012). At low concentrations exogenous H_2S has dramatic physiological effects that improve survival in changing conditions. Mice exposed to 80 ppm H_2S, in otherwise normal room air, enter into a suspended-animation-like state in which basal metabolic rate is depressed and core body temperature is maintained only slightly above ambient (Blackstone et al., 2005; Volpato et al., 2008). Mice exposed to low H_2S survive in otherwise lethal hypoxia (Blackstone and Roth, 2007), and H_2S improves outcome in a variety of mammalian models of I/R spanning multiple organ systems, including myocardial infarct, hepatic I/R, and lung injury from smoke inhalation (Szabó, 2007; Nicholson and Calvert, 2010; King and Lefer, 2011).

The mechanisms by which H_2S signaling integrates with hypoxia are not well understood. Pharmacological inhibitors of K_{ATP} channels and protein kinase C (PKC) abrogate the protective effect of NaHS, the ionized form of H_2S, in a neuronal cell culture model of hypoxic injury (Tay et al., 2010). Similarly, the vasodilatory effects of NaHS depend partially on plasma membrane K_{ATP} subunit SUR2 (Liang et al., 2011). The ability for H_2S to stimulate rat K_{ATP} channels heterologously expressed in HEK293 cells requires specific cysteine residues (Jiang et al., 2010), suggesting that H_2S directly sulfhydrates the K_{ATP} channel to modulate its activity. However, endogenously produced H_2S post-translationally modifies up to 80% of cellular proteins (Mustafa et al., 2009), and elucidating the functionally relevant targets of H_2S in different contexts is a major challenge. In addition to

K_{ATP} channels, H_2S has been proposed to directly activate mitochondrial energy production in smooth muscle of mice (Fu et al., 2012). Similar activity has been reported for ciliated mussel gills (Doeller et al., 1999) and isolated chicken liver mitochondria (Yong and Searcy, 2001), suggested that the ability to stimulate cellular energy production may be a conserved features of H_2S (Theissen et al., 2003; Olson, 2012). Cardioprotective effects of H_2S administration in murine models of myocardial ischemia require the Nrf2 transcriptional factor (Calvert et al., 2009, 2010). *C. elegans* require the Nrf2 homolog *skn-1* to survive H_2S, and some early transcriptional changes in H_2S depend on *skn-1* (Miller et al., 2011). SKN-1 is important for the response to various oxidative stresses, though the gene products that are regulated can vary depending on context (An and Blackwell, 2003; Oliveira et al., 2009; Li et al., 2011). SKN-1 is required for increased stress resistance and lifespan resulting from inhibiting either TOR or IIS (Tullet et al., 2008; Robida-Stubbs et al., 2012), and it is also required in the two ASI neurons for increased lifespan by DR (Bishop and Guarente, 2007).

The transcriptional response to H_2S requires *hif-1* in *C. elegans*, suggesting a potential mechanistic link between the response to hypoxia and H_2S. HIF-1 is stabilized and accumulates in the nucleus upon exposure to H_2S in *C. elegans* (Budde and Roth, 2010). Similarly, NaHS induces expression and accumulation of HIF in rat endothelial cells (Liu et al., 2010). Increased expression of *hif-1* target genes and survival in H_2S requires CYSL-1, which binds to EGL-9 and is proposed to inhibit its ability to hydroxylate HIF-1 (Budde and Roth, 2010; Ma et al., 2012). CYSL-1 is member of the cystathionine β-synthase/cysteine synthase family of pyridoxal-5′-phosphate (PLP)-dependent enzymes that has *O*-acetylserines ulfhydrylase activity *in vitro* (Ma et al., 2012). All of the transcripts that accumulate after 1 h exposure to H_2S require *hif-1* (Miller et al., 2011). However, it is not yet clear how H_2S effects on HIF contribute to protection in hypoxia. The *hif-1*-mediated response is essential for animals to survive exposure to H_2S (Budde and Roth, 2010), whereas *hif-1(ia04)* mutant animals can survive 24 h exposure to hypoxia (Miller and Roth, 2009). Moreover, there is curiously little overlap between gene products that require *hif-1* to accumulate in response to hypoxia and H_2S (Miller et al., 2011). The source of this variation has not been determined, but could reflect different tissues of activity, other cooperating transcription factors, or context-dependent effects on HIF-1 activity deriving from other signaling events.

H_2S increases lifespan and thermotolerance in *C. elegans* (Miller and Roth, 2007), and overexpression of dCBS, a H_2S-producing enzyme in the transsulfuration pathway, modestly increases lifespan in *Drosophila* (Kabil et al., 2011). Pharmacological inhibition of dCBS and RNAi-mediated knockdown of dCBS abrogates increased lifespan by DR (Kabil et al., 2011). These experiments suggest the possibility that H_2S signaling also integrates with nutrient sensing pathways. In *C. elegans* the effects of H_2S on lifespan require the conserved sirtuin, *sir-2.1* (Miller and Roth, 2007). Sirtuin activity is intricately linked with metabolic adaptations to stress, as its activity can be modulated by changes in redox state and metabolic status (Yang et al., 2006; Schwer and Verdin, 2008; Weyrich et al., 2008; Longo, 2009; Yu and Auwerx, 2009; Donmez and Guarente, 2010; Haigis and Yankner, 2010). In mammals, the SIRT1 sirtuin deacetylates and activates HIF (Lim et al., 2010). This suggests the possibility that *sir-2.1* activates *hif-1*, leading to physiological responses to H_2S that increase lifespan.

CONCLUSION

Signaling pathways that mediate responses to decreased O_2, food deprivation, and H_2S are integrated with fundamental aspects of cellular physiology and metabolism. As a result, these (and other) stress responses depend on the initial state of the organism. Anything that changes the physiological state – such as aging or previous stress exposure – will necessarily change response(s) to subsequent stresses. In this way, stress responses can be considered to be path dependent: the initial conditions determine the magnitude and trajectory of the response. A greater understanding of the systems biology of stress responses will provide insight into how physiological systems change with age, and may suggest new strategies to delay age-associated disruptions in homeostasis.

Many important questions remain to be answered that will advance our understanding of mechanisms that underlie how context-dependent stress responses are coordinated. For example, we understand little about how conserved factors such as AMPK and HIF have different effects in different conditions. The physiological basis for H_2S signaling effects physiological functions, including lifespan and stress response are relatively unexplored. Similarly, the mechanisms by which proteostasis networks are integrated with conditional stress responses are not well understood. In order to address questions requires that both genetic and environmental conditions can be precisely controlled experimentally. The power of genetically tractable model organism systems provides great promise in this regard, as do unbiased approaches that have the potential to reveal novel regulators in these responses. Moreover, these studies will reveal neuroendocrine signaling factors that coordinate the organism-wide response to changing conditions.

Insufficient or inappropriate responses to hypoxia contribute to the progression of many human diseases, suggesting that it may be possible to exploit context-dependent physiological responses for clinical benefit. For example, the observation that the fasting response protects normal cells from chemotherapeutic agents more than cancerous cells led to the simple idea of using fasting to improve the efficacy of chemotherapeutics (Raffaghello et al., 2008; Powell-Coffman, 2010; Lee et al., 2012). This promising study demonstrates the importance of understanding how diverse stress responses are coordinated with each other and is an excellent example of the promise of this emerging research area.

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Neuronal responses to physiological stress

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Physiological stress can be defined as any external or internal condition that challenges the homeostasis of a cell or an organism. It can be divided into three different aspects: environmental stress, intrinsic developmental stress, and aging. Throughout life all living organisms are challenged by changes in the environment. Fluctuations in oxygen levels, temperature, and redox state for example, trigger molecular events that enable an organism to adapt, survive, and reproduce. In addition to external stressors, organisms experience stress associated with morphogenesis and changes in inner chemistry during normal development. For example, conditions such as intrinsic hypoxia and oxidative stress, due to an increase in tissue mass, have to be confronted by developing embryos in order to complete their development. Finally, organisms face the challenge of stochastic accumulation of molecular damage during aging that results in decline and eventual death. Studies have shown that the nervous system plays a pivotal role in responding to stress. Neurons not only receive and process information from the environment but also actively respond to various stresses to promote survival. These responses include changes in the expression of molecules such as transcription factors and microRNAs that regulate stress resistance and adaptation. Moreover, both intrinsic and extrinsic stresses have a tremendous impact on neuronal development and maintenance with implications in many diseases. Here, we review the responses of neurons to various physiological stressors at the molecular and cellular level.

Keywords: stress responses, neuronal homeostasis, aging, developmental stress

INTRODUCTION

Stress is an inherent component of the natural world that applies to virtually all the biological systems. Biological stress signifies any condition that forces living systems away from a physiological steady state, and its impact is closely connected to the nature of elements that shape living organisms. As stress can be applied to many different levels of biological organization, the term has been used in many different contexts to date. "Physiological stress" is referred to as the primary biological stress and can be defined as any external or internal condition that challenges the homeostasis of a cell or an organism. Taking into account the different possible sources of biological stress, we can conceive three different aspects of physiological stress: environmental stress, intrinsic developmental stress, and aging (**Figure 1**).

ENVIRONMENTAL STRESS

Biological systems are designed to develop and live in a variety of changing environments. During evolution many different adaptations have been developed to provide organisms with the ability not only to survive but also to reproduce under different, often hostile conditions. Such adaptations are associated with specific structures and behaviors tailored to a specific environment. At the molecular level different strategies exist that are used by cells and systems to respond and adapt to environmental changes such as changes of oxygen availability and temperature fluctuations. Environmental variations exceeding certain levels define "environmental stress."

INTRINSIC DEVELOPMENTAL STRESS

An additional cause of physiological stress can be assigned to developmental events. As living organisms develop, they face a variety of challenges associated with morphogenesis and changes in inner chemistry. Indeed, rapid development of embryos causes massive internal changes to an organism as it grows and changes morphology. Different developmental events can cause different stressful conditions with some being harsher than others. Therefore adaptation to developmental stress is equally crucial for the survival of individuals and species.

AGING

When development completes and organismal maturation is achieved, environmental stress is only one aspect of the physiological stress that challenges individuals. Aging constitutes another burden that living organisms have to cope with during their life. Even though aging has often been considered as the deteriorative result of different stresses, it can also be seen as an extra layer of stress throughout life due to the thermodynamic properties of biological materials that lead to the stochastic accumulation of molecular damage over time. The capacity of each organism to cope with aging and other stresses defines its longevity. Thus, functional decline through aging, which occurs even under physiologically perfect environmental conditions, may partly constitute the impact of entropy on organisms and reveals possible imperfections in homeostatic mechanisms.

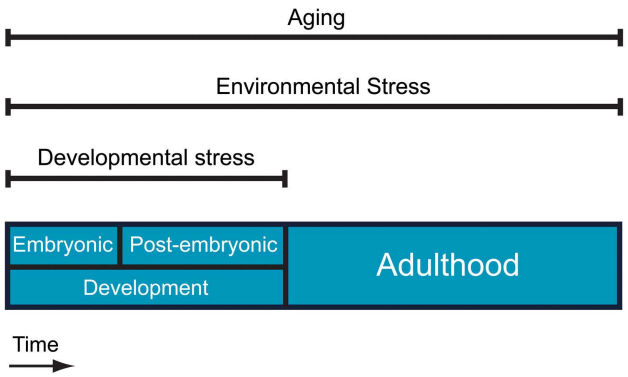

FIGURE 1 | Different aspects of physiological stress. Aging and environmental stress are present throughout life whereas intrinsic developmental stress applies only during embryonic and post-embryonic development.

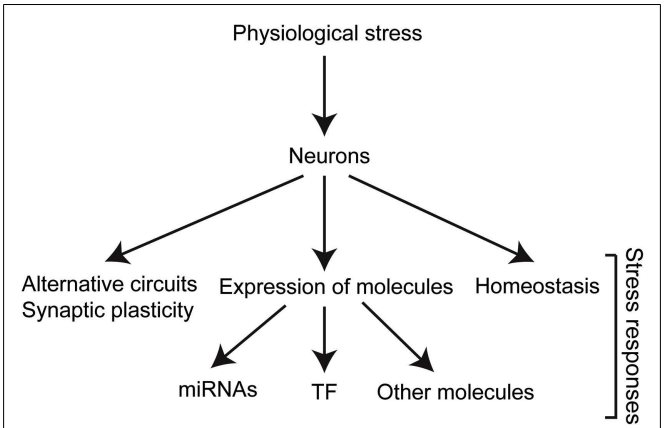

FIGURE 2 | Stress triggers biological responses in different levels of organization. The different molecules involved dictate the changes required for adaptation, and therefore survival and reproduction. TF, transcription factors.

ROLE OF NEURONS

Research over the years has identified neurons as major players in stress responses. Neurons do not only receive and process information from the environment but they also have an important direct impact on different aspects of the stress response (**Figure 2**). In order for neurons to fulfill their roles in stress responses, specific molecules are temporally regulated in response to changes in internal or external conditions.

AIM OF THE REVIEW

This review aims to provide examples that demonstrate the important role of neurons in physiological stress responses as well as the impact of physiological stress on neurons at the intercellular, cellular, and molecular level.

NEURONAL RESPONSES

ENVIRONMENTAL STRESS AND NEURONAL CIRCUITS

Hypoxia

Sensory neurons form cellular networks through which information from the environment is processed. In lower organisms these networks are relatively simple and stereotypic, and therefore they constitute attractive models to study the effects of physiological stress on neuronal information processing. From studies in *C. elegans*, in which the entire neuronal circuitry has been mapped (White et al., 1986), it has been found that stress can alter the processing of sensory information. In particular, under hypoxia, a latent circuit is engaged in the processing of gustatory information that is not normally used in normoxic conditions (Pocock and Hobert, 2010). In contrast, the aerotaxis neuronal circuit becomes simplified and less flexible after a hypoxic insult in the same organism (Chang and Bargmann, 2008). Interestingly, food-sensing and O_2-sensing circuits in *C. elegans* can be altered by a natural gene variation underlining the specific adaptation of the neuronal circuits of different strains in diverse local environments (Cheung et al., 2005). Such plasticity described here alters behavioral responses that may provide the organism with advantages under stress. In higher organisms, where neuronal networks are extremely complex, there is a lack of information as to whether stress can alter the information flow through alternative neuronal networks in a similar way. However, in rats and other animals the so-called "cross phrenic phenomenon" has been observed where a latent respiratory motor pathway is activated by hypoxia, mediating faster recovery from spinal injury (Zhou et al., 2001). Together these examples show the functional plasticity of neuronal circuits and how they can alter information processing in response to environmental stress.

Preconditioning

The functionality of neuronal circuits under harsh environmental conditions can also be dependent on the prior exposure to different stresses (Robertson, 2004). Neurons that have been exposed previously to acute sub-lethal stress appear to retain a memory that allows them to survive and respond to higher doses of this stress than before their initial exposure. This phenomenon is called "preconditioning" or "neurohormesis" (Mattson and Cheng, 2006). Characteristic examples are the enhanced thermotolerance of neurons by prior heat shock in *Drosophila* (Karunanithi et al., 1999), in locusts (Dawson-Scully and Meldrum Robertson, 1998; Wu et al., 2001), and in *C. elegans* (Kourtis et al., 2012). Other examples include neuroprotection by prior hypoxic insult to subsequent ischemic conditions in mice (Miller et al., 2001), gerbils (Kitagawa et al., 1991), and in neuronal cell culture (Bruer et al., 1997). Recently it was shown that in piglets, ischemic preconditioning of a distant ischemic tolerant tissue protects the brain against ischemic injury, a phenomenon that is called "remote ischemic preconditioning," and highlights the complexity of preconditioning mechanisms (Jensen et al., 2011). Interestingly, preconditioning of neurons can also be achieved by low doses of toxins naturally present in fruits and vegetables (Mattson and Cheng, 2006). In addition, exposure to a stress can induce tolerance to a different stress, a phenomenon that is called "cross-tolerance." For example, in locusts, prior exposure to anoxia induces thermotolerance in neurons that control flight (Wu et al., 2002). In rats, prior exposure to high temperature enhances tolerance to spinal cord ischemia

(Zhang et al., 2000). Finally, heat stress in murine cortical cell cultures enhance tolerance to combined oxygen and glucose deprivation (Snider et al., 1998). Preconditioning of neuronal circuits consist of an adaptive mechanism that uses prior experience to better confront hostile conditions. Moreover, stress cross-tolerance is a natural demonstration of the existence of common response mechanisms to different stresses such as high temperature and lack of oxygen.

Synaptic and neuronal network remodeling/plasticity

Another way that neurons respond to physiological stress is by altering their synaptic strength (functional plasticity) and/or connectivity pattern (structural plasticity) in a manner that promotes adaptation. In contrast to organisms like *C. elegans* where the neuronal connectivity appears to be stereotypical between individuals (White et al., 1986), the adult brain of higher animals exhibits a remarkable level of plasticity and the neuronal networks of certain regions can be altered under different conditions during and after their development (Pascual-Leone et al., 2011). Although this phenomenon has been mainly associated with learning and memory, it can also occur as a response to external stimuli and contributes to the homeostasis of the nervous system. For instance, sensory experience promotes the synaptic integration of new neurons into olfactory circuits in the mouse (Arenkiel et al., 2011). Moreover, chronic intermittent hypoxia alters the synaptic properties in a hub sensory circuit in rats (Kline et al., 2007), and in the peripheral chemoreceptor cells in the mollusk *Lymnaea stagnalis* synapses develop *in vitro* that exhibit a form of short-term synaptic plasticity in response to hypoxia (Bell et al., 2007). Finally, it is also characteristic that short-term plasticity of synapses is strongly dependent on temperature (Klyachko and Stevens, 2006). These examples show functional and structural plasticity of the nervous system in response to external stimuli.

ENVIRONMENTAL STRESS AND INDIVIDUAL NEURONS

Hypoxia

In addition to different responses that neurons exhibit upon particular stresses, they also exhibit various stress-related phenotypes. Because neurons are highly active cells, they require high amounts of oxygen in order to survive and function. Thus, hypoxia can have a tremendous impact on the physiology of animal brains. Limited oxygen availability during development (Lipton, 1999), at birth (Arpino et al., 2005; Gozzo et al., 2009), and later in life (Lipton, 1999) can cause irreversible damage to neuronal tissue. Hypoxia can also have an effect on axon outgrowth in a rat neuronal cell line (O'Driscoll and Gorman, 2005). In addition, specific *in vivo* developmental defects of individual neurons caused by hypoxia have been demonstrated in *C. elegans*. In this model, axon guidance and neuronal migration is defective in specific types of neurons under hypoxic conditions as a result of hypoxia inducible factor-1 hypoxia inducible factor (HIF)-1 stabilization (Pocock and Hobert, 2008). A recent study has also shown that similar defects are observed in the central nervous system of zebrafish via a similar pathway, providing strong evidence that this mechanism is conserved (Stevenson et al., 2012). Thus, hypoxia can have a pleiotropic impact on diverse neurons in different organisms.

Heat shock response

Increase in temperature (hyperthermia) is also known to affect many cell types. However, neurons are particularly susceptible to elevations in temperature, and organismal death under high temperature can be a result of neuronal malfunction before other cells fail (Robertson, 2004). High temperature mainly causes protein misfolding, which triggers cell autonomous and cell-non-autonomous responses (Ramirez et al., 1999). Certain sensory neurons are responsible for sensing the optimal temperature in freely moving animals as well as for the mediation of thermonociception (Clark et al., 2007; Liu et al., 2012). An interesting study in *C. elegans* has shown that such sensory neurons play a critical role in the cell-non-autonomous heat shock response of somatic cells (Prahlad et al., 2008). In addition, these neurons have also been shown to regulate a response to chronic stress caused by intracellular accumulation of misfolded proteins in remote somatic cells (Prahlad and Morimoto, 2011). Finally, the ciliated ASI chemosensory neurons in the same organism remotely regulate the proliferation vs. differentiation decision in gonads by secreting DAF-7/TGFβ when environmental conditions are favorable (Dalfo et al., 2012). In these ways, sensory neurons regulate systemic stress non-cell autonomously by integrating environmental inputs.

DNA damage response

Genotoxic factors such as UV and other electromagnetic radiations can also cause serious damage to neurons by damaging both their nuclear (Ide et al., 2000) and mitochondrial DNA (LeDoux et al., 2007). In contrast to other cell types that undergo cell cycle checkpoint arrest upon DNA damage, neurons seem to engage components of the cell cycle machinery in response to such insults (Park et al., 1997), as well as in response to other stresses such as ischemic hypoxia (Li et al., 1997; Timsit et al., 1999). Such cell cycle entry of neurons upon stress has been correlated with the apoptotic death after extensive DNA damage (Park et al., 1998; Herrup et al., 2004; Kruman et al., 2004). Moreover, entry of neurons into the cell cycle has also been correlated to neuronal cell death as an early disease related process (Herrup, 2012).

The majority of information on different DNA repair mechanisms available in cells comes from studies in non-neuronal mammalian cell systems. Despite the existence of diverse DNA repair pathways, base excision repair (BER) and nucleotide excision repair (NER) are the major mechanisms responsible for repairing oxidative-induced and UV-induced damage respectively, in both nuclear and mitochondrial DNA (Seeberg et al., 1995; Lagerwerf et al., 2011). Details of NER (Lagerwerf et al., 2011) and BER (Robertson et al., 2009) pathways have been recently reviewed. The gradual maturation of such repair mechanisms in neurons is shown by the fact that mature neurons appear to be more resistant to UV- and IR-induced DNA damage than their younger counterparts (Romero et al., 2003; Shirai et al., 2006). Neurons are also more resistant to IR-induced apoptosis compared to neuronal precursor cells (Kameyama and Inouye, 1994) and other cell types (Li et al., 1996). This highlights the importance of efficient DNA damage response (DDR) mechanisms to maintain mature post-mitotic cells, like neurons, which cannot be replenished (Romero et al., 2003). IR irradiation also affects multiple behavioral outputs of different species by affecting neurons

(Sakashita et al., 2010 and references herein). Particularly in *C. elegans*, IR differentially affects neuron subtypes (Sakashita et al., 2010), which could also suggest that DNA repair efficiency varies between neuron types. Endogenous DNA damage also occurs in neurons. Neurons are highly active cells producing high levels of reactive oxygen species (ROS) that results in increased DNA damage in nuclear and mitochondrial DNA (LeDoux et al., 2007; Barzilai et al., 2008). Responses to endogenous DNA damage are crucial to enable correct neuronal development, and subsequent neuronal maintenance (LeDoux et al., 2007; Lee and McKinnon, 2007; Barzilai et al., 2008). The importance of the DDR in the absence of external mutagenic factors is also supported by genetic conditions in humans that cause developmental defects (O'Driscoll and Jeggo, 2006; Barzilai et al., 2008). Finally, neurons can remotely protect other tissues from insults such as ionizing irradiation. A striking example comes from *C. elegans* where DNA damage-induced apoptosis in the worm gonad is negatively regulated by a pathway involving HIF-1. Specifically, HIF-1 acts in ASJ amphid sensory neurons to upregulate the tyrosinase family member TYR-2. TYR-2 is subsequently secreted from these neurons and downregulates CEP-1, the homolog of p53, in gonads thereby suppressing radiation-induced apoptosis (Sendoel et al., 2010). It is thus clear that neurons are especially susceptible to both exogenous and endogenous genotoxic reagents and cells can react in both a cell autonomous and non-cell autonomous manner to promote survival.

INTRINSIC DEVELOPMENTAL STRESS AND THE NERVOUS SYSTEM

Stressful conditions in the internal somatic microenvironment of organisms can be caused by development (Simon and Keith, 2008). Even though development has been recognized as an additional layer of stress, there is limited knowledge as to how it influences the nervous system. Developmental stress is unique in its nature by being stereotypical during embryonic and postembryonic life. Therefore, the responses to this stress have to be embedded into neurons and in some cases may serve as a necessary part of their development. The most well studied stressor during development is intrinsic hypoxia. In the developing embryo, hypoxic regions naturally occur as a result of limited O_2 distribution (Simon and Keith, 2008) where the major hypoxia regulator, HIF-1 plays extensive roles (Dunwoodie, 2009). Interestingly, low oxygen levels during development are important for differentiation of many cells and tissues (Morriss and New, 1979; Maltepe and Simon, 1998; Simon et al., 2002) and studies in neuronal cell culture suggest that this might also be true for neurons (Morrison et al., 2000; Studer et al., 2000). Therefore, it seems that development uses an inherent stressful condition, such as embryonic hypoxia, as a signal to form various structures. Thus, it has been suggested that O_2 functions as a developmental morphogen (Simon and Keith, 2008). In addition, the observed hypoxic tolerance of developmentally immature neurons compared to mature neurons is indicative of the adaptation of neurons to developmentally derived hypoxia (Bickler and Buck, 1998).

Neurons can also help the embryo to overcome developmental stress at the behavioral level. In the pond snail *Helisoma trivolvis* the cilia-driven rotational behavior of early embryos facilitates gas exchange with the surrounding liquid and is regulated by a pair of serotonergic sensory-motor cells that sense oxygen levels (Kuang and Goldberg, 2001; Kuang et al., 2002). This embryonic behavior has also been observed in other species such as in the pond snail *Lymnaea stagnalis* (Byrne et al., 2009). Other stressors can also be present during development. For example, oxidative stress is produced as a result of routine adult neurogenesis (Walton et al., 2012). The developing brain has adopted a variety of different defenses against developmentally derived oxidative stress (Ikonomidou and Kaindl, 2011), such as differential expression of antioxidant systems during brain development (Aspberg and Tottmar, 1992). Finally, the nervous system is subjected to mechanical stress via movement and from the increasing mass of the brain during development (Van Essen, 1997; Benard and Hobert, 2009). This developmentally derived mechanical stress results in a variety of neuronal responses at different levels (Benard and Hobert, 2009). The above examples describe the existence of developmental stress as an important aspect of development and stress biology. Neurons not only adapt to stress but they also *require* specific stressors for correct development to occur.

AGING AND THE NERVOUS SYSTEM

Aging is perceived as the deteriorative effect of time on different structures of living organisms and many theories have been developed to date to explain how aging evolved in different organisms (Kirkwood and Austad, 2000). Early studies showed a relation between sensory neurons and longevity (Apfeld and Kenyon, 1999). Different types of sensory neurons have been found to regulate *C. elegans* lifespan in both positive and negative manner, emphasizing the underlying complexity of such regulation (Alcedo and Kenyon, 2004; Bishop and Guarente, 2007; Lee and Kenyon, 2009; Shen et al., 2010a,b). Neurons can impact on longevity in a non-cell autonomous manner. For example, upon neuronal specific mitochondrial stress a cue from the nervous system in *C. elegans* induces the mitochondria-specific unfolded protein response in intestinal cells, thus increasing the lifespan of the animals (Durieux et al., 2011). In addition, neuroprotection plays a critical role in longevity and aging (Murakami, 2007) and the regulation of aging via neurons appears to be conserved in *Drosophila* (Parkes et al., 1999; Libert et al., 2007). This association between sensory neurons and longevity shows the non-cell autonomous impact that environmental cues have in organismal aging.

Aging is also known to impact on individual neurons. Beyond pathological conditions, e.g., Alzheimer's disease and Parkinson disease, which have been well documented over the years (Yankner et al., 2008; Hung et al., 2010), neurons undergo important morphological and functional changes during normal aging. Invertebrate models have been extensively used and have revealed a number of age-related neuronal events. For example, *C. elegans* touch receptor and cholinergic neurons display age-dependent morphological defects such as cytoskeletal disorganization, axon beading, and defasciculation (Pan et al., 2011). Moreover, unexpected ectopic branching of neurites has been recently observed in *C. elegans* neurons as a result of aging (Tank et al., 2011). Although this branching was linked to impaired mechanosensory perception and decreased mobility, it seems to be regulated independently of organismal lifespan (Tank et al., 2011). Based on this notion, we could hypothesize that neuronal branching plays a survival role

in aged worms in the wild, but not under laboratory conditions and that it is not just a result of aging. This hypothesis would also explain why the branching is regulated by age-related pathways, such as the Jun kinase and the insulin/IGF-1 pathways (Tank et al., 2011). As these pathways are known to affect neuronal plasticity across species (Sherrin et al., 2011; Antoniou and Borsello, 2012; Fernandez and Torres-Aleman, 2012), it is not surprising that they also affect *C. elegans* neurite branching. Finally, the protection of neurons from aging relies heavily on the general lifestyle of individuals, e.g., diet and exercise, which highlights further the complexity of aging mechanisms (Stranahan and Mattson, 2012).

In higher organisms normal aging influences different parts of the brain at different rates (Woodruff-Pak et al., 2010) and impacts on synaptic connectivity of specific areas of the brain. For example, in the olfactory bulb in mice, the synaptic density of olfactory sensory neurons decreases with age in the glomerular layer but not the external plexiform layer (Richard et al., 2010). Interestingly, other neuronal characteristics, as well as neuronal populations are unaffected in the same region during aging (Richard et al., 2010). Such a selective effect of aging on different synaptic populations is not well understood. However, synaptic dysfunction during aging is conserved and it has been observed in different monkey species (Page et al., 2002; Duan et al., 2003) and in rats, where a study estimated a 27% decrease in axodendritic synapse number in the middle molecular layer of the dentate gyrus in 25 month old individuals compared to those of 3 months old (Bondareff and Geinisman, 1976). The relation between aging-related neuronal phenotypes and cognitive impairment is also not clear. An interesting study in *Drosophila* has recently made a functional connection between memory loss and impairment in specific neurons during normal aging (Tonoki and Davis, 2012). The authors were also able to retrieve lost memories due to aging by prior stimulation of these neurons (Tonoki and Davis, 2012). Despite the high number of different neuronal defects associated with aging, relatively few neurons die during normal aging (Herndon et al., 2002; Burke and Barnes, 2006). However, a recent study has shown that there is a significant and specific loss of hyperploidic neurons (neurons that contain more than a diploid number of chromosomes) with aging in the cerebral cortex of normal human brain (Fischer et al., 2012). Such specific neuronal loss is yet to show whether it contributes to age-related impairments. In general, neuronal decline rather than neuronal loss seems to be responsible for the negative manifestations of normal aging such as memory loss.

The cause of neuronal changes during aging is not completely understood. A common suspect appears to be increased oxidative stress and is in accordance with the "oxidative stress theory of aging" (Gerschman et al., 1954; Harman, 1956; Cadet, 1988). Oxidative stress is the negative impact of ROS on different aspects of cellular function and can lead to molecular defects such as DNA and mitochondria damage. It is characteristic that genes associated with stress responses and DNA repair are upregulated in the aging human brain (Lu et al., 2004). In normal aging, generation of ROS is elevated due to alterations in neuronal calcium handling and changes in lipid peroxidation (Stranahan and Mattson, 2012). These molecular events can lead to suppression of adult neurogenesis and implementation of alternative plasticity mechanisms to compensate for the damaged tissue (Stranahan and Mattson, 2012). Neurons are particularly vulnerable to oxidative stress, which can lead to neuronal cell death associated with many age-related neurodegenerative diseases (Coyle and Puttfarcken, 1993; Andersen, 2004). Nevertheless, recent studies question the role of oxidative stress in aging (Doonan et al., 2008; Yen et al., 2009; Van Raamsdonk and Hekimi, 2010, 2012; Hekimi et al., 2011). In addition, although it is not known whether any neurons are involved, mild elevations of ROS can be beneficial for a longer lifespan in *C. elegans* (Lee et al., 2010).

Thus, in general, aging is actively regulated by the nervous system and aging in turn influences neuronal properties. The nature of aging as a stressor appears to go beyond the result of environmental stress and can extend to an inevitable decline of repair mechanisms possibly due to physical entropy.

NEURONAL HOMEOSTASIS

Much of the ability of nervous system to fulfill its role relies on its homeostatic capability. This process is known as "homeostasis" and has been defined as "the maintenance of the constancy of the internal environment" (Turrigiano and Nelson, 2004). Regardless of changes in their environment, the structural and functional integrity of neurons must be conserved throughout life. When homeostatic mechanisms go awry, neurons decline and become unable to respond to external disturbances. This leads to many age-related neurodegenerative diseases, as well as other neuronal defects (Ramocki and Zoghbi, 2008). During development, neurons and neuronal networks put in place a variety of regulatory mechanisms in order to maintain their function despite changes in their microenvironment (Turrigiano and Nelson, 2004). The nervous system is also subjected to a variety of physical stresses throughout life. For example, the addition of new cells into adult neuronal circuits during neurogenesis tends to destabilize the functionality of circuits and dictates homeostatic adaptations in different levels (Meltzer et al., 2005). Neurons can also be challenged from physical body movements, muscle contractions, and injury, all of which have to be confronted by homeostatic mechanisms (Benard and Hobert, 2009). To this end neurons utilize extracellular matrix components, cell adhesion molecules and cytoskeletal proteins to maintain architectural integrity (Benard and Hobert, 2009). Thus, homeostatic mechanisms seem to act in the opposite direction to aging and disease in a complex and dynamic manner.

KEY MOLECULES INVOLVED

The regulation of protein expression under stress is complex and includes mechanisms such as epigenetic gene regulation, transcriptional regulation, and post-transcriptional regulation. Many molecules have been reported in many different species to mediate the cellular responses to stress. However, only a fraction of them have specific roles in neurons during these responses (**Table 1**). We summarize here studies for some key molecules involved in neuronal stress responses.

TRANSCRIPTION FACTORS

Hypoxia inducible factors

Hypoxia inducible factors are the key modulators of hypoxic stress responses. They function as heterodimers consisting of

Table 1 | Molecules involved in neuronal responses to different stresses in various organisms.

Molecule type	Name	Organism	Role	Reference
Transcription factors	bZIP transcription factors and related targets	*M. mu*[1]	ER stress response, hypoxia-induced neuronal death	Halterman et al. (2010)
	Activator protein-1 (AP1)	*H. sa*[2], *M. un*[3]	Hypoxia	McGahan et al. (1998), Domanska-Janik et al. (1999)
	P53	*H. sa*	DNA damage response, oxidative stress	Culmsee and Mattson (2005)
	RORα (retinoid-related orphan receptor-α)	*M. mu*	Hypoxia	Jolly et al. (2011)
	Nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)	*H. sa*	Various stresses including hypoxia	Qiu et al. (2001), Massa et al. (2006)
	SKN-1	*C. el*[4]	Oxidative stress, longevity	An and Blackwell (2003), Bishop and Guarente (2007)
Phosphatidylinositol 3-kinase-related kinases (PIKKs)	ATPase associated diverse cellular activities (AAA+) proteins RUVBL1 and RUVBL2 (RUVBL1/2)	*H. sa*	DNA damage response	Izumi et al. (2012)
	ATM protein kinase	*H. sa*	DNA damage response, synaptic plasticity	Abraham (2001), Li et al. (2009a), Tian et al. (2009)
	Ataxia- and Rad3-related neuron (ATR)	*R no*[5]	DNA damage response	Ye and Blain (2010)
	DNA-dependent protein kinase catalytic subunit (DNA-PKcs)	*M mu*	DNA damage response	Chechlacz et al. (2001)
	Cyclin dependent kinase 5 (Cdk5)/p35 complex	*M. mu*, *H. sa*	Hypoxia. DNA damage response	Antoniou et al. (2011), Zhu et al. (2011)
Signaling molecules	TNF-α, NRF2, and CREB	*R. no*	Preconditioning, oxidative stress	Shih et al. (2005), Saha et al. (2009)
	Tumor suppressor warts/lats (Wts)	*D. me*[6]	Dendritic maintenance	Emoto et al. (2006)
	Neurotrophins	*H. sa*	Neuronal maintenance	Henderson (1996)
	Unfolded protein response (UPR) system	*H. sa*	ER stress	Sammeta and McClintock (2010), Hoozemans and Scheper (2012)
	AMP-activated protein kinase (AMPK)	*H. sa*, *C. el*	Axogenesis during metabolic stress, neuronal plasticity, longevity	Schulz et al. (2007), Potter et al. (2010), Williams et al. (2011)
Extracellular components	DGN-1, ANC-1	*C. el*	Neuronal maintenance	Johnson and Kramer (2012)
	DIG-1	*C. el*	Neuronal maintenance	Benard et al. (2006), Burket et al. (2006)
	Collagen VI	*M. mu*	Neuroprotection after UV exposure	Cheng et al. (2011)
Histone deacetylate	Sirtuin (silent mating type information regulation 2 homolog)	*H. sa*, *C. el*	Oxidative stress	Gan and Mucke (2008)
Ion channel	Thermo transient receptor potential (thermoTRP)	*H. sa*	Thermo-avoidance	Dhaka et al. (2006)

[1]*Mus musculus*, [2]*Homo sapiens*, [3]*Meriones unguiculatus*, [4]*Caenorhabditis elegans*, [5]*Rattus norvegicus*, [6]*Drosophila melanogaster*.

an oxygen regulated α and a stable β subunit. The HIF heterodimer binds to the promoter of target genes via hypoxia response elements (HREs) with the consensus sequence G/ACGTG (**Figure 3A**; Majmundar et al., 2010). These target genes regulate a vast array of processes that enable cellular adaptation to hypoxia. HIFα is primarily regulated by oxygen-dependent prolyl hydroxylase-domain enzymes (PHDs) that lead to its degradation via the von Hippel–Lindau tumor suppressor protein (VHL)

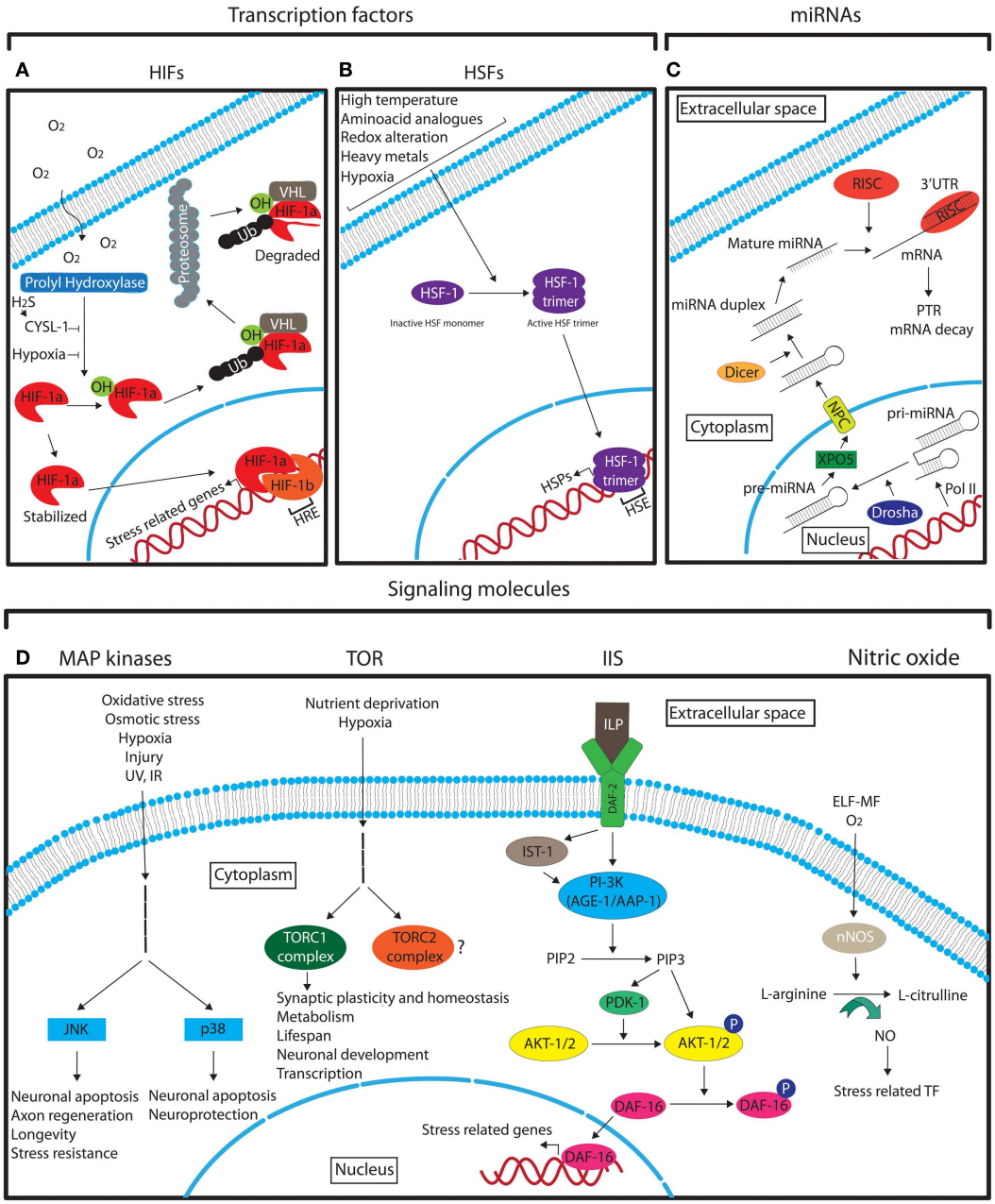

FIGURE 3 | Schematic representation of the different main molecular pathways that are involved in neuronal stress response. See the text for detailed description. **(A)** The role of HIFs in hypoxia response. **(B)** The role of HSF-1 in stress response. **(C)** The biogenesis of miRNAs. **(D)** The main signaling pathways that mediate the neuronal response to different stresses. Legend: arrows indicate the pathway flow and/or the positive effect of an element onto another. Blunted arrows indicate inhibition. Question mark denotes lack of information. Ub, ubiquitin; TF, transcription factors.

under normoxia (Epstein et al., 2001). In hypoxic conditions PHD activity is diminished and HIFα is stabilized (**Figure 3A**; Epstein et al., 2001; Majmundar et al., 2010). A recent study in *C. elegans* has also implicated the homolog of sulfhydrylases/cysteine (CYSL-1) in stabilizing HIF-1α in neurons via EGL-9, the worm PHD homolog, as a response to hypoxia-derived intracellular hydrogen sulfide (H_2S; **Figure 3A**; Ma et al., 2012).

Various studies have shown a specific role of HIF-1 in neurons. Depletion of HIF-1α in the mouse brain and in neuronal cell cultures causes increased cell damage and lower survival rate after cerebral ischemia (Baranova et al., 2007). In rat cortical neurons, HIF-1α appears to play a protective role in early steps of responses to mild hypoxia (Lopez-Hernandez et al., 2012). HIF-1α has recently been shown to regulate prion protein expression in hippocampal neuronal cells to protect from cell damage (Jeong

et al., 2012). Moreover, it is not clear whether HIF-1α plays a central role in hypoxic preconditioning as different studies argue for (Grimm et al., 2005; Shao et al., 2007; Ara et al., 2011) and against (Li et al., 2011) such a role. Although HIF-1 retains neuroprotective and anti-apoptotic properties, there is experimental evidence that point to a deteriorative impact of stabilized HIF-1α on neuronal tissue. For example, HIF-1 stabilization causes axon guidance and neuronal migration defects in *C. elegans* (Pocock and Hobert, 2008) and promotes neurodegeneration in neonatal rat brain (Jiang et al., 2012). In addition, HIF-1 in *C. elegans* negatively regulates lifespan extension by dietary restriction, acting in the serotonergic neurons ADF and NSM (Chen et al., 2009). However, studies from other groups report that in the same organism HIF-1 promotes longevity (Mehta et al., 2009; Zhang et al., 2009; Lee et al., 2010). This discrepancy was addressed in two more recent studies where it was shown that HIF-1 regulates longevity in a temperature-dependent manner (Hwang and Lee, 2011; Leiser et al., 2011). Thus, it is possible that the role of HIF-1 in longevity is context-dependent and may involve different neurons or other cells under the different conditions. While *C. elegans* neurons have also been recently found to sense and respond to hypoxia in a HIF-independent manner (Park et al., 2012), HIFs appear to be the main factors mediating neuronal responses to hypoxia through the regulation of many downstream effectors. This is despite the fact that neuroprotective properties of HIF may cause side effects on other aspects of neuronal physiology.

Heat shock factors and heat shock proteins

Heat shock factors (HSFs) are stress-inducible transcription factors that upon induction positively regulate the expression of heat shock proteins (HSPs) through direct binding to their promoters containing heat shock elements (HSEs; NGAAN; Shamovsky and Nudler, 2008). The master regulator of heat shock response is the HSF-1, which under normal conditions is in a monomeric inactive form (Shamovsky and Nudler, 2008). Upon different stress conditions HSF-1 forms an active trimer that enters the nucleus in order to activate HSPs (**Figure 3B**; Shamovsky and Nudler, 2008). The regulation of HSPs by HSFs is highly conserved from yeast to mammals (Liu et al., 1997). HSPs function as molecular chaperones to facilitate the proper folding of other cellular proteins. Protein misfolding can originate under normal cellular conditions, and under different stresses. It has also been shown that over-excitation of motor neurons can cause protein misfolding in post-synaptic muscle cells in *C. elegans* (Garcia et al., 2007). The induction of HSFs in neurons and in other cell types is not only stimulated by hyperthermia as its name implies, but also by other stresses, such as, hypoxia, alterations in the intracellular redox environment, and exposure to heavy metals and amino acid analogs (Morimoto et al., 1997). HSPs have also been shown to be upregulated in ischemic preconditioning (Liu et al., 1993; Kato et al., 1995). The neuroprotective properties of HSF-1, the principal regulator of heat shock response in *C. elegans*, have been demonstrated in two recent studies where upregulation of HSF-1 suppresses the defective neuronal phenotypes of a Machado–Joseph disease mutant model (Teixeira-Castro et al., 2011) and HSF-1 and the small heat shock protein HSP-16.1 mediate cytoprotection by heat preconditioning (Kourtis et al., 2012). In addition to HSPs, HSFs can induce the transcription of other proteins with various functions (Akerfelt et al., 2010). Some of them have neuroprotective functions under stress. For example, under heat shock in *Drosophila*, HSFs induce the expression of the NAD synthesis enzyme, nicotinamide mononucleotide adenylyltransferase (NMNAT), which is critical for neuronal maintenance under stress (Ali et al., 2011).

The expression of HSF-1 in neurons appears to be strictly controlled (Dirks et al., 2010) and the increased susceptibility of neurons to heat shock treatment is associated with the delayed onset of HSF-1 expression (Batulan et al., 2003; Kern et al., 2010). However, HSPs may have a more neuronal specific role under normal conditions, as some HSPs are constitutively more highly expressed in neurons than other cells (Chen and Brown, 2007). The beneficial role of the expression of HSPs was recently recognized (Rordorf et al., 1991) and can be induced by chemical compounds serving as medical drugs against different degenerative diseases (Katsuno et al., 2005; Chow and Brown, 2007). Many HSFs and HSPs are also up- and down-regulated during normal development of different species (Akerfelt et al., 2010), which may suggest a protective role for these proteins with regards to intrinsic developmental stress. Moreover, HSPs are related to normal and abnormal embryonic development (Evans et al., 2005; Brown et al., 2007) and the expression of HSPs has shown to be phase- and tissue-specific (Loones et al., 1997; Masuda et al., 1998). HSFs and HSPs are also implicated in aging. Hsp22, when overexpressed in motor neurons was shown to increase the lifespan of *Drosophila* by 30% (Morrow et al., 2004) and the flies maintained their locomotory activity longer and were more resistant to oxidative stress and hyperthermia (Morrow et al., 2004). In *C. elegans* HSF-1 promotes longevity by acting in neurons and other tissues (Lithgow et al., 1995; Hsu et al., 2003; Morley and Morimoto, 2004). Finally, the heat shock response of AFD and AIY thermosensory neurons in *C. elegans* involves upregulation of Hsp70 protein (Prahlad et al., 2008). It is thus apparent that HSFs and HSPs are involved in the core of the response mechanisms against many different stresses with particular importance in neuronal cell function.

SIGNALING MOLECULES

Mitogen-activated protein kinases

Mitogen-activated protein (MAP) kinases are essential signal transduction molecules that mediate the response to environmental cues in virtually all cell types and play key roles in cellular functions such as differentiation, cell survival, and apoptosis (Gehart et al., 2010). These and other general roles of certain MAP kinases in neurons was demonstrated several years ago (Fukunaga and Miyamoto, 1998). Sub-families of MAPKs have been recognized to date such as the extracellular signal-regulated kinases (ERK1/2), ERK5 (also known as BMK1 or MAPK7), the Jun amino-terminal kinases (JNK) 1–3, and the p38 kinases (p38α, β, γ, and δ; Gehart et al., 2010). The exact content and features of the different MAPK pathways have been described elsewhere (Dhillon et al., 2007). However, the general scheme of MAPK signaling follows the sequence "stimulus – G-protein – MAPKKK – MAPKK – MAPK-final response" (Dhillon et al., 2007). The stress-activated MAPK pathways are essentially the JNK and the p38 kinases (**Figure 3D**; Dhillon et al., 2007).

The JNK pathway can be induced by oxidative and osmotic stress, UV radiation, and other DNA-damaging agents to modulate the Activator protein-1 (AP1) and other transcription factors, such as HSF-1 and HIF-1 (Park and Liu, 2001; Antoniou and Borsello, 2012) in order to regulate the cellular stress responses. Neuronal apoptosis after stress is positively regulated by the JNK pathway (Weston and Davis, 2002; Biteau et al., 2011) and JNK/MAPK signaling is also involved in axon regeneration after injury in *C. elegans* (Hammarlund et al., 2009; Yan et al., 2009; Li et al., 2012). Moreover, the JNK pathway in neurons promotes organismal longevity by activating neuroprotective mechanisms in *Drosophila* (Lee et al., 2009; Biteau et al., 2011; Takahama et al., 2012), and in *C. elegans* (Oh et al., 2005). Finally, the JNK pathway can act by modulating FOXO transcription factors and antagonizes Insulin/IGF-1 factors to regulate different aspects of stress resistance and aging (Wang et al., 2005; Neumann-Haefelin et al., 2008).

p38 kinase pathway can be induced by stresses such as hypoxia, oxidative stress, IR, and UV irradiation and it is mainly implicated in the induction of neuronal apoptosis under different stresses (Horstmann et al., 1998; Namgung and Xia, 2000; Choi et al., 2004; Guo and Bhat, 2007). The p38 pathway also appears to play a role in neuroprotection against ischemia after isoflurane preconditioning (Zheng and Zuo, 2004). However, the p38 pathway has other roles in neurons beyond stress responses (Takeda and Ichijo, 2002).

Target of rapamycin

Target of rapamycin (TOR) is an evolutionary conserved serine/threonine kinase important predominantly in regulating cell growth and proliferation, with implications in many different aspects of development, aging, and disease (Wullschleger et al., 2006). TOR functions as a sensor of extracellular signals, including stressors such as hypoxia and nutrient deprivation, and is found in two functionally distinct complexes, namely TORC1 and TORC2 (**Figure 3D**). Inhibition of TOR signaling increases lifespan in many organisms, including mice and *Drosophila*, partly by reducing mRNA translation (Harrison et al., 2009; Bjedov et al., 2010). Even though mammalian TOR is ubiquitously expressed, it plays an extensive role in neuronal development and plasticity (Jaworski and Sheng, 2006; Chong et al., 2010; Hoeffer and Klann, 2010). TOR has also been recently found to be important for synaptic homeostasis in *Drosophila* (Penney et al., 2012) and for synaptic plasticity after ischemia in rats (Ghiglieri et al., 2010). mTOR may also be involved in the regulation of HIF-1alpha in the developing rat brain with hypoxia-ischemia (Chen et al., 2012). However, the role of TOR signaling in mediating stress responses in neurons has not been fully elucidated.

Insulin/IGF-1 signaling. The insulin/IGF-1 pathway is a very well characterized pathway that regulates aging and longevity in many different species (van Heemst et al., 2005). The complexity of Insulin/IGF-1 signaling (IIS) pathway has been greatly increased during evolution (van Heemst et al., 2005). In the nematode *C. elegans*, where the involvement of this pathway in aging was first discovered and extensively studied, the insulin-like growth factor receptor DAF-2 is activated by insulin-like peptides (ILP; 40 encoded in worm genome) that are primarily expressed in neurons (Pierce et al., 2001; Li et al., 2003; Husson et al., 2007; Cornils et al., 2011). After ligand binding, the signal is transduced directly or via the insulin receptor substrate homolog protein-1 (IST-1) to the phosphatidylinositol 3-kinase (PI-3K) consisting of the catalytic subunit AGE-1 (aging alteration-1) and the regulatory subunit AAP-1 (phosphoinositide kinase AdAPter subunit), which converts phosphatidylinositol 4,5-bisphosphate (PIP_2) into phosphatidylinositol 3,4,5-trisphosphate (PIP_3; van Heemst, 2010). PIP_3 activates the 3-phosphoinositide-dependent protein kinase-1 (PDK1) and the protein kinases B (known as AKT-1/2), leading to the phosphorylation of DAF-16, a homolog of the mammalian FoxO family of transcription factors (van Heemst, 2010). Phosphorylated DAF-16 is retained in the cytoplasm whereas unphosphorylated DAF-16 enters the nucleus to regulate a battery of stress response genes (**Figure 3D**; Lin et al., 2001; Lee et al., 2003a; Murphy et al., 2003).

Although the insulin receptor was found expressed in neuronal tissue (Havrankova et al., 1978; Unger et al., 1989), and its distribution appears to be enriched in particular brain areas (Schulingkamp et al., 2000), its role in neurons was not clear. As neurons can take up glucose without the involvement of insulin or insulin receptor, these cells were believed to be "insulin insensitive." Cline and colleagues provided the first *in vivo* evidence that the insulin pathway regulates neuronal circuit function and synaptic maintenance in the central nervous system of *Xenopus* tadpoles (Chiu et al., 2008). Previous studies had also demonstrated the role of insulin signaling in the regulation of lifespan in *C. elegans* neurons (Kenyon et al., 1993; Kimura et al., 1997; Apfeld and Kenyon, 1998; Wolkow et al., 2000), in *Drosophila* neuroendocrine cells (Tatar et al., 2001), and in the mammalian brain (Kappeler et al., 2008). A number of other studies have identified roles of the neuronal insulin pathway in energy homeostasis (Konner et al., 2011; Freude et al., 2012), synaptic plasticity (Oda et al., 2011; Costello et al., 2012), and neuronal apoptosis following hypoxic insult (Liu et al., 2011). Interestingly, IIS has also been shown to regulate salt chemotaxis learning in *C. elegans* (Tomioka et al., 2006). The insulin pathway can also act on neurons non-cell autonomously to regulate neuronal aging (Pan et al., 2011) and has also been shown to act in neurons to suppress organismal survival under hypoxia (Scott et al., 2002). In general, the insulin pathway has extended roles in neuronal tissues of many organisms that can span from the regulation of aging to hypoxia sensitivity.

Nitric oxide

Nitric oxide (NO) is a free radical important for cell signaling with a number of physiological roles such as synaptic plasticity and neurotransmission. The enzyme responsible for NO production in neurons is the neuronal nitric oxide synthase (nNOS) that mediates the generation of L-citrulline from L-arginine (**Figure 3D**; Zhou and Zhu, 2009). nNOS responds to oxygen levels and is upregulated by hypoxia in different neurons (AbuSoud et al., 1996; Prabhakar et al., 1996). NO regulates a variety of stress-related transcription factors such as HIF-1 and NF-κB (Contestabile, 2008; Keswani et al., 2011) and can lead to neuronal death under different stress conditions (Brown, 2010). Interestingly, NO in neurons can also be induced by extremely low frequency magnetic fields (ELF-MF) in the rat brain, a usual stressor

emanated from electrical devices (Cho et al., 2012). Therefore, NO appears to be an important inorganic molecule for neuronal stress responses.

MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNA molecules with a length of approximately 22 nucleotides that act as post-transcriptional regulators of gene expression (Ebert and Sharp, 2012). They have the ability to fine-tune expression to ensure stability under sudden external or internal perturbations or, if needed, enforce a new gene expression program that enables an organism to tolerate a new environment (Ebert and Sharp, 2012). miRNAs are transcribed, mostly by RNA polymerase II (Pol II), as capped and polyadenylated primary miRNAs (pri-miRNAs) that fold in extended hairpin structures. The pri-miRNAs are cleaved in the nucleus by the RNase III enzyme Drosha, creating the shorter precursor miRNA (pre-miRNA; Lee et al., 2003b). The pre-miRNA is transported by exportin-5 (XPO5) via the nucleopore complex (NPC; Yi et al., 2003) out of the nucleus (Lee et al., 2011) where is further processed by the RNase II enzyme, Dicer, and is incorporated into an Argonaute-containing RNA-induced silencing complex (RISC; Lee et al., 2002). In the RISC complex, the miRNA associates with a specific target mRNA by imperfectly base-pairing with its 3′ UTR and mediates post-transcriptional repression (PTR) or decay of specific mRNA targets (**Figure 3C**; Lee et al., 2002, 2003b, 2011; Pasquinelli, 2012). Robustness in systems that control cell fate, developmental transitions, and stress responses is required under changing conditions as fluctuations in the internal or external environment can be fatal for an organism. miRNAs can generate rapid and reversible responses and, in this way, are ideal molecules for mediating stress responses. As deletion of individual miRNAs (Miska et al., 2007) or whole miRNA families in *C. elegans* (Alvarez-Saavedra and Horvitz, 2010) have little to no effect on viability and development and as most of the miRNA knock-out mice do not show any gross phenotypes (Park et al., 2010), it is believed that the main function of miRNAs may be to buffer gene expression when an organism is challenged under stressful conditions. Supporting this idea Zhang and colleagues showed that deletion of *mir-71* impaired the long-term survival of nematodes during starvation-induced L1 diapause (Zhang et al., 2011b). In addition, miR-7 was shown to be essential for the maintenance of regulatory stability under temperature stress during development of a *Drosophila* sensory organ (Li et al., 2009b). miRNAs are abundantly expressed in the nervous system and a relation between miRNAs and neuronal responses to stress has been demonstrated in different model systems. Most recently, *mir-71* has also been associated with an increase in lifespan in *C. elegans* (Boulias and Horvitz, 2012). This study showed that expression of *mir-71* in neurons alone was sufficient to promote germline-mediated longevity and proposed a model in which *mir-71* mediates lifespan-extending signals through the DAF-16/FOXO transcription factor in the nervous system. The direct mechanism by which the internal stress is sensed by the neurons is not clear; however the function of miR-71 was shown to be partly dependent on expression of TCER-1 (Boulias and Horvitz, 2012), a transcription elongation factor shown to promote the transcriptional activity of DAF-16 in the intestine (Ghazi et al., 2009). Systemic stress can also be triggered by alcohol and neuronal adaptation to this stress was shown to cause a rapid increase in miR-9 expression in neurons (Pietrzykowski et al., 2008). This led to transcription of a voltage-activated potassium channel isoform associated with an increase in alcohol tolerance. This process may represent a general mechanism of neuronal adaptation to alcohol and suggests that miR-9 has an important role in neuronal plasticity (Pietrzykowski et al., 2008). A study in primary rat hippocampal neuronal cells has also shown that under hypoxia, the miR-130 family is highly expressed (Saito et al., 2011). In particular, miR-130a appears to decrease DDX6 protein levels, the normal function of which is to restrict HIF 1α (HIF-1α) mRNA to P-bodies of hippocampus neuronal cells of mice (Saito et al., 2011). Under hypoxia, HIF-1α mRNA is released from these foci and the protein can regulate oxygen homeostasis (Saito et al., 2011). The expression of miRNAs in response to stress has also been demonstrated to be cell specific. In a primary cell culture-based assay, astrocytes and neurons were subjected to oxygen-glucose deprivation to mimic ischemia, which is an essential feature of traumatic brain injuries (Ziu et al., 2011). In this model, different panels of miRNAs were upregulated in the two cell types, indicating that different neurons utilize different biochemical pathways to respond to physiological stress (Ziu et al., 2011). Together these studies suggest that miRNAs are implicated in neuronal stress responses. However, further research is expected to address more questions as to how miRNAs regulate neuronal responses to stress, and determine their impact on organismal homeostasis.

DISCUSSION

The physical world is built around principles that dictate the usage and management of energy. These principles are all connected by the basic laws of physics, and obey the same basic restrictions and limitations. We can think of living organisms as biological engines using their structures to utilize energy in order to survive and reproduce in a certain environment. The negative biological impact of stress on living systems relies on the inability of the latter to function and continue utilizing energy under certain conditions. This may either reflect the limitations of certain basic principles when, for example, no life can thrive at a temperature of absolute zero (−273°C), or a lack of adaptation of a particular organism to a given environment when, for example, an elephant cannot survive in Antarctica, while a polar bear cannot survive in the African savannah. Apart from such extreme mismatches between life and environmental conditions, there are milder ones that define stress as we discuss here. Stressful conditions often differ between organisms as different species have adapted to different environments. The extent to which each species has adopted defense mechanisms against stressors may reflect the extent of the presence of the relevant conditions during evolution.

Intrinsic developmental stress and aging can also count as selective factors for genes that give a survival advantage in the course of evolution, in the same manner as external environmental stress does. Stress derived from development may have led organisms to adopt particular developmental programs that lead to the final structures; and aging may have selected molecular pathways that

lead different organisms to achieve a health span that fits their ecological role. Considering the cellular properties of neurons and their documented implication in stress responses, the nervous system could constitute a "hot-spot" for the evolutionary adaptation of organisms to different stresses. In this sense, stress can be seen as a driving force of biodiversity in the long term that may have been particularly applied on the different levels of neuronal organization to select for the variety of the adaptation mechanisms to stress we presented here. However, the role of neurons on aging of higher organisms is not yet well understood. Much of our knowledge in this field comes from invertebrate models where it is characteristic that long-lived animal mutants show tolerance in different stresses (Kourtis and Tavernarakis, 2011). Yet there is clearly an additional level of active regulation of aging and longevity by neurons that is beyond a simple resistance to environmental stress.

During the life of an organism, stress conditions subject a burden that needs to be confronted and overcome in order to survive and reproduce. We have described mechanisms that have been developed for this purpose; however, stress often leads to disease and death. This stress can be termed as "pathophysiological stress" and it is beyond the scope of this review. Moreover, bacterial and other infections consist a form of environmental stress that leads to neuronal inputs of immune responses, which have been discussed elsewhere (Rosas-Ballina and Tracey, 2009).

Despite the plethora of harsh conditions present in the life of an organism, living systems are remarkably adept at coping with internal and external stress and preserve their homeostatic balance. A big part of this ability relies on the nervous system as we described in this review. As neurons sense fluctuations in conditions, they use this information to orchestrate appropriate defense and adaptation responses at different levels (**Figure 2**). In addition, neurons can systemically act on other cells to regulate their response to stress. The importance of the role of the nervous system in stress responses is simply highlighted by the well-known ability of neurons to quickly respond to environmental changes. This ability integrates external inputs to the several different lines of defense that span from cell protection to behavioral strategies. The extent to which neurons have adapted to different stresses during their development is also demonstrated by the fact that developmentally immature neurons are more resistant to hypoxia (Romero et al., 2003; Shirai et al., 2006) than their mature counterparts, whereas mature neurons are more resistant to UV and IR than immature ones (Bickler and Buck, 1998). This resistance of neurons to different stresses at different developmental stages may reflect the exposure of the organisms to these stresses in the course of their life.

There is also an inherent element of stress in natural systems that is connected to aging. Regardless of how perfect an environment is and how well an organism is adapted to this environment, eventually the organism will functionally decline. Instead of considering aging as an event that comes late in the biological "equation" of living systems, we propose that aging is an extra layer of stress that applies throughout life and is related to the physical entropy (**Figure 1**). As energy cannot be transformed from one form to another without a qualitative loss (second law of thermodynamics), every single chemical reaction performed by a cell may contribute to aging. Similar ideas have been supported by others (Hayflick, 2007 and references therein).

Currently, aging is defined as a post-reproductive process precluding the selection for and transmission of the relevant genes to the next generation. However, a number of studies have now shown that many molecular pathways have an impact on organismal longevity and that these pathways are conserved among species. Our hypothesis that aging occurs before, during and after reproduction, could provide a basis for the evolution of a genetic program regulating aging. At the same time, the stochastic nature of aging due to physical entropy could explain the variations in lifespan and in expression of biomarkers of aging between genetically identical individuals, for example in cloned worm populations (Herndon et al., 2002). However, other explanations have been provided for such variations like epigenetic modifications occurring in early development and adulthood (Bell and Spector, 2011; Steves et al., 2012).

In addition to the active neuronal responses under certain stresses, neurons can experience several side effects under stressful conditions. The distinction between an active neuronal response and a stress-derived neuronal defect is often not clear. In the laboratory environment a potential beneficial advantage of a neuronal phenotype under a stressor can be missed due to the absence of the condition under which this phenotype promotes survival, and which is present in the natural habitat of the organism. However, strong deteriorative impact of stress on different structures can be recognized in many cases as we described.

The study of intrinsic developmental stress and its significance to embryonic and neuronal development is admittedly technically challenging. Very few studies have clearly shown the impact of such stress on normal development (Zhang et al., 2011a) and fewer of them have focused on neurons (Benard and Hobert, 2009). However, the information derived from these studies provide the basis for further investigations and provide a first glance as to the importance of the different aspects of intrinsic developmental stress on normal development.

In the field of human and murine biology, "stress" has mainly been associated with the psychological reaction of an individual to stressful external stimuli, and has been connected to the central nervous system, which generates sentiments like fear and anxiety. Although this consists a very important aspect of the biology of "stress," it is extremely complex in its origin and manifestation. Therefore, we think that studying primary "stress" in a reductionist manner using models like *C. elegans* will provide us with more detailed information on how neurons survive and respond under "stress," and how they are affected by it. Supportive of this approach are many examples where the psychological stress involves similar mechanism to other more basic aspects of stress (Yao et al., 2007).

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Calcium homeostasis in aging neurons

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The nervous system becomes increasingly vulnerable to insults and prone to dysfunction during aging. Age-related decline of neuronal function is manifested by the late onset of many neurodegenerative disorders, as well as by reduced signaling and processing capacity of individual neuron populations. Recent findings indicate that impairment of Ca^{2+} homeostasis underlies the increased susceptibility of neurons to damage, associated with the aging process. However, the impact of aging on Ca^{2+} homeostasis in neurons remains largely unknown. Here, we survey the molecular mechanisms that mediate neuronal Ca^{2+} homeostasis and discuss the impact of aging on their efficacy. To address the question of how aging impinges on Ca^{2+} homeostasis, we consider potential nodes through which mechanisms regulating Ca^{2+} levels interface with molecular pathways known to influence the process of aging and senescent decline. Delineation of this crosstalk would facilitate the development of interventions aiming to fortify neurons against age-associated functional deterioration and death by augmenting Ca^{2+} homeostasis.

Keywords: endoplasmic reticulum, Golgi, long-term potentiation, ion channel, mitochondria, neurodegeneration, neurotransmitter, synaptic plasticity

INTRODUCTION

Fluctuations in intracellular calcium concentration act as signals for a variety of processes in neurons. Most notably, Ca^{2+} is the major trigger of neurotransmitter release, a process that has been thoroughly investigated over the past decades (Neher and Sakaba, 2008). Moreover, it has also become clear that Ca^{2+} is essential for a variety of other neuronal functions, including neuronal excitability (Marty and Zimmerberg, 1989), integration of electrical signals (Llinas, 1988; Marty and Zimmerberg, 1989), synaptic plasticity (Malenka et al., 1989), gene expression (Szekely et al., 1990), metabolism (McCormack and Denton, 1990), and programmed cell death (Chalfie and Wolinsky, 1990). Given its central role in processes that are fundamental to the excitable nature of neurons, Ca^{2+} homeostasis is tightly regulated in these cells (see **Table 1** for a summary of the key effectors of Ca^{2+} homeostasis, in neurons). Here, we briefly overview the main mechanisms neurons use in order to achieve an intricate regulation of the intracellular concentration of Ca^{2+}. In addition, we discuss the accumulating evidence on the potential role of deregulated Ca^{2+} homeostasis in aging and disease of the nervous system.

MECHANISMS OF NEURONAL CALCIUM HOMEOSTASIS RELEVANT TO AGING AND DEGENERATION

Ca^{2+} INFLUX THROUGH THE PLASMA MEMBRANE

Plasma membrane Ca^{2+} channels allow the passive influx of calcium ions down their electrochemical gradient. These channels are categorized into two major groups depending on the mechanism controlling their transition between the open and closed conformations: channels gated by voltage (also known as voltage-operated Ca^{2+} channels, VOCC), and channels gated by ligand binding, in neurons usually L-glutamate (**Figure 1**; **Table 1**).

Voltage-gated Ca^{2+} channels are multi-protein complexes comprising several different subunits: α_1, $\alpha_2\delta$, β_{1-4}, and γ (Takahashi and Catterall, 1987; Catterall et al., 1990). The α_1 subunit is the largest and it contains the conduction pore, the voltage sensors, and gating apparatus, and most of the known sites of channel regulation by second messengers, drugs, and toxins. The α_1 subunits are associated with distinct auxiliary protein subunits (Catterall et al., 1990): the intracellular β subunit, the transmembrane, disulfide-linked $\alpha_2\delta$ subunit complex, and the γ subunit, a component of skeletal muscle Ca^{2+} channels also expressed in heart and brain having four transmembrane segments. Although these auxiliary subunits modulate the functional properties of the Ca^{2+} channel complex, the pharmacological and physiological diversity of Ca^{2+} channels arises primarily from the existence of multiple α_1 subunits. These are encoded by 10 distinct genes in mammals, further divided into three subfamilies based on sequence similarity (Catterall et al., 1990; Snutch and Reiner, 1992; Ertel et al., 2000). Division of Ca^{2+} channels into these three subfamilies is phylogenetically ancient, as single representatives of each are found in the *Caenorhabditis elegans* genome. Recently, calcium homeostasis modulator 1 (CALHM1), a glycosylated membrane protein expressed throughout the brain, was identified as the pore-forming subunit of a unique plasma membrane Ca^{2+}-permeable voltage-gated ion channel (Ma et al., 2012).

Based on the characteristics of channel composition, distinct classes of Ca^{2+} currents have been described (Tsien et al., 1988). In summary, N-type, P/Q-type, and R-type Ca^{2+} currents are induced upon strong depolarization (Tsien et al., 1991) and are pharmacologically blocked by specific toxins derived from snail and spider venoms (Miljanich and Ramachandran, 1995). N-type and P/Q-type Ca^{2+} currents are observed primarily in neurons where they initiate neurotransmission at most fast conventional synapses (Catterall et al., 1990; Olivera et al., 1994; Dunlap et al., 1995). More specifically, the CaV2 subfamily members (CaV2.1, CaV2.2, and CaV2.3) conduct P/Q-type, N-type, and R-type

Table 1 | Summary of different Ca^{2+} channels, buffers and sensors, their subcellular localization and function.

	Sub-cellular localization	Function
Channels		
Voltage-gated Ca^{2+} channels	Plasma membrane	Influx of Ca^{2+} into the cell
NMDA receptor		
PMCA, ATP driven Ca^{2+} pump		Efflux of Ca^{2+} from the cell
NCX, "Na^{+}/Ca^{2+} exchanger"		
SERCA 1, 2a, 2b, 3	ER and Golgi	Influx of Ca^{2+} into the ER or Golgi
Inositol 3-phosphate (InsP3) receptors	ER	Efflux of Ca^{2+} from the ER
Ryanodine receptors (RyRs)		
NAADP receptors		
polycystin-2 channels		
presenilin 1 and 2		
SPCA 1a, 1b, 1c, 1d, 2	Golgi	Influx of Ca^{2+} into the Golgi
Ca^{2+} uniporter	Mitochondria	Influx of Ca^{2+} into mitochondria
NCX mitochondrial Na^{+}/Ca^{2+} exchanger		Efflux of Ca^{2+} from mitochondria
mPTP		
Buffers		
Calreticulin	ER	Reversible sequestering of Ca^{2+}
Calsequestrin		
Endoplasmin		
BiP/grp78		
Reticulocalbin		
CREC family proteins		
Calretinin	Cytosol, mainly CNS GABAergic interneurons	
Calbindin		
Parvalbumin		
Nucleo-calbindin	Golgi	
Glycerophosphate dehydrogenase	Mitochondrial	
Aralar ARE		
Sensors		
Calmodulin	Cytosol	Translation of graded Ca^{2+} concentration changes into graded signaling responses via interaction with Ca^{2+} sensitive enzymes
Recoverins	Cytosol, photoreceptors	
Guanylyl cyclase activating protein 1 (GCAP1)		
Frequenins	Cytosol, CNS neurons	
Visinin-like proteins		
Kv channel interacting proteins (KChIPs)		

Ca^{2+} currents, respectively (Catterall et al., 1990; Snutch and Reiner, 1992; Olivera et al., 1994; Ertel et al., 2000). Ca^{2+} entering neurons through the CaV2.1 and CaV2.2 channels is primarily responsible for initiating synaptic transmission at conventional fast synapses (Olivera et al., 1994; Dunlap et al., 1995). CaV2.2 channels are most prevalent at synapses formed by neurons of the peripheral nervous system. In contrast, CaV2.1 channels play a major role at most synapses formed by neurons of the mammalian central nervous system. However, in some central synapses, including a subset of inhibitory interneurons of the hippocampus (Poncer et al., 1997), CaV2.2 channels are predominant in neurotransmitter release.

Ca^{2+} entry through a voltage-gated Ca^{2+} channel initiates neurotransmission by triggering vesicular release (Stanley, 1993). Ca^{2+}-triggered synaptic vesicle exocytosis depends on the assembly of the SNARE complex, in which the vesicle-associated

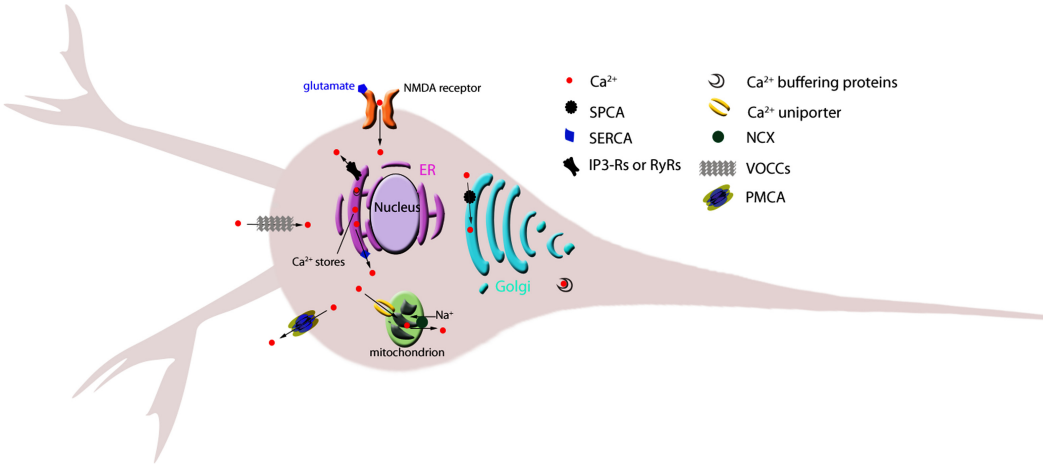

FIGURE 1 | Schematic representation of the main Ca^{2+} homeostatic machinery components in neurons. Individual, key components of calcium homeostatic mechanisms discussed in the text are shown. Arrows indicate direction of ion flux. ER, endoplasmic reticulum; IP3-R, inositol 3-phosphate receptor; NCX, sodium calcium exchanger; NMDA, *N*-methyl-D-aspartate; PMCA, plasma membrane Ca^{2+} ATPase; RyR, ryanodine receptor; SERCA, sarco(endo)plasmic reticulum Ca^{2+} ATPase; SPCA, secretory-pathway Ca^{2+}-ATPase; VOCC, voltage-operated calcium channel.

v-SNARE protein synaptobrevin (VAMP) interacts with two plasma membrane-associated t-SNARE proteins, SNAP-25 and syntaxin-1 (Sollner et al., 1993; Bajjalieh and Scheller, 1995; Sudhof, 1995, 2004). Maturation into a release-ready SNARE complex requires synaptotagmin, an integral Ca^{2+}-binding protein of the synaptic vesicle membrane that provides Ca^{2+}-dependent regulation of the fusion machinery. Ca^{2+} influx into the presynaptic terminal binds to the Ca^{2+} sensor, synaptotagmin, and the SNARE complex changes conformation from a *trans* to a *cis* state, resulting in the fusion of apposing membranes and the release of neurotransmitter. Neurotransmitter release occurs in two phases: a fast synchronous (phasic) component and a slow asynchronous (tonic) component (Hubbard, 1963; Barrett and Stevens, 1972; Rahamimoff and Yaari, 1973; Goda and Stevens, 1994; Atluri and Regehr, 1998). Both forms of transmission are Ca^{2+} dependent. Synchronous release driven by the precisely timed presynaptic Ca^{2+} current results in a large, fast postsynaptic response (Llinas et al., 1981; Sabatini and Regehr, 1996), whereas the slower asynchronous component, resulting from residual Ca^{2+} remaining in the terminal after an action potential, provides a basal or tonic level of neurotransmitter release at many synapses (Atluri and Regehr, 1998; Lu and Trussell, 2000; Hagler and Goda, 2001).

In addition to voltage-gated channels, a number of Ca^{2+} channels on the plasma membrane of neurons are activated by the interaction of ligands with their own plasma membrane receptors. The most prominent such ligand in the nervous system is L-glutamate, by far the most widespread excitatory transmitter in the vertebrate central nervous system. L-glutamate activates two general classes of receptors, the "ionotropic" receptors, which are ionic channels, and the G-protein coupled "metabotropic" receptors. Of these, the ionotropic receptors mediate the direct penetration of Ca^{2+} into the cell. Three forms of ionotropic receptors have been characterized and named after their most widely used agonists. These are the kainate (KA) receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, and the *N*-methyl-D-aspartate (NMDA) receptors. The channels formed by AMPA and KA receptors are primarily permeable to Na^+ and K^+ and exhibit a rather low conductance to Ca^{2+} (Mayer and Westbrook, 1987). By contrast, the NMDA receptors have a considerably higher conductance and are permeable to Na^+ and Ca^{2+} (MacDermott et al., 1986). These receptors do not mediate rapid synaptic transmission, their contribution being primarily to the slow component of excitatory postsynaptic currents. At the resting plasma membrane potential they are powerfully inhibited by Mg^{2+}, whose block is reversed by plasma membrane depolarization (Nowak et al., 1984). Thus, the rapid increase of membrane depolarization following the activation of KA/AMPA receptors by glutamate released into the synaptic cleft reduces the inhibition of NMDA receptors by Mg^{2+}. Therefore, the excitatory postsynaptic potential produced by activation of an NMDA receptor highly increases the concentration of Ca^{2+} in the cell. The Ca^{2+} in turn functions as a key second messenger in various signaling pathways. The ability of the NMDA receptor to act as a "coincidence receptor," requiring the concomitant presence of its ligand and membrane depolarization in order to be activated, explains many aspects of its functional involvement in long-term potentiation (LTP) and synaptic plasticity, a process associated with memory and learning as discussed later.

EFFLUX OF CALCIUM THROUGH THE PLASMA MEMBRANE

Two major plasma membrane mechanisms are responsible for the extrusion of Ca^{2+} from cells (**Figure 1**; **Table 1**). One is the ATP-driven plasma membrane Ca^{2+} pump (PMCA) and the other is the Na^+/Ca^{2+} exchanger (NCX), a complex similar to that discussed later for the removal of Ca^{2+} from the mitochondrial matrix into the cytoplasm (Baker and Allen, 1984; Carafoli and Longoni, 1987; Blaustein, 1988). Unlike in mitochondria, plasma membrane NCX has the inherent ability to move Ca^{2+} into or out of the cell depending on the prevailing conditions. When the

system is acting to remove Ca^{2+}, energy is supplied by the electrochemical gradient that ultimately results from the activity of the plasma membrane Na^+/K^+ ATPase (Na^+ pump).

Plasma membrane Ca^{2+} pump has a higher affinity for Ca^{2+} ($K_d = 100$ nM) but a very slow turnover, whereas NCX has a much lower affinity ($K_d = 1000$ nM) but a higher turnover. Both types of transporters are co-expressed in neurons and in astrocytes (DiPolo and Beauge, 1983; Juhaszova et al., 2000). However, the precise role that each plays in removing excess Ca^{2+} loads under different physiological and pathophysiological conditions remains rather unclear. A major difference is the fact that they exhibit distinct subcellular localization patterns. In particular, some if not all of PMCA found in neurons seems to be localized very close to the neurotransmitter release sites (active zone) of the presynaptic terminals, whereas NCX is excluded from these sites and present in a more dispersed fashion on the rest of the neuron (Juhaszova et al., 2000; Blaustein et al., 2002). Therefore, the PMCA may help keep active zone Ca^{2+} very low, and function to re-prime the neurotransmitter release mechanism following activity. NCX, on the other hand, is believed to efflux Ca^{2+} that has diffused away from the active zone and perhaps been temporarily sequestered by the endoplasmic reticulum (ER). Moreover, the discovery of a multitude of PMCA isoforms and alternative splice variants (Strehler and Treiman, 2004; Strehler et al., 2007), as well as recent results on PMCA "knockout" mice and PMCA mutants (Prasad et al., 2007), show that at least some PMCAs play a more specific role in local Ca^{2+} handling. In addition, a growing number of specific PMCA-interacting proteins have been identified with regulatory, targeting, and signaling functions. These findings support a new paradigm, whereby PMCAs are not only responsible for global Ca^{2+} homeostasis but are dynamic participants in spatially defined Ca^{2+} signaling. The main regulator of PMCA function is Ca^{2+} calmodulin (Ca^{2+}-CaM; Werth et al., 1996). In the absence of CaM, the pumps are autoinhibited by a mechanism that involves the binding of their C-terminal tail to the two major intracellular loops. Activation requires binding of Ca^{2+}-CaM to the C-terminal tail and a conformational change that displaces the autoinhibitory tail from the major catalytic domain. Release of autoinhibition may be facilitated by means other than CaM binding, including by acidic phospholipids, protein kinase A- or C-mediated phosphorylation of specific (Ser/Thr) residues in the C-terminal tail (Werth et al., 1996), partial proteolytic cleavage of the tail (e.g., by calpain or caspases), or dimerization via the C-terminal tail (for a detailed review see Di Leva et al., 2008). Different PMCA isoforms show significant differences in their regulation by kinases and CaM. Interestingly, loss of PMCA function was reported to lead to an increase in the levels of intracellular Ca^{2+}, causing apoptotic death of cerebellar and spinal cord neurons (Kurnellas et al., 2007).

INTRACELLULAR CALCIUM HOMEOSTASIS IN NEURONS

Ca^{2+} homeostasis in the ER

The ER, a complex system of endomembranes, is present in all neurons and extends from the nucleus to the soma, dendrites, and dendritic spines, and down the axon to the presynaptic terminals. Particularly relevant for neuronal function is the ability of the ER to act as a dynamic Ca^{2+} store, able to actively accumulate Ca^{2+} and to release it in response to physiological stimulation. As such, the ER contains a variety of channels, buffers, and sensors dedicated to Ca^{2+} homeostasis (**Figure 1**; **Table 1**). In general, Ca^{2+} exits the ER through several types of Ca^{2+} release channels, such as inositol 3-phosphate (InsP3) receptors, ryanodine receptors (RyR), nicotinic acid adenine dinucleotide phosphate (NAADP) receptors, and polycystin-2 channels [the relative of transient receptor potential (TRP) proteins]. In neurons, the NAADP receptors were reported to exist in brain microsome preparations (Bak et al., 1999) and Ca^{2+} release from these channels was described in neurons from the buccal ganglion of aplysia (Chameau et al., 2001), yet their relevance in vertebrate neurons remains unclear. Regarding the TRPs, although they are expressed by neurons, there is so far no evidence for their involvement in Ca^{2+} homeostasis in these cells. Therefore, in neurons, Ca^{2+} exit from the ER occurs mainly through the inositol 3-phosphate receptors (IP3-Rs) and the Ca^{2+} activated RyR, both forming large tetrameric channel proteins. Both receptor families are comprised of multiple members that display distribution patterns that are both temporally and spatially regulated in neurons. For example, there are three RyRs, all of which can be activated by Ca^{2+} on the cytosolic side with differential sensitivities (RyR1 > RyR2 > RyR3). All three members have been detected in neurons, with distinct patterns that change during development and postnatal growth. For example, postnatally, RyR1 is highly expressed in cerebellar Purkinje cells, RyR3 in the hippocampus, striatum, and diencephalon, while many neurons co-express more than one RyR isoform (Hakamata et al., 1992; Lai et al., 1992; Furuichi et al., 1994; for review also see Berridge, 1998; Hertle and Yeckel, 2007). Regarding their sub-cellular localization, RyRs have been seen in all parts of neurons, including the soma, axons, dendrites, and even the spine apparatus of excitatory neurons. Similarly, there are three InsP3R isoforms with different sensitivities to Ca^{2+}, and further diversity may arise from alternative splicing of InsP3R1. InsP3R1 is the main isoform in neurons in the brain, while InsP3R3 is mainly found in the spinal cord and in glial cells (Berridge, 1998).

Propagating Ca^{2+} waves is the most dramatic expression of Ca^{2+} release from the ER, reflecting the Ca^{2+}-induced Ca^{2+} release (CICR) mode, where elevated cytoplasmic Ca^{2+} induces further Ca^{2+} release. Ca^{2+} waves in neurons were described more recently, after the notion had first been established using *Xenopus* oocytes (Lechleiter et al., 1991; Parker and Ivorra, 1991). Given the functional compartmentalization of neurons, Ca^{2+} waves take up different properties depending on their spatial localization and neuronal type diversity. For example, synaptically activated Ca^{2+} waves preferentially initiate at branch points of dendrites (Nakamura et al., 2002; Larkum et al., 2003; Fitzpatrick et al., 2009) and are mediated by the IP3-Rs (Nakamura et al., 1999). Such waves have been observed in pyramidal neurons of the rodent CA1 and CA3 regions of the hippocampus (Miller et al., 1996; Kapur et al., 2001), in layers 2 and 3 of the cortex (Larkum et al., 2003; Hagenston et al., 2008) and principal neurons of the amygdala (Power and Sah, 2008), all regions heavily involved in memory and learning. Relevant to the cognitive decline and memory loss associated with aging, synaptically induced Ca^{2+} waves are functionally linked to synaptic plasticity, a process known to require a

rise in the postsynaptic concentration of Ca^{2+}. More specifically, there are several cases where synaptically activated Ca^{2+} release from stores was shown to induce LTP (Yeckel et al., 1999), though it remains controversial as one study challenged this conclusion (Mellor and Nicoll, 2001).

In addition to the channels discussed above, some studies have suggested that presenilin 1 and 2, beyond constituting the proteases in the γ-secretase complex, also function as Ca^{2+} leak channels in the ER, either by themselves, or indirectly by increasing the activity of IP3-Rs and RyRs (Pack-Chung et al., 2000). In particular, presenilin 2 was shown to interact with sorcin, a cytoplasmic calcium-binding protein that modulates the activity of RyRs (Pack-Chung et al., 2000). Interestingly, in some mutations of presenilin 1 and 2 that are responsible for familial Alzheimer's disease, disruption of intracellular Ca^{2+} homeostasis by the ER is the major measurable cellular consequence (Nelson et al., 2010), as discussed later on.

Calcium uptake into the ER lumen results from the function of Ca^{2+} pumps of the P-type sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) family. This family includes three members (SERCA1–3), as well as two splice isoforms of SERCA2. While SERCA2b is ubiquitously expressed, SERCA2a and SERCA3 are found almost exclusively in cerebellar Purkinje neurons. Inhibition of the SERCA pumps results in a relatively slow emptying of ER Ca^{2+} stores, with Ca^{2+} exiting the ER through poorly described pathways (Camello et al., 2002). Ca^{2+} buffering in the ER lumen is achieved by specific Ca^{2+}-binding proteins. In neurons, the most abundant of these is calreticulin and calsequestrin, while some others such as endoplasmin, BiP/grp78, and proteins of the CREC family also participate in Ca^{2+} buffering. A main difference of ER Ca^{2+} buffers is that, unlike their cytosolic counterparts, they have a low affinity for Ca^{2+} to allow the maintenance of high intra-ER Ca^{2+} levels.

Ca^{2+} homeostasis in the Golgi

Ca^{2+} uptake in the Golgi apparatus involves two groups of Ca^{2+} pumps: the well characterized SERCAs, discussed above, and a less characterized group of ATPases that were described as secretory-pathway Ca^{2+}-ATPases (SPCAs; Shull, 2000; **Figure 1**; **Table 1**). The SPCAs in addition supply the Golgi lumen with Mn^{2+}, which is needed for many enzymatic reactions in this compartment. Mammalian SPCA was originally cloned from rat using a probe derived from sequences of the ATP-binding site of SERCA1 and SERCA2 (Gunteski-Hamblin et al., 1992). The corresponding human gene (*ATP2C1*) was described by two independent groups (Hu et al., 2000; Sudbrak et al., 2000). Alternative processing of *ATP2C1* results in four SPCA1 proteins with C-termini differing in length and specific amino acid sequence (Hu et al., 2000; Sudbrak et al., 2000; Fairclough et al., 2003), SPCA1a, SPCA1b, SPCA1c, and SPCA1d. Ishikawa et al. (1998) later described a second human SPCA isoform, named SPCA2. Its human gene (*ATP2C2*) was independently described in 2005 by two groups (Vanoevelen et al., 2005; Xiang et al., 2005). The widespread expression pattern of SPCA1 and the observation that homozygous loss of a functional *ATP2C1* gene do not seem to be viable suggest that SPCA1 is a housekeeping enzyme. The tissue and cellular expression of SPCA2 appears to be more restricted than that of SPCA1, and based on mRNA data it is expressed in the brain, among other tissues (Vanoevelen et al., 2005; Xiang et al., 2005). It is now well established using a range of different cell types that the endogenous SPCA1 is specifically located in the Golgi compartment (Behne et al., 2003; Van Baelen et al., 2003; Reinhardt et al., 2004; Ramos-Castaneda et al., 2005). The relative contribution of SERCAs and SPCAs to the total uptake of Ca^{2+} into the Golgi apparatus seems to be cell-type-dependent. The highest dependence on SPCAs occurs in human keratinocytes (Callewaert et al., 2003). This finding is important for explaining the physiopathology of the skin-related Hailey–Hailey disease.

While the potentially specific roles of SPCAs in neurons are poorly understood, our own recent findings (Kourtis et al., 2012) suggest that SPCA1 is both necessary and sufficient in mediating the neuroprotective function of heat preconditioning in a model of heat stroke-induced neurodegeneration. Notably, this mechanism is evolutionarily conserved as it is preserved from *C. elegans* to mammals. This finding invites the speculation that SPCAs may have a more general neuroprotective role, whose relevance to other forms of neurodegeneration and aging remains to be examined.

Ca^{2+} homeostasis by mitochondria

Beyond their main role in the cell to produce NADH and ATP, it is now well accepted that mitochondria also function as Ca^{2+} buffers (**Figure 1**; **Table 1**). As proton pumping creates an inside-negative membrane potential in mitochondria, Ca^{2+} tends to be drawn into the mitochondrial matrix following its electrochemical gradient. This influx is mainly achieved by the mitochondrial Ca^{2+} uniporter whose conductance is dependent on both intracellular Ca^{2+} concentration and energy demand. At high cytosolic Ca^{2+} concentrations and low ATP/ADP ratio more Ca^{2+} is conducted, whereas at low cytosolic Ca^{2+} concentration and high ATP/ADP ratio less Ca^{2+} is conducted. Intricately enough, increasing mitochondrial Ca^{2+} concentration activates the enzymes of the Krebs cycle, thus causing increased ATP production. As mitochondrial Ca^{2+} buffering is more energy efficient compared to expelling Ca^{2+} through the plasma membrane or into the ER, this mechanism is considered of high relevance for neurons in situations when ATP and oxygen demands reach high levels, such as in the case of repeated axon potentials (Contreras et al., 2010).

Calcium is expelled from the mitochondrial matrix into the cytosol mainly by the mitochondrial sodium calcium exchanger (NCX; three Na^+ for one Ca^{2+}), in conditions of low ATP demand and oxygen consumption, or through a mitochondrial proton/Ca^{2+} exchanger (two or more H^+ per Ca^{2+}). Indirect experiments with isolated mitochondria under pathological conditions or Ca^{2+} overload suggest an additional, higher conductance route, through the transient opening of the mitochondrial permeability transition pore (mPTP). However, the physiological relevance of mPTP in Ca^{2+} homeostasis remains controversial and is not supported by genetic ablation studies (Ichas et al., 1997; Baines et al., 2005). In addition to its contribution in disease, which is discussed later, new roles for mitochondrial Ca^{2+} homeostasis are also emerging for normal neuron physiology. For example, it was recently described that olfactory sensory neurons require mitochondrial Ca^{2+} mobilization in order to encode intensity

(Fluegge et al., 2012). Therefore, aberrant mitochondrial Ca^{2+} homeostasis in these neurons converts them into simple signal detectors and impairs their function in olfaction.

Calcium buffers and sensors

A large set of proteins with ability to bind Ca^{2+} specifically and reversibly provide yet another level of control in Ca^{2+} homeostasis by acting as sensors or buffers (**Figure 1**; **Table 1**). A large family of these Ca^{2+}-binding proteins is the one containing EF-hand Ca^{2+} binding domains. These motifs consist of two 10–12 residue long alpha helices, oriented perpendicularly against each other, separated by a 12-residue long loop region. EF-hand domains often exist as multiple pairs generating a wide structural and functional variability within this large family of proteins (Kretsinger, 1980). A prominent member of this family, calmodulin, serves as a Ca^{2+} sensor that translates graded changes of intracellular Ca^{2+} concentration into a graded signaling response by interacting with various Ca^{2+}-sensitive enzymes.

Another set of EF-hand-containing proteins, represented by calretinin, calbindin, and parvalbumin, function as Ca^{2+} buffers. These proteins are predominantly expressed by the inhibitory GABAergic interneurons of the central nervous system in specific patterns, therefore contributing to the diversification of these interneurons into distinct subtypes (Van Brederode et al., 1990). A multitude of studies has demonstrated that these proteins modulate the Ca^{2+} levels locally in the presynaptic active zone or at postsynaptic densities. Moreover, they are thought to actively and differentially participate in modulating neuronal vulnerability to different types of stress. In hippocampal primary cultures, neurons expressing calbindin are less vulnerable to oxidative stress-induced apoptosis because they recover Ca^{2+} concentration more effectively after stimulation, whereas in cortical neurons this is true for calretinin-containing neurons (Mattson et al., 1991). Similarly, genetic over-expression of parvalbumin in mice rescues motorneurons from injury-induced cell death (Dekkers et al., 2004).

It is generally thought that the transduction of the Ca^{2+} signal by EF-hand proteins consists a series of conformational changes that occur after Ca^{2+} has become bound. However, it is important to also mention that there are some exceptions, as no significant conformational changes after Ca^{2+} binding have been described for at least two of the EF-hand proteins, such as parvalbumin itself and calbindin, which are thus likely to act instead only as temporal Ca^{2+} buffers. Although most EF-hand proteins reside in the cytosol (and in the nucleoplasm), reticulocalbin is localized in the lumen of the ER (Tachikui et al., 1997). On the other hand, Cab45 (Scherer et al., 1996) and nucleobindin are localized in the Golgi apparatus (Lin et al., 1998) and glycerophosphate dehydrogenase (Pilstrom and Kiessling, 1972) and Aralar are located on the outer face of the inner mitochondrial membrane (del Arco and Satrustegui, 1998; Del Arco et al., 2000).

Another group of Ca^{2+}-binding proteins, collectively known as intracellular neuronal calcium sensors (NCS; Braunewell and Gundelfinger, 1999; Burgoyne and Weiss, 2001), includes five subfamilies: the recoverins and guanylyl cyclase activating proteins (GCAPs), which are primarily expressed in retinal photoreceptor cells and have established roles in the regulation of photo-transduction; the frequenins, visinin-like and Kv-channel-interacting proteins (KChIPs), which are widely expressed in central neurons. One key feature of most NCS is N-terminal acylation: several members of the family are N-terminally myristoylated. Binding of Ca^{2+} to recoverin, and presumably to other NCS proteins, changes their conformation, exposing the myristoyl residue and hydrophobic portions of the molecule, making them available for membrane (or target protein) interaction. The Ca^{2+}-myristoyl switch could be a mechanism that affects the compartmentation of signaling cascades in neurons and/or the transmission of Ca^{2+} signals to their membranes (Braunewell and Gundelfinger, 1999; Burgoyne and Weiss, 2001). Although the functions of the last three families are not clearly defined, it has been shown that they interact with multiple target proteins and with nucleic acids as well (Carrion et al., 1999). KChIP3 encodes the protein calsenilin, shown recently to interact with presenilin 1 and 2, two proteins whose mutations result in familial Alzheimer's disease (AD; Buxbaum et al., 1998; Buxbaum, 2004). Relevant to the neurodegenerative phenotype of AD pathology, this interaction was shown to modulate the proteolytic processing of presenilins. In addition, two other NCS proteins, recoverin and GCAP1 have been involved in degenerative diseases of the retina. Mutations in the GCAP gene have been associated with autosomal dominant cone dystrophy. One of the defects has been related to constitutive activation of guanylyl cyclase that is not properly inactivated by high levels of Ca^{2+}, characteristic of physiological dark conditions, eventually leading to degeneration of cone cells (Dizhoor et al., 1998; Sokal et al., 1998). The other condition [GCAP1(P50L); Sokal et al., 2000] is a milder form of autosomal dominant cone dystrophy in which the mutation reduces the Ca^{2+}-binding ability of GCAP1. Recoverin has been identified as the autoantigen in a degenerative disease of the retina called cancer-associated retinopathy (CAR), in which patients lose vision due to degeneration of photoreceptors (Polans et al., 1991; Polans et al., 1995).

BRAIN AGING AND THE "CALCIUM HYPOTHESIS"

The potential contribution of altered Ca^{2+} homeostasis at least to some aspects of brain aging and neurodegeneration was first put forward by Khachaturian in the 1980s, with the formulation of the "Ca^{2+} hypothesis of aging" (Gibson and Peterson, 1987; Disterhoft et al., 1994; Khachaturian, 1994). Early findings in the field that corroborated this hypothesis examined the major transport pathways of Ca^{2+} during aging and found that at least in some types of neurons, such as the principal cells in the hippocampal CA1 region, there is an increased Ca^{2+} influx mediated by increased VOCC activity in aged neurons (Landfield and Pitler, 1984; Thibault and Landfield, 1996). Similarly, Ca^{2+} extrusion through the PMCA was found to be decreased in aged neurons (Michaelis et al., 1996). Subsequently, the focus shifted toward the intracellular mechanisms of Ca^{2+} homeostasis and their deregulation during aging. Several studies demonstrated that there is an increased release of Ca^{2+} from the ER stores through both the InsP3 and RyR receptors (Thibault et al., 2007), leading to the proposal that release from the RyR receptor may be a useful biomarker of neuronal aging. Below, we will consider in more detail findings

that relate to two key elements of aging: aberrant synaptic plasticity and neurodegeneration.

ROLE OF CALCIUM IN SYNAPTIC PLASTICITY AND NEURONAL EXCITABILITY DURING AGING

Aging of the brain is manifested in humans by a progressive cognitive decline associated with weakening of the ability to process new information and of the executive function. The most dramatic effect is notably observed on the function of episodic memory, including spatial memory. The cognitive decline associated with normal aging is not attributed to significant neuronal loss (Gallagher et al., 1996), but is rather thought to result from changes in synaptic connectivity and plasticity. There is a general consensus that memory and learning are molecularly encoded by mechanisms controlling synaptic plasticity in several brain areas. Among these, the afferent pathways of the hippocampus are the most relevant, but other areas such as the amygdale, the visual, somatosensory and prefrontal cortices, and the subiculum also play important roles in processing, integration, and consolidation of new information. Using mainly the hippocampus, numerous studies have deciphered a major role for Ca^{2+} in the two major forms of synaptic plasticity, LTP (Bliss and Collingridge, 1993) and long-term depression (LTD). LTP represents an increase in synaptic transmission, induced by pattern stimulation of afferent fibers and it is the main process proposed to underlie memory formation. On the other hand, LTD is a means of decreasing synaptic strength, contributing to the loss of synaptic contacts and associated with increased forgetfulness during aging (Foster, 1999, 2007; Zhou et al., 2004; Shinoda et al., 2005). Age-related changes in LTP and LTD underline the functional significance of altered synaptic plasticity for cognitive function (Foster and Norris, 1997; Foster, 1999; Foster and Kumar, 2002).

Relevant to the role of Ca^{2+} deregulation in memory loss, the critical event leading to induction of LTP appears to be the large influx of calcium ions into the postsynaptic spine. Importantly, LTP is blocked by injection of intracellular Ca^{2+} chelators such as EGTA (Lynch et al., 1983) or BAPTA (Mulkey and Malenka, 1992) and conversely, LTP is induced when the postsynaptic cell is loaded with calcium (Malenka et al., 1988). Therefore, it is well established that a significant elevation of postsynaptic Ca^{2+} concentration is both necessary and sufficient for the induction of hippocampal LTP (Bliss and Collingridge, 1993). In contrast, a modest rise in Ca^{2+} concentration results in induction of LTD through activation of protein phosphatases that dephosphorylate AMPA receptors (Artola and Singer, 1993; Lisman, 1989, 1994). Due to the differential level of Ca^{2+} fluctuation involved in the generation of the various forms of synaptic plasticity, the stimulation patterns for the induction of LTP and LTD constitute high- and low-frequency stimulation, respectively.

In general, the effect of aging on synaptic plasticity can be summarized by several key observations: First, the threshold for induction of LTP increases such that higher stimulation frequencies or more induction sessions are required in older animals in order to achieve the same level of potentiation. Second, the threshold for induction of LTD is lowered in aged animals, facilitating its prevalence. Furthermore, the maintenance of LTP is disrupted such that the enhanced transmission decays more rapidly in aged animals. In contrast, LTD and depotentiation, or erasure of LTP, are increased in aged animals due to a lowering of the threshold stimulation needed for induction of synaptic depression (Norris et al., 1996; Foster and Norris, 1997; Kamal et al., 2000; Vouimba et al., 2000). Thus, the age-related decline in synaptic transmission (Barnes, 1994) may reflect a shift in the LTP/LTD balance, with insufficient LTP induction and maintenance and excessive synaptic depression (Foster et al., 2001).

In most of the synapses that support LTP (in the hippocampus and elsewhere), the postsynaptic increase in calcium is mediated through the activation of the NMDA receptor. As already mentioned earlier, NMDA receptor activation allows the influx of calcium only when the receptor is occupied by L-glutamate and concomitantly the postsynaptic membrane is depolarized. Emerging evidence indicates that the synaptic plasticity shift during aging results from changes in the source of Ca^{2+} such that Ca^{2+} influx through NMDARs is reduced (Lehohla et al., 2008; Bodhinathan et al., 2010) and Ca^{2+} influx through L-type VDCCs is increased (Barnes, 1994; Norris et al., 1996; Thibault and Landfield, 1996; Shankar et al., 1998; Potier et al., 2000). The increase could arise from altered gene or protein expression (Herman et al., 1998), or phosphorylation changes of the L-type Ca^{2+} channels (Norris et al., 2002; Davare and Hell, 2003). Interestingly, the L-type Ca^{2+} channel blocker nimodipine counteracts age-related learning impairment in rabbits (Deyo et al., 1989; Kowalska and Disterhoft, 1994), rodents (Levere and Walker, 1992), non-human primates (Sandin et al., 1990), and elderly patients with dementia (Ban et al., 1990; Tollefson, 1990).

Additionally, aged neurons show a multitude of defects in Ca^{2+} homeostasis, including enhanced release of Ca^{2+} from the ER (Kumar and Foster, 2004; Gant et al., 2006), diminished Ca^{2+} extrusion through the plasma membrane ATPase (Michaelis et al., 1996; Gao et al., 1998), reduced cellular Ca^{2+} buffering capacity due to impairment of the SERCA pumps (Murchison and Griffith, 1999), and diminished mitochondrial Ca^{2+} sink capability (Murchison and Griffith, 1999; Xiong et al., 2002). The overall result is an increase of Ca^{2+} loads which negatively impact neuronal excitability (Landfield and Pitler, 1984; Khachaturian, 1989; Matthews et al., 2009). Moreover, such an increase in intracellular Ca^{2+} concentration increases the threshold frequency for induction of LTP (Shankar et al., 1998; Ris and Godaux, 2007), and enhances the susceptibility to induction of LTD (Norris et al., 1996; Kumar and Foster, 2005), ultimately explaining the age-associated deficits in learning and memory. In line with this notion, administration of the cell permeable Ca^{2+} chelator BAPTA, ameliorates impaired presynaptic cytosolic and mitochondrial Ca^{2+} dynamics in hippocampal CA1 synapses of old rats (Tonkikh and Carlen, 2009), and enhances spatial learning (Tonkikh et al., 2006).

In the context of LTP induction, a key early finding was the observation that postsynaptic entry of calcium leads to activation of Ca^{2+}/calmodulin complex-dependent kinase II (CaMKII), one of the most abundant proteins in neurons comprising 1–2% of the total protein. Although it is expressed both pre- and postsynaptically, its expression is particularly high in the postsynaptic density, where it is ideally located to respond to changes in calcium concentration. There are more than 30 isoforms of CaMKII and numerous substrates, many of which are located in the

postsynaptic density (Fink and Meyer, 2002). CaMKII is generally considered a mediator of primary importance in linking transient calcium signals to neuronal plasticity. Importantly, observations by Silva et al. (1992a,b,c) indicated that deletion of the CaMKII gene in mice results in impaired LTP and aberrant spatial memory. Moreover, activation of CaMKII is significantly reduced in aged hippocampal neurons (Mullany et al., 1996). The data obtained from studies on rodents have to a large extent, been paralleled by similar findings in other organisms, indicating that several models expressing various forms of synaptic plasticity exhibit a requirement for CaMKII activation. For instance, CaMKII knockout in *Drosophila* exhibits impaired associative learning, while motor and sensory systems remain unaffected (Joiner and Griffith, 1999). Similarly, knockout of *unc-43* (a gene encoding the CaMKII analog in *C. elegans*) affects the stability of synapses and general neuronal physiology, ultimately culminating in altered function of olfactory neurons (Sagasti et al., 2001).

Beyond activating the CaMKII signaling cascade, Ca^{2+} also acts as a second messenger that is responsible for the activity-dependent transcription of several key genes (West et al., 2001). The products of these genes are necessary in order to convert the effects of transient stimuli into long-term changes in brain function, a process that is required for the formation of memories. Of the neural-selective activity-dependent genes, brain-derived neurotrophic factor (BDNF) is activated by calcium influx through L-type VOCCs (L-VOCCs) acting on the transcription of *BDNF* from promoter III (West et al., 2001). BDNF is among the most relevant calcium targets for the modulation of memory. BDNF transcription is up-regulated dramatically by membrane depolarization *in vitro* (Ghosh et al., 1994; Tao et al., 1998) and by induction of LTP, and associative learning (Ernfors et al., 1991; Patterson et al., 1992; Tokuyama et al., 2000). Moreover, loss of BDNF is associated with impaired LTP among other synaptic defects. It is also well established that BDNF transcription is largely decreased during aging (Tapia-Arancibia et al., 2008), and that epigenetic induction of BDNF transcription in aged subjects significantly ameliorates the cognitive and memory defects associated with aging (Zeng et al., 2011). A summary of the perturbations of Ca^{2+} homeostasis associated with nervous system aging is shown in **Table 2**.

ROLE OF CALCIUM IN AGING-RELATED NEURODEGENERATION

Aging is the greatest risk factor for the development of neurodegenerative disorders. These include a diverse collection of pathologies characterized by the late onset and gradual loss of specific neuronal subpopulations in motor, sensory, or cognitive systems. Despite major intrinsic differences in the etiology of each disorder, deregulated Ca^{2+} homeostasis has emerged as a common underlying mechanism of neuronal loss in AD, Parkinson's (PD) diseases, amyotrophic lateral sclerosis (ALS), and other neurodegenerative disorders (Mattson, 2007; Bezprozvanny, 2009).

Alterations of Ca^{2+} homeostasis may be in some cases directly responsible for neuronal death. Persistently increased levels of intracellular Ca^{2+} can result in severe phenotypes in neurons, culminating to neuronal death and degeneration (Siman et al., 1989; Celsi et al., 2009). This process is often specifically mediated or even initiated by the diminished capacity of mitochondria to buffer Ca^{2+}. An example where there is ample evidence that altered mitochondrial Ca^{2+} homeostasis mediates neuronal loss is ALS, an adult onset disease, with incidence increasing with age. ALS is characterized by selective and progressive degeneration of motorneurons in the spinal cord and brain, leading to weakness, atrophy, and paralysis of voluntary muscles. Mutations in superoxide dismutase (SOD1) are the most common genetic factors responsible for about 20% of familial ALS cases (Rosen et al., 1993). SOD1 is a ubiquitously expressed enzyme that converts superoxide to hydrogen peroxide in order to protect cells against oxidative stress. While there is still no consensus as to how mutant SOD1 causes selective toxicity to motorneurons, increasing evidence suggests that the mechanisms largely concentrate on the dysfunction of ER and mitochondrial Ca^{2+} homeostasis (Bacman et al., 2006; Hervias et al., 2006; Magrane et al., 2009; Shi et al., 2010).

Table 2 | Perturbations of Ca^{2+} homeostasis in the aging nervous system.

Ca^{2+} deregulation associated with aging of the nervous system	Reference
Increased Ca^{2+} influx mediated by voltage-dependent calcium channels	Landfield and Pitler (1984), Thibault and Landfield (1996)
Decreased Ca^{2+} extrusion through the plasma membrane pump (PMCA)	Michaelis et al. (1996), Gao et al. (1998)
Increased release of Ca^{2+} from the ER stores through both the InsP3 and RyR receptors	Thibault et al. (2007)
Reduced Ca^{2+} influx through NMDARs	Lehohla et al. (2008), Bodhinathan et al. (2010)
Increased Ca^{2+} influx through L-type VDCCs	Barnes (1994), Norris et al. (1996), Thibault and Landfield (1996), Shankar et al. (1998), Potier et al. (2000)
Phosphorylation changes of the L-type Ca^{2+} channels	Norris et al. (2002), Davare and Hell (2003)
Increased release of Ca^{2+} from the ER	Gant et al. (2006), Kumar and Foster (2004)
Impairment of the SERCA pumps	Murchison and Griffith (1999)
Diminished mitochondrial Ca^{2+} sink capability	Murchison and Griffith (1999), Xiong et al. (2002)
Reduced activation of CaMKII in hippocampal neurons	Mullany et al. (1996)
Reduced Ca^{2+}-dependent transcription of genes such as BDNF	Tapia-Arancibia et al. (2008)

At the level of the ER, a recent paper implicates the Ca^{2+} buffering protein calreticulin in the death of motorneurons in a model of ALS (Bernard-Marissal et al., 2012). More specifically, fast fatigable motorneurons selectively activate an ER stress response that drives their early degeneration, while a subset of mSOD1 motorneurons shows exacerbated sensitivity to activation of the motorneuron-specific Fas (transmembrane TNF receptor superfamily member 6) and nitric oxide (NO) pathway. However, the links between the two mechanisms and the molecular basis of their cellular specificity remained unclear. This paper demonstrates that Fas activation causes reduced levels of calreticulin specifically in mSOD1 motorneurons. Decreased expression of calreticulin is both necessary and sufficient to trigger SOD1(G93A) motorneuron death through the Fas/NO signaling pathway, and represents an early event that precedes muscle denervation and is restricted to vulnerable motor pools.

At the mitochondrial level, altered Ca^{2+} handling also appears early on, before motorneuron degeneration is manifested, suggesting that it is actively involved in disease pathogenesis. SOD1, which is a predominantly cytosolic protein, also localizes to the ER and mitochondria (Jaarsma et al., 2001; Okado-Matsumoto and Fridovich, 2001; Higgins et al., 2002; Mattiazzi et al., 2002), predominantly in the intermembrane space and less so on the outer membrane (Pasinelli et al., 2004; Vande Velde et al., 2008) and matrix (Vijayvergiya et al., 2005). By mechanisms that are still poorly understood, mutant SOD1 induces increased Ca^{2+} uptake by mitochondria, as convincingly demonstrated in mitochondria isolated from the brain and spinal cord of SOD1 mutant mice (Damiano et al., 2006). This defect appears to be neuron-specific, as liver cells from the same mutants retain unaffected mitochondrial Ca^{2+} homeostasis. Impaired Ca^{2+} handling by mitochondria is thought to be the primary cause of the abnormally high concentration of intracellular Ca^{2+} observed in ALS motorneurons (Carri et al., 1997; Kruman et al., 1999), making them vulnerable to degeneration (Kim et al., 2002, 2007).

Mitochondrial Ca^{2+} overload is associated with activation of cell death pathways (Bernardi et al., 1999) and is observed in many pathological conditions in addition to ALS (Honda and Ping, 2006; Norenberg and Rao, 2007). The mechanisms responsible for Ca^{2+} overload are not entirely clear; however, their elucidation could provide a base for significant pharmacological interventions in the future. Theoretically, defects of the mitochondrial NCX could be involved in causing Ca^{2+} overload in ALS, although this putative mechanism remains to be directly explored. Another potential factor contributing to Ca^{2+} overload could be the functional and physical link between mitochondria and ER. Transfer of Ca^{2+} from the large stores in the ER to mitochondria depends on the relative positioning of these two organelles, and it is thought to occur at Ca^{2+} "hotspots", sites where ER and mitochondrial membranes are in close physical contact (Rizzuto et al., 1999). Shortening the distance between the two organelles was shown to result in increased accumulation of Ca^{2+} in mitochondria, causing cell death (Csordas et al., 2006). Since mutant SOD1 accumulates both in ER (Kikuchi et al., 2006; Urushitani et al., 2006) and mitochondrial (Liu et al., 2004) membranes, it is plausible that the structure of these calcium hotspots is altered in mutant neurons, leading to abnormal handling of Ca^{2+} between the two organelles.

Whatever the mechanism of the increased Ca^{2+} accumulation in mitochondria, activation of cell death by mitochondrial Ca^{2+} overload involves the opening of the mPTP, followed by release of cytochrome *c*, and downstream activation of apoptosis. Cytochrome *c* released into the cytosol can further propagate apoptotic signaling by binding to the IP3-R on the ER, desensitizing its autoinhibition by calcium and thus causing further calcium release from ER stores (Boehning et al., 2003). Ablation of cyclophilin D (CypD), a modulatory component of the mPTP, delays the opening of mPTP (Basso et al., 2005) and has a protective effect against neuronal death in models of ischemia (Baines et al., 2005; Schinzel et al., 2005). In ALS, it was also reported that loss of CypD in SOD1 mutant mice delays the onset of the disease and significantly extends lifespan (Martin et al., 2009). Moreover, two studies using the immunosuppressant cyclosporin A, which binds to CypD to inhibit mPTP, in mutant SOD1 mice, suggest that inhibition of mPTP may be of benefit to ALS (Keep et al., 2001; Kirkinezos et al., 2004).

Another mechanism whereby Ca^{2+} contributes to the activation of cell death is by stimulating the production of mitochondrial reactive oxygen species (ROS). Oxidative stress caused by the damaging effect of ROS to proteins, lipids, and DNA, is a common feature of aging-related diseases, including ALS (Floyd and Hensley, 2002; Lin and Beal, 2006). Mitochondrial dysfunction (Wei, 1998), and particularly mitochondrial Ca^{2+} overload (Petrosillo et al., 2004), increases ROS production. In particular, increased levels of mitochondrial Ca^{2+} enhance cytochrome *c* release through a mechanism involving ROS-mediated oxidation of cardiolipin (Vercesi et al., 1997; Iverson and Orrenius, 2004). Notably, lipid peroxidation (Mattiazzi et al., 2002) and dissociation of cytochrome *c* from the mitochondrial inner membrane (Kirkinezos et al., 2005) have been reported in mutant SOD1 mice, but also in PD (Beal, 2003), and AD (Green and Kroemer, 2004;Lin and Beal, 2006; Kawamoto et al., 2012; Lee et al., 2012a).

Alzheimer's disease is perhaps the most widespread neurodegenerative disorder of the elderly, with most familiar cases attributed to several mutations in presenilin 1 and 2, genes whose protein products are responsible for the proteolytic cleavage of the amyloid precursor peptide (APP). The mechanism by which presenilin mutations cause AD involves increased production of Aβ1–42 which aggregates and damages neurons. This view has been recently expanded by emerging findings suggesting that perturbed ER Ca^{2+} homeostasis significantly contributes to the dysfunction and degeneration of neurons in AD (Kipanyula et al., 2012). For example, recent work indicates that there is impaired Ca^{2+} uptake by mitochondria in the dentate gyrus of a mouse model of AD (Lee et al., 2012b). This can be explained to some extent by the novel role proposed by at least two groups for presenilins as regulators of Ca^{2+} homeostasis in the ER (Pack-Chung et al., 2000; Yoo et al., 2000). Interestingly, mutations in presenilin 1 that cause early onset familial AD, increase the pool of ER Ca^{2+} available for release, and enhance Ca^{2+} release from the ER through IP3- and RyR receptors (Chan et al., 2000; Guo et al., 1996, 1999; Cheung et al., 2010; Leissring et al., 2000). Future research should clarify the specific contributions of perturbed ER Ca^{2+} handling to the cellular events that underlie synaptic dysfunction and neuronal degeneration in AD. While elevated pools of ER

Ca^{2+} resulting from mutations in presenilins have been widely documented in a range of cell culture and animal models, the molecular basis of this alteration remains unknown and is potentially a key field for the development of novel pharmacological targets.

In addition to direct effects on neuronal survival, altered Ca^{2+} homeostasis is also likely to contribute to the initiation or progression of the neurodegenerative process by enhancing neuronal vulnerability to metabolic and other stressors (Toescu and Verkhratsky, 2004; Toescu and Vreugdenhil, 2010). One such example is the population of basal forebrain cholinergic neurons, a group of neurons that are selectively vulnerable to pathology and loss early in AD, as well as in a number of other neurodegenerative disorders of the elderly. In the primate, including man, these neurons are rich in the Ca^{2+} buffer protein calbindin. Notably, there is a substantial loss of calbindin in the course of normal aging and a further loss in AD(Iacopino and Christakos, 1990). Significantly, cholinergic neurons that had lost their calbindin in the course of normal aging were those that selectively degenerated in AD, while calbindin-containing neighboring neurons were virtually resistant to the process of tangle formation, a hallmark of the disease (Riascos et al., 2011). Another study reported that over-expression of calbindin in presenilin 1 mutant neurons was sufficient to prevent apoptosis (Guo et al., 1998). Similarly, a dramatic reduction in the Ca^{2+} buffering protein calbindin levels has been described in brains of PD patients (Iacopino and Christakos, 1990) and dopaminergic (DA) neurons expressing higher levels of calbindin, or other Ca^{2+} buffers such as calretinin and parvalbumin, were shown to be resistant to degeneration in PD (Yamada et al., 1990; Tsuboi et al., 2000). These findings are consistent with earlier findings suggesting that calbindin-positive hippocampal neurons are more resistant against oxidative stress (Mattson et al., 1991), although other Ca^{2+} buffer proteins seem to confer resistance to stress in different neuronal subpopulations. Understanding the mechanisms underlying such an instructive function of Ca^{2+} buffer proteins is of great importance as there may be a yet unidentified crosstalk with major signaling cascades. More work in this direction would greatly enhance our ability to selectively intervene in order to modulate the vulnerability of distinct neuronal populations.

Similar to ALS and AD, PD is another case where Ca^{2+} deregulation has recently attracted a lot of attention. PD is characterized by motor defects resulting from the selective loss of DA neurons in the substantia nigra and intracellular accumulation of cell aggregates known as Lewy bodies, mostly composed of α-synuclein. The idea that mitochondria could be directly involved in the pathogenesis of PD comes from the early accidental observation that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an inhibitor of the mitochondrial respiratory chain complex I, causes Parkinson-like symptoms (Langston and Ballard, 1983). Later on, it was also demonstrated that DA neurons from PD patients show massive accumulation of mitochondrial DNA (mtDNA) deletions that impair the function of the respiratory chain complexes (Exner et al., 2012), thus increasing the probability of dysfunctions in these organelles.

Some clues as to the selective vulnerability of this population arise from the fact that DA neurons of the substantia nigra display unusual physiological properties. First, unlike most other neurons in the brain, they are autonomously active, generating regular action potentials in the absence of synaptic input (Grace and Bunney, 1983). This pacemaking activity is thought to maintain physiological levels of dopamine in regions they innervate, particularly the striatum (Romo and Schultz, 1990). To drive this pacemaking activity, these neurons rely, at least in part, on a rare form of L-type Ca^{2+} channels (Bonci et al., 1998; Ping and Shepard, 1996; Puopolo et al., 2007) comprised of the Cav1.3 pore-forming subunit (Striessnig et al., 2006; Chan et al., 2007). This leads to typically elevated intracellular Ca^{2+} concentrations under physiological conditions (Wilson and Callaway, 2000; Chan et al., 2007). Second, DA neurons of the substantia nigra display an elaborate axonal network (Matsuda et al., 2009), supporting orders of magnitude more synapses compared to a cortical pyramidal neuron (Arbuthnott and Wickens, 2007). As a result, the mitochondrial density in their somatic and dendritic regions is very low compared to other neuronal types (Liang et al., 2007). Taken together, these characteristics are thought to contribute to an intrinsic state of increased metabolic stress, where increased load of intracellular Ca^{2+} is met by a depleted mitochondrial network.

Additional genetic factors could increase the rate at which mitochondrial Ca^{2+} homeostasis is compromised in these already vulnerable neurons. At least 13 gene loci and 9 genes have been linked to both autosomal dominant and recessive forms of PD (Lesage and Brice, 2009). Mutations in three proteins encoded by these genes, namely, parkin (PARK2), DJ-1 (PARK7), and PINK1 ($PARK_6$), are associated with recessive early onset forms of PD, whereas mutations in α-synuclein (PARK1–4) and LRRK2 ($PARK_8$) are responsible for dominant forms of familial PD. Mitochondrial dysfunction has been described for mutants of all these genes (Lesage and Brice, 2009).

Recent papers have started to explore in more detail the possibility of Ca^{2+} handling by the PD-related proteins. DJ-1 is a multitask protein that, in addition to its main role as an antioxidant (Taira et al., 2004), is also involved in maintaining cytosolic basal Ca^{2+} concentration values to permit depolarization-induced Ca^{2+} release from the sarcoplasmic reticulum in muscle cells (Shtifman et al., 2011). Moreover, DJ-1 was shown to protect DA neurons from Ca^{2+}-induced mitochondrial uncoupling and ROS production during physiological pacemaking (Guzman et al., 2010).

Regarding α-synuclein, it has been described that it can modulate Ca^{2+} influx from the extracellular milieu by enhancing the plasma membrane ion permeability (Danzer et al., 2007) either through their direct insertion into the plasma membrane and the formation of a pore (Lashuel et al., 2002) or through the modulation of plasma membrane Ca^{2+} permeability (Furukawa et al., 2006). The actual mechanisms through which α-synuclein aggregation and Ca^{2+} dysfunction influence each other are not clear, however, a functional interplay is unambiguous: Increased intracellular Ca^{2+} promotes α-synuclein aggregation, which in turn could promote intracellular Ca^{2+} increase (Nath et al., 2011). A recent study suggests that using its C-terminal domain, α-synuclein controls mitochondrial calcium homeostasis by enhancing ER–mitochondria interactions (Cali et al., 2012). As these

results were obtained *in vitro* using non-neuronal cell lines, their relevance to DA neuron physiology and pathology remains to be examined.

As to PINK1, its direct role in regulating cellular, and most specifically mitochondrial Ca^{2+} fluxes, has been recently proposed starting with the observation that the co-expression of mutant PINK1 in a cellular model of PD-expressing mutated α-synuclein exacerbated the observed mitochondrial defects, that is, increased mitochondrial size with loss of cristae and reduced ATP levels (Marongiu et al., 2009). The proposed mechanisms of PINK1 action was based on a deregulation of mitochondrial Ca^{2+} influx. As by blocking mitochondrial Ca^{2+} uptake, it was possible to restore the original phenotype (Marongiu et al., 2009), thus suggesting that mutant PINK1 could reinforce α-synuclein pathology by acting on converging pathways affecting mitochondrial function. Other studies have further investigated the role of PINK1 in mitochondrial Ca^{2+} metabolism, but the results are controversial. In one case, it was proposed that PINK1 absence caused an impairment of mitochondrial Ca^{2+} efflux, probably affecting the mitochondrial Na^{+}/Ca^{2+} exchanger activity and thus resulting in mitochondrial Ca^{2+} overload, ROS production, and impaired respiration (Gandhi et al., 2009). In another very recent study, PINK1 depletion has instead been shown to impair mitochondrial Ca^{2+} uptake and consequently to affect energy metabolism (Heeman et al., 2011). However, consistently, numerous reports showed that PINK1-deficient cells have impaired mitochondrial membrane potential and enhanced sensitivity to the toxic effects of mitochondrial complex I inhibitors (Wood-Kaczmar et al., 2008), as well as enhanced Ca^{2+} vulnerability (Akundi et al., 2011).

OUTLOOK

Given the fundamental importance of Ca^{2+} homeostasis in the biology of all cells, it is not completely surprising that more and more studies suggest that deregulated Ca^{2+} is actively involved in the course of normal aging and in diverse pathological conditions. A general message arising from these studies is that in the nervous system Ca^{2+} signaling and homeostasis should be examined in view of the amazing cellular diversity exhibited by the nervous system. The machinery controlling Ca^{2+} homeostasis is similarly diverse among neurons, uniquely suited to the needs of each neuronal subtype. Taken together, the intrinsic differences of neurons in morphology, connectivity, proteome and Ca^{2+} homeostatic machinery are very likely to collectively and synergistically contribute to the selective vulnerability of distinct neuronal populations to different causes of senescence. The more we understand the interplay of Ca^{2+} homeostatic mechanisms with the intrinsic qualities of different neurons, the closer we will get to developing cell-specific therapies.

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Mitochondrial deficiency: a double-edged sword for aging and neurodegeneration

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For decades, aging was considered the inevitable result of the accumulation of damaged macromolecules due to environmental factors and intrinsic processes. Our current knowledge clearly supports that aging is a complex biological process influenced by multiple evolutionary conserved molecular pathways. With the advanced age, loss of cellular homeostasis severely affects the structure and function of various tissues, especially those highly sensitive to stressful conditions like the central nervous system. In this regard, the age-related regression of neural circuits and the consequent poor neuronal plasticity have been associated with metabolic dysfunctions, in which the decline of mitochondrial activity significantly contributes. Interestingly, while mitochondrial lesions promote the onset of degenerative disorders, mild mitochondrial manipulations delay some of the age-related phenotypes and, more importantly, increase the lifespan of organisms ranging from invertebrates to mammals. Here, we survey the insulin/IGF-1 and the TOR signaling pathways and review how these two important longevity determinants regulate mitochondrial activity. Furthermore, we discuss the contribution of slight mitochondrial dysfunction in the engagement of pro-longevity processes and the opposite role of strong mitochondrial dysfunction in neurodegeneration.

Keywords: aging, insulin/IGF-1, mitochondria, neurodegeneration, oxidative stress, TOR

INTRODUCTION

Eukaryotic cells have adopted an elaborated set of molecular mechanisms that prevent the accumulation of aberrant macromolecules (Kirkwood, 2005; Douglas and Dillin, 2010; Kourtis and Tavernarakis, 2011). Over time, these protective responses decline and make cells more vulnerable to stressful conditions. The consequent dysfunction of tissues and organs can prompt to the development of pathologies that compromise survival. For many years, the age-related decline was considered simply a passive and inevitable process. Conversely, it is now clear that aging is a biological process, which like many others is subjected to the regulation of well-defined signaling pathways (Kenyon, 2010; Bano et al., 2011; Martin, 2011). Most of these molecular cascades control metabolism, proliferation, stress resistance, and cell maintenance. Although their contribution to longevity was firstly described in simple model organisms with a relatively short lifespan, like yeast and invertebrates, a large number of findings in mammals support that they are evolutionary conserved and likely relevant in humans (Fontana et al., 2010).

Aging has a significant impact in our modern human society, as it is associated with the increased susceptibility to pathologies. Intensive studies in the last years have shown that most of the mechanisms involved in longevity influence also the onset of sporadic forms of brain disorders (Mattson, 2006; Mattson and Magnus, 2006; Bishop et al., 2010). The complex network of interactions intimately links various signaling pathways and molecular players that, together, contribute to such neurological conditions. Among them, mitochondria have a fundamental role in neuronal function and decline in their activity accelerates the onset and progression of age-related dysfunction (Nunnari and Suomalainen, 2012; Rugarli and Langer, 2012). Interestingly, while mild mitochondrial impairment extends the lifespan in various organisms as different as yeast, invertebrates and mice, significant suppression of mitochondrial activity compromises animal survival. Similarly, whilst mitochondrial deficiency or uncoupling can partially delay neuronal degeneration as a result of excitotoxic injury or toxins, loss-of-function mutations in genes encoding certain mitochondrial proteins can negatively disturb neural circuits and ultimately lead to cell death. Here, we review the advances in understanding some of the molecular mechanisms that regulate certain aspects of aging, such as age-related mortality. We also dedicate particular attention to the contribution of mitochondria to the signaling pathways involved in this important biological process. Moreover, we address the controversial opposite role of mitochondrial dysfunction in the onset of brain pathologies.

LONGEVITY PATHWAYS

THE INSULIN/IGF-1 SIGNALING PATHWAY

The insulin/IGF-1 signaling pathway is one of the main pathways regulating aging in organisms ranging from invertebrates, like *Drosophila melanogaster* and *Caenorhabditis elegans*, to mammals. The role of this pathway in longevity was initially identified in the nematode *C. elegans* through the discovery of mutants that decrease the activity of the pathway and extend the lifespan of the organism (Kenyon et al., 1993). The existence of such mutations supported the concept of molecular factors underlying aging. Among them, mutations in the gene *age-1* extend the

chronological lifespan of the nematode (Friedman and Johnson, 1988). This gene encodes the *C. elegans* ortholog of the class I phosphoinositide 3-kinase (PI3K) and is a key enzyme in the Insulin/IGF-1 signaling pathway. It catalyzes the production of phosphatidylinositol-3,4,5-trisphosphate (Morris et al., 1996) that serves as a second messenger for the activation of downstream kinases. AGE-1/PI3K is activated by the sole insulin/IGF-1 receptor DAF-2, which belongs to the tyrosine kinase receptor family and is a master regulator of metabolism. Mutations in the *daf-2* gene almost double the lifespan of nematodes (Kenyon et al., 1993), mainly through the activation of the transcription factors DAF-16/FOXO, SKN-1/Nrf, and HSF-1 (Hsu et al., 2003; Tullet et al., 2008; **Figure 1**). In animals with reduced insulin/IGF-1 signaling, the nuclear translocation of DAF-16/FOXO, SKN-1/Nrf, and HSF-1 promotes the expression of various target genes involved in stress resistance, proteostasis, defense reaction and metabolism (Narasimhan et al., 2009). Interestingly, enhanced transcription in certain tissues contributes differently to the aging of somatic tissues. For example, specific expression of *daf-16* in the intestine – the main adipose tissue in nematodes – extends the lifespan of *daf-16*; *daf-2* double mutants, although it is not sufficient to completely restore the same survival as in the *daf-2* mutant animals (Libina et al., 2003). Notably, the activity in one tissue, like in the case of the intestine, can regulate DAF-16-mediated longevity pathways in others in a feedback loop that controls post-mitotic cell senescence (Murphy et al., 2007). In this context, the intestinal DAF-16/FOXO coordinates the rate of aging of the whole organism in response to signals from the reproductive and nervous systems. Block of germ cell proliferation in animals lacking functional gonad increases the lifespan through the DAF-16/FOXO accumulation in the intestinal nuclei and the consequent gene transcription (Lin et al., 2001; Arantes-Oliveira et al., 2002). Remarkably, loss-of-function of the microRNA *mir-71* in the nervous system suppresses intestinal DAF-16-dependent gene expression and therefore germline-mediated longevity (Boulias and Horvitz, 2012), further underlying the complexity of the signals that dictate how long an organism is going to live.

The prominent role of the insulin/IGF-1 signaling pathway in longevity is evolutionary conserved across species. In *D. melanogaster*, mutations in the sole insulin/IGF-1 receptor (dINS) or the insulin receptor substrate *chico* extend the lifespan through the activation of the FOXO transcription factor (Clancy et al., 2001; Tatar et al., 2001; Slack et al., 2011). Similarly to nematodes, FOXO overexpression in the fat body is sufficient to increase the lifespan of flies (Giannakou et al., 2004; Hwangbo et al., 2004). In mice, haploinsufficiency of the insulin-like growth factor type 1 receptor (Igf1r) significantly increases the lifespan compared with wild-type littermates (Holzenberger et al., 2003). Although the recent findings argue the increased longevity of Igf1r-deficient mice (Bokov et al., 2011), it is accepted that mild reduction of the insulin/IGF-1 signaling throughout the body or even restricted at the central nervous system can increase the lifespan of mice (Taguchi et al., 2007). Even in humans, accumulating evidence suggests that lower insulin/IGF-1 signaling is beneficial for longer survival (van Heemst et al., 2005). It is noteworthy to mention that single nucleotide polymorphisms in FOXO3A gene are strongly associated with human longevity (Willcox et al., 2008). Likewise, a study on centenarians demonstrated that heterozygous mutations in the highly polymorphic *Igf1r* are correlated with longevity in humans (Suh et al., 2008).

Decreased activity of the insulin/IGF-1 signaling pathway enhances the resistance to exogenous and endogenous oxidative stress in nematodes as well as in mice (Holzenberger et al., 2003; Hsu et al., 2003). This is the result of the DAF-16/FOXO reprogramming process and the consequent synthesis of chaperones and other anti-oxidant factors. Recent evidence suggests that mitochondria are also important for the resistance of *daf-2* mutant nematodes through the production of reactive oxygen species (ROS). ROS are a by-product of oxidative phosphorylation but apart from their toxic effect in high concentrations, they can also act as signaling molecules. At least in nematodes, impairment of the insulin/IGF-1 pathway increases mitochondrial activity and, as a consequence, ROS production. ROS mediate a retrograde response resulting to up-regulation of genes encoding antioxidant enzymes. Importantly, AMP-activated protein kinase (AMPK) is required to sense the intracellular energetic status associated with enhanced oxidative stress. As a result, AMPK up-regulates L-proline mitochondrial catabolism while reduces glucose metabolism, further contributing to the ROS generation (Ristow and Zarse, 2010; Zarse et al., 2012). In parallel, while AMPK controls mitochondrial respiration in impaired glucose conditions, SKN-1(Nrf) and PMK-1(p38) up-regulate the transcription of specific genes and induce the protective stress resistance response. Clearly, AMPK is a fundamental intracellular checkpoint as it adapts intracellular metabolism and catabolism to the energetic needs of the organism. In *C. elegans*, AMPK is required for the extension of lifespan in mutants with reduced insulin/ IGF-1 signaling, as it controls the lipid storage and the fat metabolism according to the energetic stress (Apfeld et al., 2004; Curtis et al., 2006; Narbonne and Roy, 2009). Overexpression of the AMP-activated protein kinase subunit AAK-2 prolongs the lifespan of the organism, whereas its loss-of-function reduces it (Apfeld et al., 2004). Notably, AMPK deficiency compromises the rapid mobilization of fat reservoirs, leading to premature lethality of dauer larvae. Recently, it was revealed that AMPK acts on catalases and regulates the levels of H_2O_2 into the cell (Xie and Roy, 2012). Similarly to ROS, sub-lethal doses of H_2O_2 signal to the nucleus through the HIF-1 transcription factor and modulate the physiology of the cell enabling the survival under stress. Some of the genes that are up-regulated favor the biosynthesis of fatty acids. In parallel, H_2O_2 blocks lipases and protects lipid stores. Moreover, AMPK modulates autophagy through the direct phosphorylation of ULK1, the mammalian ortholog of Atg1, as a required step for survival when nutrients are insufficient (see next paragraph and Lee et al., 2010a; Egan et al., 2011; Kim et al., 2011).

Although it is currently unknown whether other mitochondria-to-nucleus signals are engaged in insulin/IGF-1 deficient organisms, the regulation of mitochondrial respiration is clearly a key component of lifespan extension and can further influence survival. As an example, increased mitochondrial fusion, by suppressing dynamin-related protein DRP-1 expression does not alter the lifespan of wild-type animals, whereas it further prolongs the survival of *daf-2* mutant nematodes (Yang et al., 2011). Similarly,

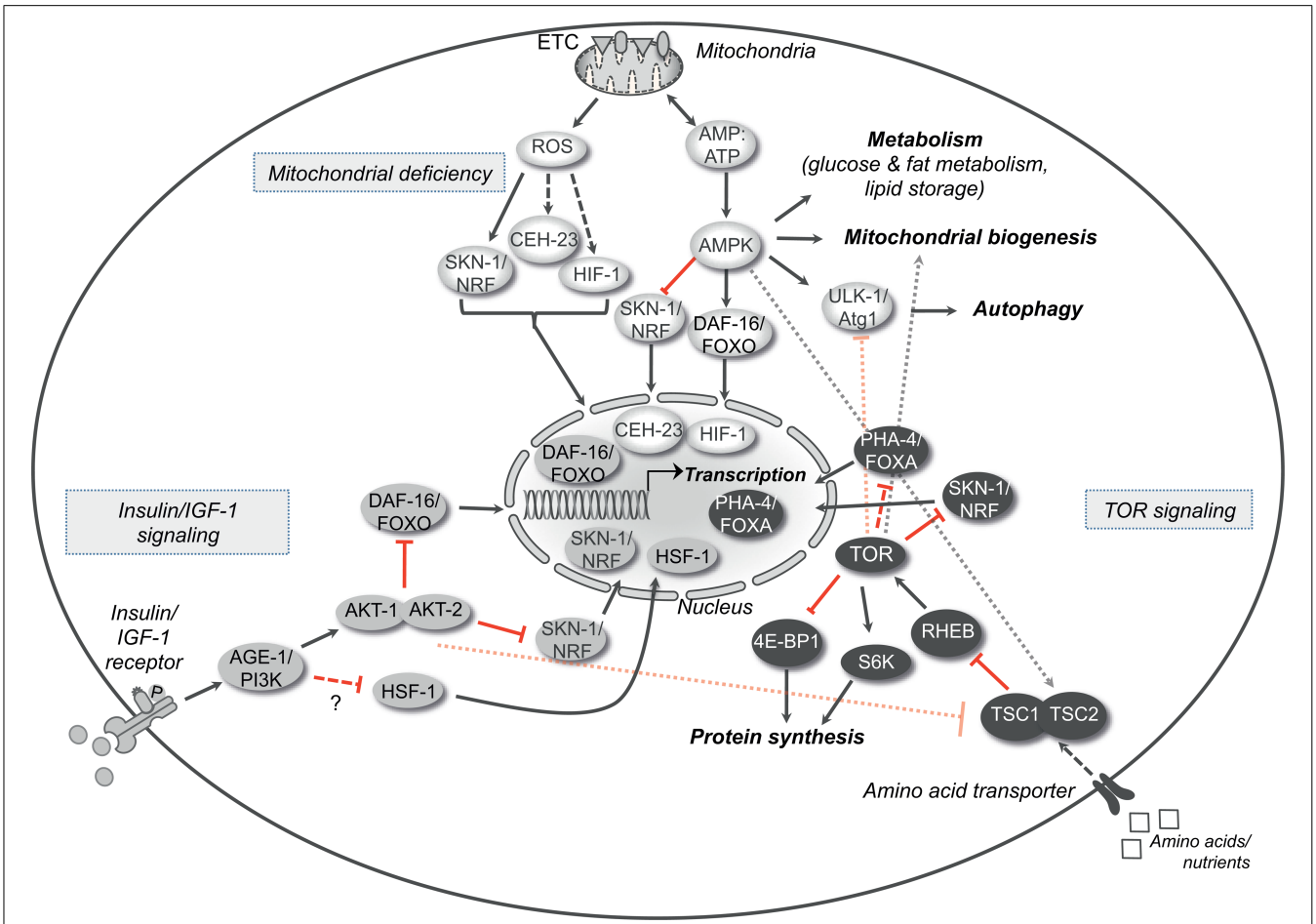

FIGURE 1 | Longevity pathways. The insulin/IGF-1 signaling, the TOR signaling and the molecular cascade that is activated by mild mitochondrial dysfunction are three important pathways that interact with each other and modulate aging in various organisms. The crosstalk between these pathways is represented in the figure. Arrows indicate positive regulatory events and red bars indicate inhibitory interactions. Dotted arrows or bars represent interactions between the different pathways, whereas dashed arrows or double bars indicate possible indirect interactions. AGE-1/PI3K, phospatidylinositol-3-kinase; DAF-16/FOXO, forkhead box O (FOXO) transcription factor; HSF-1, heat shock response transcription factor-1; SKN-1/Nrf, skin in excess transcription factor 1/NF-E2-related factor; TSC1/2, tuberous sclerosis complexes 1 and 2; RHEB, Ras homolog enriched in brain; TOR, target of rapamycin kinase; S6K, S6 kinase; 4E-BP1, eukaryotic initiation factor 4E-binding protein; PHA-4/FOXA, forkhead box A (FOXA) transcription factor; ROS, reactive oxygen species; ATP, adenosine-5′-triphosphate; CEH-23, homeobox transcription factor; HIF-1, hypoxia-inducible transcription factor 1; AMPK, adenosine monophosphate-activated protein kinase; ULK-1/Atg1, serine-threonine kinase ortholog of the autophagy related kinase 1 (Atg1).

increased mitochondrial proliferation in insulin/IGF-1 deficient animals as a result of some genetic lesions, like prohibitins, causes a twist in cellular metabolism and further extends the lifespan of nematodes (Artal-Sanz and Tavernarakis, 2009).

THE TOR SIGNALING PATHWAY

The serine/threonine kinase "target of rapamycin" TOR (mTOR in mammals) has drawn large attention for its pleiotropic effects on aging through the control of multiple downstream pathways (Ravikumar et al., 2010; **Figure 1**). TOR senses the availability of amino acids and nutrients into the cell and regulates cell growth, proliferation, and metabolism accordingly. In the presence of growth factors, like insulin and IGF-1, Akt kinase is activated and controls the function of the TSC1/TSC2 complex, a negative regulator of mTOR. Post developmental TOR inhibition extends the lifespan of many different organisms, ranging from yeast to mammals (Vellai et al., 2003; Jia et al., 2004; Kapahi et al., 2004; Kaeberlein et al., 2005; Harrison et al., 2009). Moreover, TOR mediates gene transcription that, at least in yeast and in *C. elegans*, is necessary for the effect on chronological lifespan (Medvedik et al., 2007; Sheaffer et al., 2008). In yeast, TOR increases the expression of the nicotinamidase gene *PNC1*, an important regulator of the NAD-dependent deacetylase Sir2, through the transcription factors Msn2p and Msn4p (Medvedik et al., 2007). In *C. elegans*, reduced TOR signaling enables the forkhead transcription factor PHA-4 to induce the expression of pro-survival factors that contribute to lifespan extension of animals under nutrient restriction (Sheaffer et al., 2008). In a variety of conditions, TOR signaling controls the expression of stress-resistance genes through the SKN-1/Nrf transcription factor and prevents the ROS formation beyond a fatal threshold due to the increased mitochondrial metabolism (Robida-Stubbs et al.,

2012; Zarse et al., 2012). In mammals, decreased mTOR activity, due to rapamycin, prevents the direct binding and coactivation of the transcription factor ying-yang 1 (YY1) with the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). At the molecular level, disruption of this complex and reduced recruitment to the promoters of genes encoding mitochondrial proteins diminishes mitochondrial biogenesis and consequently oxidative phosphorylation (Cunningham et al., 2007).

Beside the regulation of gene expression, TOR pathway contributes to aging through its role in protein synthesis: TOR activates the ribosomal subunit S6 kinase (RS6K) and in parallel inhibits the 4E-BP1, which is a negative regulator of translation, resulting in increased protein synthesis. Block of protein synthesis through inhibition of RS6K or the initiation of translation 4E protein (eIF4E), which is the target of 4E-BP inhibitor, leads to lifespan extension in various organisms (Kapahi et al., 2004; Kaeberlein et al., 2005; Hansen et al., 2007; Pan et al., 2007; Syntichaki et al., 2007; Selman et al., 2009). In S6K knockout mice they have found activation of pathways regulated by PGC-1α and AMPK in some tissues, like the liver, adipose tissue, or muscles (Selman et al., 2009). These pathways modulate mitochondrial biogenesis. In yeast, Sch9/S6K regulates mitochondrial oxygen consumption and mutant strains, either for TOR or Sch9/S6K, up-regulate both nuclear and mitochondrial genes encoding proteins of oxidative phosphorylation (OXPHOS; Pan and Shadel, 2009). In flies kept under dietary restriction (DR), the 4E-BP boosts mitochondrial activity due to enhanced translation of nuclear-encoded mitochondrial genes, whereas inhibition of the electron transport chain prevents the lifespan extension (Zid et al., 2009).

By all means, the integration of the cellular status depends on the crosstalk between the different pathways and intracellular sensors. Nutrient or growth factor deprivation promotes the catabolic process autophagy through TOR. Autophagy is a homeostatic process likely developed in unicellular organisms as an adaptive survival response to harsh conditions (Yorimitsu and Klionsky, 2005; Singh and Cuervo, 2011). It is important for the turnover of intracellular macromolecules and damaged organelles and, it is widely considered as a potential anti-aging mechanism. Thus, in the case of mitochondria, TOR not only regulates mitochondrial biogenesis but also regulates mitochondrial turnover though macroautophagy (mitophagy). During this process the cytosolic material is engulfed by double-membrane vesicles and targeted to the lysosome for degradation. This quality control mechanism protects from the intracellular accumulation of dysfunctional organelles and, therefore, from eventual oxidative stress as a result of inefficient oxidative phosphorylation. TOR modulates autophagy through a cascade of events that alters the phosphorylation status of the serine/threonine kinases ULK-1/ULK-2, the mammalian orthologs of Atg1, and the association between Ambra1 and Beclin-1, favoring the recruitment of autophagy-related proteins to the nascent phagophore (Rubinsztein et al., 2011). Notably, reduction of TOR activity increases autophagy, which is required for the lifespan extension in TOR-deficient animals and insulin/IGF-1 defective mutants (Vellai et al., 2003; Hansen et al., 2008; Toth et al., 2008; Bjedov et al., 2010). According to this view, block of autophagy abolishes the extension of lifespan in the *daf-2* mutants independently of the DAF-16/FOXO transcription factor, although with a less pronounced effect compared to *daf-16* loss-of-function (Melendez et al., 2003; Hansen et al., 2008). Possibly, enhanced autophagy promotes longevity only in those conditions in which the engagement of the nuclear expression machinery directs raw material deriving from catabolic processes to newly synthesized biomolecules. The role of TOR pathway in aging is further supported by studies showing that the TOR inhibitor rapamycin prolongs the lifespan of different organisms through changes in the protein synthesis and autophagy (Kapahi et al., 2004; Kaeberlein et al., 2005; Hansen et al., 2008; Toth et al., 2008; Harrison et al., 2009; Bjedov et al., 2010). Interestingly, rapamycin treatment protects from some age-related pathologies, such as cancer, and extends the lifespan of mice, even when the feeding begins during adulthood. This might lead to the development of pharmacological interventions targeting mTOR signaling, which could theoretically delay some of the age-related phenotypes and prevent age-related disorders (Harrison et al., 2009).

MITOCHONDRIAL DEFICIENCY AND OXIDATIVE STRESS

One of the first and most accepted aging theories, called the "free radical theory of aging," proposes that loss of protective mechanisms and enhanced ROS-dependent macromolecule's damage create a vicious cycle that leads to progressive deterioration of the intracellular systems (Harman, 1956). At the cellular level, insufficient handling of oxidative stress induces senescence and ultimately death. According to this theory, mitochondria contribute as the main source of intracellular ROS, which then cause age-related decline of respiration through damage of the ETC subunits (Kirkwood, 2005). The gradual leakage of the mitochondrial electron transport system is the main endogenous source of reactive radicals that sustains this deleterious feedback loop. In addition, as a consequence of uncontrolled oxidative stress, mitochondrial DNA (mtDNA) accumulates many mutations or deletions. Interestingly, studies in cell lines have revealed that mitochondria with impaired ETC or mtDNA mutations can produce even more ROS further increasing the ROS overload of the cell (Indo et al., 2007). Other studies have shown that mutations in the mtDNA accumulate during aging and, at least in mice, can accelerate certain age-related phenotypes (Melov et al., 1995a,b; Welle et al., 2003). However, whether mtDNA mutations are the cause or the consequence of aging is still a matter of debate. In a mouse model expressing an error-prone version of the catalytic subunit of the mtDNA polymerase, accumulation of mtDNA mutations leads to respiratory dysfunction and premature aging (Trifunovic et al., 2004). Interestingly, these animals do not show increased ROS production indicating that their accelerated aging might be linked to respiratory deficiency rather than oxidative stress (Trifunovic et al., 2005). In line with these observations, the use of vitamins, natural antioxidants, does not have any effect in the life expectancy in humans (Bjelakovic et al., 2008; Chong-Han, 2010). Even more intriguing is the fact that nematode mutants for the superoxide dismutase (SOD) genes show prolonged rather than decreased lifespan, despite the significant oxidative damage (Van Raamsdonk and Hekimi, 2009). All these indications raise

questions whether oxidative stress is the main cause of aging or it is simply the result of extended mitochondrial dysfunction (Hekimi et al., 2011).

MITOCHONDRIAL FUNCTION AND AGING: HOW TO LIVE LONGER

Efficient oxidative phosphorylation is critical for the normal cellular function as it provides most of the intracellular energy. Paradoxically, slight mitochondrial dysfunction exerts a beneficial effect on the lifespan in many organisms. Indeed, RNA interference (RNAi) or mutations in genes encoding certain subunits of the electron transport chain (ETC) cause mild mitochondrial defect and promote longevity. One example is the *clk-1* gene encoding a mitochondrial hydroxylase necessary for the ubiquinone biosynthesis and therefore important for an effective ETC. Both in *C. elegans* and in mice, mutation or haploinsufficiency of *clk-1* decreases the oxidative phosphorylation rate and prolongs significantly the lifespan (Lakowski and Hekimi, 1996; Felkai et al., 1999; Liu et al., 2005). In nematodes, mitochondrial deficiency is associated with a delayed developmental rate, reduced adult size, and lower fecundity. The lifespan extension requires AMPK activity and the engagement of autophagy, whereas it is independent of the insulin/IGF-1 signaling pathway (Curtis et al., 2006; Toth et al., 2008; **Figure 1**). Longevity is also increased by altering mitochondrial function through silencing of genes encoding other mitochondrial proteins beside the ETC, as long as the treatments occur during development (Felkai et al., 1999; Dillin et al., 2002; Lee et al., 2003). However, null mutations in genes encoding ETC components severely compromise survival. Taken together, mitochondrial dysfunction can improve the fitness and survival of an organism up to a certain threshold beyond which toxicity is reached and viability is compromised (**Figure 2**). Even when restricted to a single tissue, like the intestine or the nervous system, mitochondrial dysfunction can extend the lifespan of the whole organism (Durieux et al., 2011). According to this model, mitochondrial stress in a limited number of cells is sufficiently sensed by surrounding tissues and modulates aging in a cell-non-autonomous manner. Although the pro-longevity signals remain to be identified, it is not excluded that ROS take part in the process, as antioxidants can limit this phenotype. Mitochondrial deficiency can engage protective pathways through gene transcription. In the case of long-lived animals, increased levels of ROS, along with decreased mitochondrial respiration, is sufficient to activate transcription factors, such as SKN-1 (An and Blackwell, 2003), CEH-23 (Walter et al., 2011), and HIF-1 (Lee et al., 2010b), that mediate the transcription of antioxidant enzymes like SOD, catalase, and glutathione transferase (**Figure 1**). These detoxifying enzymes can maintain the ROS levels below a certain threshold, protecting the cellular structures from extensive damage. This type of retrograde signaling is called mitochondrial hormesis (Ristow and Zarse, 2010) and is in accordance with the basic concept that the exposure of an organism to mild stress results in an adaptive or hormetic response (Calabrese and Baldwin, 2002). Beside the increased resistance to stress, another possible scenario might include the engagement of alternative metabolic pathways that sustain cellular functions (Liu and Butow, 2006). In support of this hypothesis, it has been found that activated AMPK induces the phosphorylation of DAF-16/FOXO and CHR-1/CREB in nematodes, mediating the expression of genes involved in metabolism and energy homeostasis (Greer et al., 2007b; Mair et al., 2011). In conclusion, similarly to other pro-longevity signaling pathways, mitochondrial deficiency could stimulate gene expression and change the consequent transcriptional profiles in response to altered ETC efficiency.

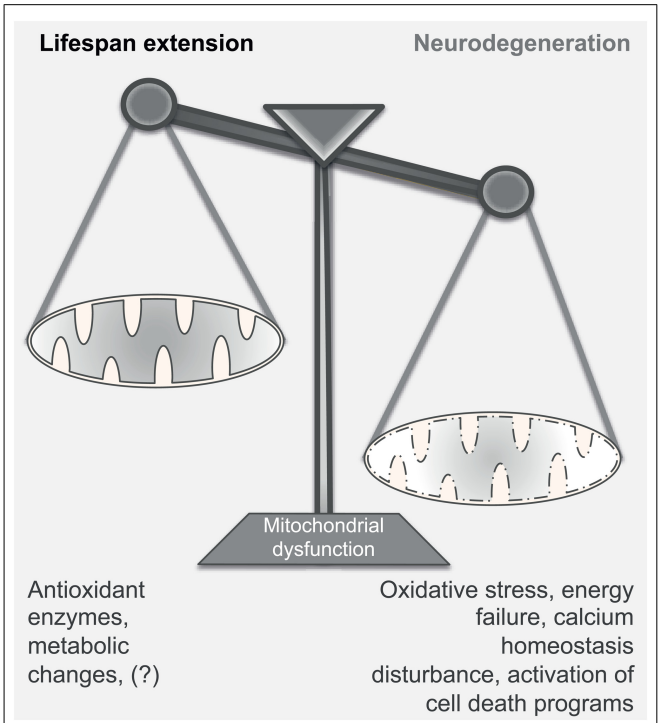

FIGURE 2 | Mitochondrial dysfunction in aging and neurodegeneration. Mitochondrial dysfunction can have either a positive or a negative effect on the normal function of the cell; mild mitochondrial dysfunction stimulates retrograde signaling, increasing – among others – the production of antioxidant enzymes and inducing metabolic changes. This adaptive response leads to the extension of the lifespan of the organism. On the contrary, severe disturbance of the mitochondrial activity may become detrimental for the survival of the cell, as it compromises energy production, induces oxidative stress and disturbs calcium homeostasis. Under these conditions, neurons undergo rapid changes of the dendritic structures and, according to the damage, can engage detrimental programs that cause cell death. Thus, strong mitochondrial inefficiency may lead to the development of neurodegenerative diseases.

MITOCHONDRIAL FUNCTION AND NEURODEGENERATION: A DELICATE BALANCE THAT CAN KILL

Mitochondria take part in a variety of heterogeneous intracellular processes. Specifically, they provide most of the cellular ATP through oxidative phosphorylation, produce ROS as side products, contribute to intracellular calcium homeostasis and under certain conditions, they can activate specific cell death programs. In neuronal cells, the abundance of mitochondria in subdomains critically regulates the density of dendritic structures, contributing to synaptic plasticity. Impairment of mitochondrial dynamics at the dendrites negatively affects the formation of new spines and leads to loss of synapses (Li et al., 2004). As it is expected,

decline of the mitochondrial activity over time can progressively perturb the intracellular environment and affect the maintenance of the surrounding tissues. Thus, it is not surprising that aging and neurodegeneration are strongly linked with mitochondrial defects. However, at which rate mitochondrial activity enables survival and, conversely, at which degree compromised organelles cause irreversible damage remain two fascinating open questions. This possible double-edged sword aspect is of particular interest as mitochondria have apparently an opposite role in these two biological processes: while their severe dysfunction provokes neurodegeneration, a slight decrease in respiration extends the lifespan in a range of organisms as diverse as yeast, invertebrates, and mammals (**Figure 2**). Whether the engagement of pro-survival programs, including those activated by slight mitochondrial deficiency, can have any protective effect in brain disorders remains still unclear.

As previously shown in animals models, the use of inhibitors of the mitochondrial respiratory complexes induces neuronal degeneration in certain brain regions and therefore resembles certain types of pathologies. For example, the use of the neurotoxins rotenone and MPTP, which mainly act at the level of the Complex I, triggers the loss of dopaminergic neurons and causes symptoms similar to the sporadic forms of Parkinson's disease (Gerlach et al., 1991; Panov et al., 2005). Similarly, the succinate dehydrogenase inhibitor 3-nitropropionic acid triggers extensive neurodegeneration in the striatum and has been used to model Huntington's disease (Brouillet et al., 1999). In support of the mitochondrial role in brain disorders, a large number of studies have demonstrated a significant association between familial forms of neurodegenerative diseases and rare mutations in genes encoding proteins related to mitochondria. Interestingly, almost one third of the mutations that are linked to brain pathologies affect proteins required for the normal mitochondrial functions (Schon and Przedborski, 2011; Exner et al., 2012). Although Alzheimer's, Parkinson's and other neurodegenerative diseases are frequently described as age-related pathologies without any genetic linkage and with distinct clinical symptoms, they all share common degenerative mechanisms that converge on mitochondria. Most of these diseases exhibit metabolic defects and increased oxidative stress. For example, in Alzheimer's disease (AD) there are significant changes in mitochondrial morphology and number (Hirai et al., 2001; Baloyannis, 2006), which are associated with reduced levels of some of the ETC subunits. Besides providing ATP, mitochondria sense localized Ca^{2+} changes and prevent the build-up of excessive intracellular Ca^{2+} that can trigger death programs. In a variety of neurodegenerative disorders, accumulation of glutamate at the synaptic cleft leads to prolonged neuronal depolarization and, through intracellular and plasma membrane Ca^{2+} permeable channels, large Ca^{2+} influx (Bano and Nicotera, 2007; Moskowitz et al., 2010). The sustained mitochondrial Ca^{2+} uptake leads to extensive mitochondrial depolarization and release of pro-death factors, which then promote caspase-dependent and independent cell death according to the intensity of the stimulus (Ankarcrona et al., 1995; Orrenius et al., 2003). Notably, at least *in vitro*, uncoupling of the mitochondrial ETC significantly reduces cell death as a result of the excitotoxic Ca^{2+} overload (Budd and Nicholls, 1996). Thus, at least for a limited period of time, mild mitochondrial dysfunction and time-limited collapse of the membrane potential can be protective against neurotoxins and favor neuronal survival.

CAN LONGEVITY PATHWAYS CONFER NEUROPROTECTION?

Despite the large number of studies on aging in model organisms, especially invertebrates, there is still an open question: can pro-longevity pathways prevent brain disorders? Although more work is required to prove the relevance in humans, new evidence suggests that low insulin/IGF-1 signaling or decreased TOR signaling has a beneficial effect in aggregate-prone animal models of neurodegenerative diseases. More specifically, *Igf1r* haploinsufficiency can reduce inflammatory response, neuronal loss and cognitive impairment associated with toxic Aβ aggregates in mouse models of AD (Cohen and Dillin, 2008; Cohen et al., 2009; Freude et al., 2009; Killick et al., 2009). Over time, decreased IGF-1 levels promote the assembly of densely packed fibrils that are less toxic compared with Aβ oligomers. In line with this, activation of the DAF-16/FOXO3a, one of the main downstream targets of the insulin/IGF-1 signaling, either genetically – encoding a nuclear targeted FOXO3a – or pharmacologically – using a specific compound called Psammaplysene A (PA) – protects both *in vitro* and *in vivo* against insults causing motor neuron disease (Mojsilovic-Petrovic et al., 2009). Similarly to the insulin/IGF-1 signaling pathway, long-term rapamycin treatment prevents cognitive deficits throughout the lifespan in mice (Ehninger et al., 2009; Halloran et al., 2012). In an AD mouse model, rapamycin improves learning and memory, ameliorates cognitive defects, and slows or blocks the progression of the disease (Caccamo et al., 2010; Spilman et al., 2010). However, even in wild type mice, rapamycin seems to have a beneficial effect in cognition, since it can ameliorate learning and memory deficits (Majumder et al., 2012). In another interesting study, it was found that the oral administration of the natural polyphenol resveratrol in mice was enough to activate the metabolic sensor AMPK and reduce the cerebral Abeta levels and their deposition in the cortex (Vingtdeux et al., 2010).

Downregulation of the insulin/IGF-1 signaling pathway, in a *C. elegans* model for Huntington's disease delays dramatically the polyQ toxicity and the protein aggregates and protects from neurodegeneration (Morley et al., 2002). In accordance with this, mice for Huntington's disease harboring only one copy of the *IRS2* – the insulin receptor substrate that control the phosphorylation of the downstream PI3K – have improved motor performance and live longer compared with their littermates (Sadagurski et al., 2011). Importantly, some of the ameliorated phenotypes are the result of improved mitochondrial activity and decreased levels of oxidative stress. Clioquinol is a metal chelator that has been extensively used as a neuroprotective drug in Alzheimer's, Parkinson's, and Huntington's models or even as a drug in patients, where it reduces the accumulation or the expression of the toxic proteins (Cherny et al., 2001; Kaur et al., 2003; Nguyen et al., 2005). This drug inhibits the activity of CLK-1, a mitochondrial protein, and mimics many of the phenotypes produced by reduction of its activity in nematodes and mice. This might indicate that clioquinol acts, at least partially, through the mitochondrial pathway that affects longevity (Wang et al., 2009).

Table 1 | Molecular pathways affecting aging and neurodegeneration.

	Aging	Neuro-degeneration	Can the longevity pathway confer Neuroprotection?
Insulin/IGF-1 signaling	+	?	YES
TOR pathway	+	?	YES
Mitochondria	+	+	?

Insulin/IGF-1 and TOR signaling pathways are two of the main longevity determinants, whose downregulation not only increases the lifespan but also protects from neurodegenerative insults. Mitochondrial function is critical both for aging and neurodegeneration: mild dysfunction is beneficial and increases the lifespan of the organism, whereas a sustained deregulation leads to cell death.

Taken together, these findings demonstrate that genetic and pharmacological interventions that diminish the PI3K/Akt or TOR signaling cascade can attenuate some of the damaging effects associated with the expression of aggregate-prone peptides. As part of the mechanism, the maintenance of mitochondrial activity and resistance to oxidative stress can delay neuronal loss in animal models of human brain disorders. In principle, we can predict the delay of at least some aspects of neurodegenerative disorders by altering those signaling cascades that directly or indirectly control mitochondrial activity and therefore regulate the progression of aging in an organism. However, more studies are required to prove the relevance of these findings in human pathology (see **Table 1**).

CONCLUDING REMARKS

Over the last years, significant progress was achieved in the field of aging and led to the identification of molecular pathways underlying this important biological process. Most of these molecular pathways are evolutionarily conserved and affect various tissues, including the central nervous system. This is reflected by changes both in the morphology and the function of the neurons, which can promote cognitive decline and the onset of neurodegenerative diseases. Interestingly, some of the mechanisms that regulate aging are linked to neurodegeneration.

Mitochondrial activity significantly contributes to aging and plays a major role in neurodegeneration. However, these organelles influence in an opposite way these processes: severe mitochondrial dysfunction triggers neurodegeneration and affects animal survival (Gerlach et al., 1991; Kong and Xu, 1998; Panov et al., 2005; Keeney et al., 2006), whereas mild mitochondrial dysfunction prolongs the lifespan of various organisms through broad metabolic changes and the build-up of protective defenses against stressful conditions (Wong et al., 1995; Feng et al., 2001; Dillin et al., 2002; Lee et al., 2003; Liu et al., 2005; Dell'agnello et al., 2007; Copeland et al., 2009). Nevertheless, there are still many open questions that must be addressed. For example, what is the limit beyond which mitochondrial deficiency causes cell death? Is mitochondrial activity a good anti-aging target? Can modulation of mitochondrial function prolong life expectancy without causing neurodegeneration? The better understanding of the molecular mechanisms underlying aging might offer opportunities to improve healthy human lifespan and in parallel to provide new therapeutic strategies for brain disorders.

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Aging and the aggregating proteome

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For all organisms promoting protein homeostasis is a high priority in order to optimize cellular functions and resources. However, there is accumulating evidence that aging leads to a collapse in protein homeostasis and widespread non-disease protein aggregation. This review examines these findings and discusses the potential causes and consequences of this physiological aggregation with age in particular in relation to disease protein aggregation and toxicity. Importantly, recent evidence points to unexpected differences in protein-quality-control and susceptibility to protein aggregation between neurons and other cell types. In addition, new insight into the cell-non-autonomous coordination of protein homeostasis by neurons will be presented.

Keywords: protein aggregation, aging, protein homeostasis, *C. elegans*, neurodegeneration, chaperones

NATURE OF PROTEIN AGGREGATION IN DISEASE

Protein aggregation is the common defining feature in neurodegenerative diseases such as Alzheimer's and Parkinson's disease as well as systemic amyloidosis. In these diseases, one or several distinct aggregation-prone polypeptides become misfolded and are packed into large insoluble hallmark structures. Disease aggregation affects proteins with very different native structures. For example, natively unfolded proteins such as tau and β-amyloid aggregate in Alzheimer's disease whereas globular proteins rich in β-sheets like transthyretin, rich in α-helices such as apolipoprotein A1 or containing both β-sheets and α-helices such as gelsolin aggregate in different types of systemic amyloidosis (Uversky et al., 2006). Despite these differences, X-ray diffraction results suggest that all these proteins adopt a very specific amyloid structure in the aggregates where they are stacked together in cross-β-sheets parallel to the fibril axis (Eisenberg and Jucker, 2012). Aggregates typically contain amyloid fibrils which grow at their ends by providing a template for the addition of further monomers. Soluble aggregation intermediates have also been identified, in particular prefibril and fibril oligomers which are recognized by different antibodies (Glabe, 2008). These structures are more reactive than the long fibrils and are generally considered more toxic to the organism. Although aggregates often contain different proteins, amyloid fibrils and oligomers are classically composed of identical proteins.

THE PROTEOME ON THE EDGE OF SOLUBILITY

The causes, consequences, and regulation of disease protein aggregation have been extensively discussed in other reviews (Soto, 2003; Ross and Poirier, 2005; Douglas and Dillin, 2010; Eisenberg and Jucker, 2012). The present mini-review will focus on recent evidence related to the disruption of protein homeostasis with age leading to widespread protein insolubility and aggregation in the absence of disease. Indeed, it is predicted that all proteins have the capacity to aggregate under specific conditions. For example, changes in pH, heating, denaturing conditions, or increased protein concentrations all tend to favor aggregation. Recently, Goldschmidt et al. (2010) predicted that the majority of proteins have short self-complementary sequences, which can initiate the formation of a steric zipper structure thus promoting aggregation. Normally, aggregation is avoided by burying these aggregation-prone regions inside the protein during the folding process. However, partial unfolding could be sufficient to uncover these regions and lead to aggregation (Chiti and Dobson, 2009).

Computational analysis indicates that the proteome is only marginally stable (Ghosh and Dill, 2010). Cells have likely optimized protein expression levels to prevent aggregation, leaving thereby little space for deviations in concentration (Tartaglia et al., 2007; Tartaglia and Vendruscolo, 2009). Indeed, this delicate balance can be easily disrupted. For example, exposing cells in culture to thermal stress prompts protein insolubility (Salomons et al., 2009). Artificially inducing macromolecular crowding coupled with increased ionic strength after exposure to high salt concentrations leads to widespread protein insolubility and rapid irreversible protein aggregation in the model organism *Caenorhabditis elegans* (Burkewitz et al., 2011).

DECREASED PROTEIN-QUALITY-CONTROL WITH AGE

In a healthy young organism, several layers of quality-control help proteins to remain functional and prevent aggregation (Balch et al., 2008). This starts with the regulation of transcriptional and translational rates as well as a tight control over the folding of newly synthesized proteins by providing different chaperones to assist the folding process (Hartl et al., 2011). After a damaged protein is deemed beyond repair, it is targeted by chaperones to the proteasomal or autophagy degradation systems (Kettern et al., 2010). In addition to the cytoplasmic protein-quality-control components, organelle-specific quality-control systems have been identified in the nucleus, endoplasmic reticulum, and mitochondria (Sidrauski et al., 1998; Haynes and Ron, 2010; Rosenbaum and Gardner, 2011). As the organism ages, this regulation of protein homeostasis becomes disrupted. In *C. elegans*, a sharp decrease in chaperone expression is correlated with the end of

the reproductive phase and leads to the aggregation of folding-defective mutant proteins (Ben-Zvi et al., 2009). In mammals, the unfolded protein response activated by ER stress is impaired with age (Brown and Naidoo, 2012). Furthermore, aging is associated with a decline in proteasome activity in a variety of tissues in rats (Anselmi et al., 1998; Keller et al., 2000). Similarly, lysosomal chaperone-mediated autophagy activity is reduced in old-aged rat livers and senescent human fibroblasts (Cuervo and Dice, 2000). Conversely, enhancing lysosomal degradation as well as overexpressing RPN11, one of the 19S proteasome subunits, suppresses disease-related protein aggregation (Tonoki et al., 2009; Yang et al., 2011). Furthermore, aging is also associated with increased oxidative stress, leading to irreversible oxidation and nitration of proteins, which impairs their degradation (Squier, 2001; Poon et al., 2006). Errors during transcription and translation could provide a further challenge to the protein-quality-control system with age (Gidalevitz et al., 2010). In addition, molecular misreading during transcription causing dinucleotide deletions plays a role in Alzheimer's and Huntington's disease (van Leeuwen et al., 1998; Lam et al., 2000; de Pril et al., 2004). All these changes with age could contribute to widespread protein aggregation.

IDENTIFYING THE AGE-RELATED AGGREGATING PROTEOME

Although protein homeostasis is disrupted with age, it was unclear to what extent this affects the stability of the proteome (Morimoto and Cuervo, 2009). Recently, increased levels of protein hydrophobicity were detected in brains from aging rats which could promote protein aggregation (Chiti and Dobson, 2006; Dasuri et al., 2010). Consequently, a study with *Drosophila* revealed the accumulation of aggregated proteins with age in different tissues (Demontis and Perrimon, 2010). These aggregated structures were detergent insoluble and appeared to be filamentous by electron microscopy, two features associated with disease aggregation. Independently, two groups set out to identify the age-related aggregating proteome in *C. elegans* using mass-spectrometry (David et al., 2010; Reis-Rodrigues et al., 2012). *C. elegans* is widely used to study the aging process as these animals have a relatively short lifespan and show many characteristic aging features observed in higher organisms (Garigan et al., 2002; Kenyon, 2005). To isolate proteins in a similar state to aggregated proteins in disease, both groups adopted sequential biochemical fractionation methods based on differential solubility, which is widely used to extract disease aggregates in the field of neurodegeneration research (Lee et al., 1999). Both groups discovered a substantial increase in the insolubility of several hundred proteins with age confirming a widespread disruption in protein homeostasis. The significant overlap in protein identities and functional categories between both studies shows that aggregation does not randomly affect the whole proteome, but rather a subset of proteins. Furthermore, computational analysis revealed that these aggregation-prone proteins have a higher propensity to form β-sheets, a driving force behind disease protein aggregation. In addition, *in vivo* analysis of several aggregation-prone proteins with fluorescent protein tags consistently showed the abnormal clumping of these proteins into aggregate-like structures where the proteins are in a highly immobile state (David et al., 2010).

Although these physiological age-related aggregates resemble disease aggregates in several aspects, it remains to be determined whether these aggregates are in an amyloid or amorphous state. Interestingly, Alavez et al. (2011) showed that the prefibrillar-oligomeric-specific antibody A11 binds specifically to structures in the aging worm in the absence of disease. This antibody recognizes a conformation characteristic of aggregation intermediates formed by diverse disease-related aggregation-prone proteins such as β-amyloid, α-synuclein, and polyglutamine (Kayed et al., 2003). These intermediates are considered as precursors to larger amyloid fibrils (Lee et al., 2011). Evidence from bacteria also suggests that a variety of proteins can aggregate into an amyloid structure. Indeed, overexpression of exogenous proteins in bacteria often leads to their aggregation and the analysis of these aggregates revealed a partial amyloid structure (Wang et al., 2008). As the authors propose, "there might be no amorphous state of a protein aggregate" and one could speculate that physiological age-related aggregates are composed of a mixture of amyloid and disordered structures.

THE CONSEQUENCES OF AGE-RELATED PHYSIOLOGICAL AGGREGATION IN NEURODEGENERATIVE DISEASE AND AGING

Aging is the main known risk factor for sporadic neurodegenerative diseases. Henceforth, an important question is whether non-disease protein aggregation may put the brain at risk for aggregation of disease proteins. Proteomic analyses of disease aggregates reveal a large number of proteins that are associated with the main hallmark disease-aggregating protein (Liao et al., 2004; Wang et al., 2005; Xia et al., 2008). Comparison with physiological age-aggregating proteins tells us that a significant proportion of these proteins can aggregate themselves without the presence of disease aggregates. Non-disease protein aggregation could initiate or accelerate disease aggregation by several mechanisms. First, physiological aggregation could titrate anti-aggregation factors away from disease-aggregating proteins. In *C. elegans* body-wall muscles, Gidalevitz et al. (2006) showed that expressing either aggregation-prone polyglutamine or mutated proteins sensitive to misfolding reduces the folding capacity in these cells leading to enhanced protein aggregation. Similarly, widespread protein insolubility caused by heat shock impaired the ubiquitin-dependent proteasomal degradation (Salomons et al., 2009). Second, the aggregation of non-disease-associated proteins could directly induce the aggregation of disease-specific proteins by a cross-seeding mechanism. Exposure of hydrophobic stretches plays an important role in promoting protein aggregation (Munch and Bertolotti, 2010). Recently, Olzscha et al. (2011) found that artificially aggregating proteins preferentially forming oligomers with exposed hydrophobic surfaces caused the most damage to the cell. These artificial aggregating proteins efficiently sequestered cellular proteins into aggregates. Similarly, the misfolding and aggregation of non-disease proteins with age could reveal previously hidden hydrophobic stretches which may promote disease protein aggregation.

It is tempting to speculate on the consequences of physiological protein aggregation in the context of aging. During aging, aggregation affects a large number of proteins, which play a role in

regulating protein homeostasis as well as preventing disease protein aggregation (David et al., 2010; Reis-Rodrigues et al., 2012). Sequestration of these proteins into aggregates could lead to a decrease in functional protein available for the cell. In addition, proteins which play a role in determining adult lifespan are over-represented in the pool of aggregation-prone proteins (David et al., 2010). Reis-Rodrigues et al. (2012) showed that reducing the levels of aggregation-prone proteins by RNA interference extends lifespan for nearly half of the candidates tested. Two different possibilities could explain these results. First, protein aggregates or the reactive misfolded proteins during aggregation are toxic for the organism, and by down-regulating expression of the aggregation-prone proteins, the protein homeostasis is restored, which leads to the lifespan extension. Second, the cellular function carried out by aggregation-prone proteins is detrimental during aging and their aggregation may be a protective mechanism. It is impossible to distinguish between these possibilities based on RNA interference experiments alone.

Finally, it remains possible that at least a proportion of physiological aggregation has no negative consequences. Numerous examples of functional aggregation have been discovered (Fowler et al., 2007). To date, 25 proteins have been identified in yeast which can switch to a prion conformation. These proteins tend to be important regulators of gene expression as well as signaling transducers. The targeted loss-of-function caused by their aggregation allows the evolution of new traits in response to environmental changes (Halfmann et al., 2012). Functional aggregation is also found in mammals. For example, peptide hormones are stored in an amyloid aggregate and are released when needed (Maji et al., 2009). Therefore certain physiological aggregation could be a mechanism to store these proteins or rapidly inhibit their function. This type of aggregation could increase with age in response to decreased demand for the active protein or be enhanced by deregulation of the mechanisms responsible for resolubilizing the aggregated proteins.

Overall, it will be important to determine whether physiological protein aggregation contributes to tissue degeneration with age or is merely a consequence of aging. In particular, the dynamics of aggregation may determine whether the net outcome is positive or negative for the organism. Combined with the decline in protein-quality-control with age, aggregation-prone proteins which tend to remain in a misfolded and soluble state would be predicted to be more harmful than those which are rapidly sequestered into compact insoluble aggregates.

CELLULAR MECHANISMS AVAILABLE TO MANAGE AGE-DEPENDENT PROTEIN AGGREGATES

An intricate protein-quality-control system normally ensures that proteins are properly folded and damaged proteins are quickly removed (Hartl et al., 2011). However, in cases of extreme stress such as proteasome failure or heat stress, a large pool of misfolded proteins rapidly accumulates in the cell and assemblies into aggregates. Throughout evolution, the cell has developed different mechanisms to deal with this aberrant protein aggregation and either resolubilize the proteins or sequester aggregates away from vital functions. In bacteria, inclusions are formed preferentially at the poles upon heat stress and are resolubilized by the AAA+ chaperone ClpB, in collaboration with heat shock protein DnaK (Winkler et al., 2010). In yeast, stress-induced misfolded proteins and amyloidogenic proteins are actively collected in different centers in the cell (Kaganovich et al., 2008). Dependent on the state of misfolding or aggregation propensity, the damaged protein is either ubiquitinated and targeted to a juxtanuclear quality control compartment (JUNQ) or directed into peripheral insoluble protein deposits (IPODs) by Hsp42 (Kaganovich et al., 2008; Specht et al., 2011). Hsp104, the yeast homolog of the prokaryote ClpB, is targeted to stress-induced aggregates by Hsp70 and promotes their disaggregation (Winkler et al., 2012). In animal cells, aggregating proteins induced by stress as well as some disease-aggregating proteins are preferentially sequestered into a structure called the aggresome (Johnston et al., 1998). Here, ubiquitinated aggregates are actively targeted to the aggresome localized at the microtubule-organizing center through the concerted action of dynein and the histone deacetylase HDAC6 (Johnston et al., 2002; Kawaguchi et al., 2003). Of note, non-ubiquitinated aggregates have also been identified in the aggresome (Garcia-Mata et al., 1999; Ben-Gedalya et al., 2011). In addition, JUNQ and IPOD structures have been observed in mammalian cells (Kaganovich et al., 2008; Weisberg et al., 2012). Metazoans lack a direct homolog of Hsp104. However, recent studies demonstrate that Hsp110 in concert with Hsp70–Hsp40 and small Hsps actively resolubilize both heat-induced and disease aggregates in metazoans (Duennwald et al., 2012; Rampelt et al., 2012).

It remains to be shown whether any of these mechanisms are involved in managing age-dependent protein aggregation. Interestingly, physiological protein aggregation has been identified in different locations in the cell including the nucleus and does not necessarily co-localize with disease-protein aggregates (David et al., 2010).

DIFFERENCES BETWEEN NEURONAL AND NON-NEURONAL REGULATION OF PROTEIN HOMEOSTASIS

In the context of disease, different tissues and cell-types are susceptible to protein aggregation. For example in patients with sporadic inclusion-body myositis, amyloid-β and tau protein aggregate exclusively in muscles together with several other proteins (Askanas et al., 2009). Conversely, in Alzheimer's disease, amyloid-β and tau aggregates are restricted to brain tissue. The reasons for this specific vulnerability remain unclear. Physiological age-related aggregates have been identified in all tissues examined including neurons. Tissue susceptibility to age-related aggregation will probably be further refined by examining more individual aggregation-prone proteins. Interestingly, results from *C. elegans* and *Drosophila* would suggest that neurons are to some extent more resistant to age-dependent protein insolubility and aggregation than muscles (David et al., 2010; Demontis and Perrimon, 2010). How could this be? Recent results show that neurons and muscles have developed different strategies to deal with protein misfolding, which change with age. Using luciferase aggregation and the subsequent recovery of luciferase activity after heat shock, Kern et al. (2010) searched for differences in chaperone capacity in neurons and muscle cells of young and aged *C. elegans*. They found that young muscles efficiently prevented protein aggregation but lost this activity with age. On the other hand, young neurons have

a delayed chaperone response but compensate by increasing disaggregation and refolding activity. With age, neurons switched to the strategy used by young muscle cells in that they actively prevent aggregation but no longer promote refolding. In contrast, old muscle cells become highly susceptible to protein misfolding and aggregation. Similarly, Hamer et al. (2010) observed that the proteasomal degradation capacity differs between muscles and neurons. Using a photoconvertible fluorescent reporter marked for degradation by ubiquitin, the authors show that young neurons rapidly removed ubiquitinated proteins through the proteasome, whereas muscles only slowly degraded proteins. Higher protein turn-over in young neurons is achieved by improving substrate recognition using the ubiquitin-binding proteasome subunit RPN10. Interestingly, the degradation rate varies greatly between neuronal cell types which may help explain differences in neuronal susceptibility to protein homeostasis disruption. With age, the rate of protein degradation decreased solely in neurons while still remaining higher than in muscles. Overall, these different strategies used by neuronal and non-neuronal cells to control protein homeostasis and how they are modified to compensate during aging may render them more or less susceptible to physiological protein aggregation.

NON-AUTONOMOUS CONTROL OF PROTEIN AGGREGATION

Neurons play an important role in coordinating protein homeostasis regulation throughout the organism in response to changes in the environment. How does this affect physiological protein aggregation in different tissues? In *C. elegans*, thermosensory AFD neurons initiate activation of the transcription factor HSF-1, driving the transcription of chaperones, in non-neuronal tissues in response to acute heat stress (Prahlad et al., 2008). However, in the absence of heat stress, these same neurons prevent the up-regulation of chaperones in non-neuronal tissues in response to chronic protein damage and aggregation (Prahlad and Morimoto, 2011). Therefore under normal conditions, *C. elegans* blunts its protein folding machinery and cannot appropriately respond to protein aggregation. Neurons potentially also play a role in coordinating the mitochondrial unfolded protein response in non-neuronal tissues. Indeed, Durieux et al. (2011) found that mitochondrial impairment only in neurons induces the mitochondrial unfolded protein response in the intestine. Furthermore, excessive neuronal signaling through cholinergic motor neurons leads to increased misfolding of folding-defective proteins and aggregation of polyglutamine in muscle cells (Garcia et al., 2007). Therefore, depending on the circumstances, neurons can modulate protein homeostasis in both directions, either by promoting or inhibiting protein aggregation.

On the other hand, the state of protein homeostasis in non-neuronal tissue can influence neuronal protein health. Indeed, up-regulating Pten/FOXO signaling specifically in fly muscles reduces the release of insulin-like peptides from the brain, which prevented age-dependent protein aggregation in the brain and other tissues (Demontis and Perrimon, 2010).

OUTLOOK

The extensive identification of proteins aggregating during aging provides us with a starting point to understand the collapse in protein homeostasis with age. It will be essential to integrate our vast knowledge on protein homeostasis regulation to identify the key factors controlling physiological protein aggregation during the aging process. Delaying aging by dietary restriction or reducing insulin/IGF-1 signaling has been shown to mitigate the proteotoxicity of disease-protein aggregation in invertebrates and mammals (Morley et al., 2002; Cohen et al., 2006, 2009; Steinkraus et al., 2008; Freude et al., 2009; Killick et al., 2009; Teixeira-Castro et al., 2011; Zhang et al., 2011). Similarly, reducing insulin/IGF-1-like signaling (David et al., 2010; Demontis and Perrimon, 2010) or using chemical compounds such as thioflavin (Alavez et al., 2011) tell us that it is also possible to modulate physiological age-related protein aggregation (also see review Alavez and Lithgow, 2011). In both *C. elegans* and *Drosophila*, age-dependent protein aggregation occurs without additional stresses or overexpression of exogenous proteins. Compared to expressing human disease-aggregating proteins in these models, examining age-dependent aggregation gives us an unparalleled opportunity to discover new physiological pathways that control aggregation. Particularly, it will be important to investigate the interplay between physiological and disease protein aggregation. A major goal will be to translate these findings into a mammalian system and use this knowledge to develop therapies to promote healthy aging in humans.

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The intersection of aging, longevity pathways, and learning and memory in *C. elegans*

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Our understanding of the molecular and genetic regulation of aging and longevity has been greatly augmented through studies using the small model system, *C. elegans*. It is important to test whether mutations that result in a longer life span also extend the health span of the organism, rather than simply prolonging an aged state. *C. elegans* can learn and remember both associated and non-associated stimuli, and many of these learning and memory paradigms are subject to regulation by longevity pathways. One of the more distressing results of aging is cognitive decline, and while no gross physical defects in *C. elegans* sensory neurons have been identified, the organism does lose the ability to perform both simple and complex learned behaviors with age. Here we review what is known about the effects of longevity pathways and the decline of these complex learned behaviors with age, and we highlight outstanding questions in the field.

Keywords: aging, *C. elegans*, insulin signaling, memory, learning, longevity, behavior, neurons

INTRODUCTION

Human learning and memory decline with age. Understanding the genetic basis of this decline could lead to preventative treatments and therapies. *C. elegans* is an ideal model organism to identify genetic pathways that regulate both aging and cognitive decline, with its established use as a model for aging (Klass, 1977; Friedman and Johnson, 1988; Hosono et al., 1989; Kenyon et al., 1993), synapse formation and function (Sulston and Horvitz, 1977; Lewis et al., 1980; Sulston et al., 1983; Rand and Russell, 1984; White et al., 1986), and neuron-mediated behaviors (Ward, 1973; Dusenbery, 1974; Chalfie and Sulston, 1981; Avery and Horvitz, 1989; Bargmann and Horvitz, 1991).

C. elegans is a small (1 mm long) transparent nematode found worldwide in rotting vegetable matter (Brenner, 1974; Felix and Braendle, 2010). The cell lineages of all 959 somatic cells in the adult hermaphrodite have been mapped (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979), as has the position and connectivity of the 302 neurons (White et al., 1986; Varshney et al., 2011). Additionally, more than 80% of *C. elegans* genes have a human ortholog (Lai et al., 2000). *C. elegans* is a well-established model for studying the genetic basis of aging. While the normal lifespan of *C. elegans* is 23 weeks, many lifespan-extending mutants have been identified. Insulin/IGF-1 signaling (IIS) and caloric restriction (CR) regulate aging in *C. elegans*, and are conserved in higher organisms (McCay and Crowell, 1934; Kenyon et al., 1993; Lakowski and Hekimi, 1998; Bluher et al., 2003; Wood et al., 2004; Suh et al., 2008; Anderson et al., 2009). Because of its simple nervous system, *C. elegans* is also a model for synapse function (reviewed in Richmond, 2007), neuron-mediated behaviors (reviewed in Hobert, 2003), and learning and memory (reviewed in Ardiel and Rankin, 2010). Here we review age-related changes in learning and memory and their regulation by insulin signaling, mitochondrial metabolism, and CR.

C. ELEGANS LONGEVITY PATHWAYS

INSULIN/IGF-1 SIGNALING

Reduction of insulin signaling was first found to increase longevity in *C. elegans* (Kenyon et al., 1993), and this evolutionarily conserved pathway has also been shown to influence lifespan in flies, mice, and humans (Clancy et al., 2001; Tatar et al., 2001; Bluher et al., 2003; Suh et al., 2008). In worms, there is a single insulin receptor tyrosine kinase homolog, *daf-2* (Kimura et al., 1997), which was originally discovered for its role in the formation of dauers (Riddle et al., 1981), an alternative developmental stage that *C. elegans* can enter in order to survive in harsh environments. *daf-2* Loss-of-function mutants have twice the lifespan of wild-type worms (Kenyon et al., 1993). The lifespan extension observed in *daf-2* worms is dependent on the forkhead box O (FOXO) protein transcription factor, DAF-16 (Kenyon et al., 1993; Ogg et al., 1997; Lin et al., 2001). When the insulin-like growth factor 1 receptor (IGFR) DAF-2 is activated, a PI3 kinase cascade is triggered that ultimately phosphorylates DAF-16/FOXO and sequesters the transcription factor in the cytoplasm (Lin et al., 2001). *age-1*, The first gene discovered to regulate longevity in *C. elegans* (Friedman and Johnson, 1988), encodes an ortholog of the p110 catalytic subunit of Class IA phosphoinositide 3-kinase (PI3K) (Morris et al., 1996). The longevity of *age-1* mutants is dependent on *daf-16* (Dorman et al., 1995), and genetic analysis showed that AGE-1/PI3K functions downstream of DAF-2/IGFR and upstream of the AKT-2/AKT-1 and PDK-1 kinases and DAF-16/FOXO transcription factor (Paradis and Ruvkun, 1998; Paradis et al., 1999). In the absence of insulin signaling, DAF-16/FOXO is localized to the nucleus, where it regulates a host of genes that promote longevity and stress resistance. The proteins involved in IIS and many of the downstream targets of DAF-16/FOXO that contribute to the phenotypic outputs of insulin signaling (Murphy et al., 2003) have been identified and characterized.

C. elegans encodes approximately 40 insulin-like peptides that can act as either DAF-2 agonists and antagonists (Pierce et al., 2001; Li et al., 2003; Murphy et al., 2003, 2007). Most of the insulin-like peptides are expressed in the neurons, though a few are expressed in the intestine as well (Pierce et al., 2001; Li et al., 2003; Murphy et al., 2007). Pierce et al. (2001) identified INS-1 as the closest homolog of human insulin, and found that overexpression of *ins-1* or human insulin antagonizes *daf-2* signaling, moderately increasing lifespan and enhancing dauer arrest. Loss of *ins-1* did not influence lifespan or dauer entry, probably due to functional redundancy among insulin-like peptides (Pierce et al., 2001). Along with influencing dauer arrest and longevity, *ins-1* has recently been implicated in regulation of many neuron-specific sensory behaviors, such as neuropeptide feedback, serotonergic signaling, and starvation-associated aversion learning (Kodama et al., 2006; Tomioka et al., 2006; Chalasani et al., 2010; Lin et al., 2010; Harris et al., 2011).

MITOCHONDRIAL METABOLISM

Through metabolic processes, mitochondria produce the most reactive oxygen species (ROS) in a majority of eukaryotic cells (Kowaltowski et al., 2009). Excessive ROS react with proteins, DNA, RNA, and lipids, causing oxidative damage (Richter et al., 1988; Grune et al., 1997; Crawford et al., 1998). (Details of the specific mechanism of ROS production are reviewed in Kowaltowski et al., 2009). Oxidative damage caused by ROS is thought to contribute to aging, though the extent and mechanism of its action is not yet known. Mutations in the *C. elegans clk-1* gene, which encodes a hydroxylase in the electron transport chain that is required for ubiquinone biosynthesis (Miyadera et al., 2001), result in extended lifespan (Wong et al., 1995). *isp-1* Encodes the iron sulfate protein of the electron transport chain, and mutation of *isp-1* decreases metabolic respiration and increases lifespan (Feng et al., 2001). Mutations in two additional genes in the electron transport chain, *mev-1*, which encodes a cytochrome *b* homolog, and *gas-1*, which encodes the major 49 kDa iron protein subunit of complex 1, show reduced resistance to ROS and shortened lifespan (Adachi et al., 1998; Ishii et al., 1998; Kayser et al., 2001, 2004). RNAi screens have identified many mitochondrial genes that regulate lifespan in a *daf-16* and *daf-2*-independent manner (Dillin et al., 2002; Lee et al., 2003). While the exact mechanisms by which metabolic mutations influence longevity remain largely unknown, lower levels of ROS-damaged proteins in *clk-1* mutants and higher levels in *gas-1* mutants support the theory that damage caused by ROS contributes to functional decline during aging (Kayser et al., 2004).

CALORIC RESTRICTION

Restricting caloric intake to 60–70% of normal levels was first shown to extend rat lifespan by McCay et al. (1935) and has since been demonstrated in many organisms, from yeast to primates (McCay et al., 1935; Weindruch, 1996; Lin et al., 2000). Many methods of calorically restricting wild-type *C. elegans* result in a lengthened lifespan, including growing worms in or on diluted bacteria (BDR) or in axenic media (ADR) (Klass, 1977; Hosono et al., 1989; Greer et al., 2007). Calorically restricting *daf-2* or *daf-16* mutants using BDR or ADR extends lifespan compared to *daf-2* or *daf-16* alone, suggesting that CR regulates aging independent of insulin signaling (Houthoofd et al., 2003; Crawford et al., 2007). Pharyngeal pumping mutants (e.g., *eat-2*) extend longevity due to defects in feeding (Raizen et al., 1995; Lakowski and Hekimi, 1998). Like direct CR, *eat-2* regulates lifespan independent of insulin signaling, as *eat-2;daf-2* double mutants live 20% longer than *daf-2* alone (Crawford et al., 2007). The PHA-4/FOXA1 transcription factor regulates CR-mediated longevity independently of its essential role in pharyngeal development (Panowski et al., 2007). Reducing *pha-4* expression does not suppress the longevity of *daf-2* or *isp-1* mutants (Panowski et al., 2007), but PHA-4 activity is required for the lifespan extension of germline-less mutants (Hansen et al., 2008; Lapierre et al., 2011), suggesting that the germline and CR pathways may converge but that they are independent of IIS and mitochondrial longevity pathways. Adult-specific expression of *pha-4* is required for the extended longevity of BDR-treated worms and of *eat-2* mutants. The full complement of molecular mechanisms required for CR-mediated longevity downstream of PHA-4/FOXA1 activity are not yet known.

SENSORY SYSTEMS IN *C. ELEGANS*

BEHAVIOR

Despite the simplicity of this invertebrate system, the *C. elegans* model permits the analysis of both simple and complex behaviors at the individual gene level (reviewed in Hobert, 2003; de Bono and Maricq, 2005). *C. elegans* can sense and respond to temperature changes, gentle and harsh touch, O_2 and CO_2 concentration, and osmolarity, and can taste soluble chemicals and smell volatile odors (Ward, 1973; Dusenbery, 1974; Hedgecock and Russell, 1975; Culotti and Russell, 1978; Chalfie and Sulston, 1981; Bargmann et al., 1990; Bargmann and Horvitz, 1991; Gray et al., 2004; Bretscher et al., 2008; Hallem and Sternberg, 2008). Worms respond to these sensations by changing their normal locomotory behavior of smooth forward movement and turns (Croll, 1975; Niebur and Erdos, 1991) to instead chemotax using biased random walk and weathervane mechanisms (Pierce-Shimomura et al., 1999; Iino and Yoshida, 2009). *C. elegans* reverse in response to negative stimuli, suppress turns in response to attractive stimuli, adjust their speed and rate of body bends, and combine multiple behaviors to respond to more complex sensory environments (reviewed in Mori, 1999; Hobert, 2003; Bargmann, 2006; Goodman, 2006).

C. elegans integrate sensory stimuli and exhibit behavioral plasticity. Well-characterized forms of non-associative behavioral plasticity include adaptation to inherently attractive odors (Colbert and Bargmann, 1995) and habituation in response to multiple taps (Rankin et al., 1990). Associative behaviors include the ability to associate feeding state with temperature (thermotaxis) (Hedgecock and Russell, 1975; Mohri et al., 2005), salt concentration (salt learning) (Saeki et al., 2001), odor (olfactory learning) (Nuttley et al., 2002; Zhang et al., 2005; Torayama et al., 2007; Ha et al., 2010; Kauffman et al., 2010), and pathogenic state (Zhang et al., 2005). In addition to both associative and non-associative learning, *C. elegans* is able to form both short-term and long-term memories, lasting as long as 24 h (Rankin et al., 1990; Colbert and Bargmann, 1995; Gomez et al., 2001; Tomioka et al., 2006;

Kano et al., 2008; Kauffman et al., 2010). While these behavioral phenotypes, as well as many of the genes regulating chemotaxis, thermotaxis, salt learning, adaptation, and habituation have been studied, very little is known about the specific molecular mechanisms involved in integrating multiple sensory signals (reviewed in de Bono and Maricq, 2005; Ardiel and Rankin, 2010).

SENSORY NEURONS

C. elegans has a simple nervous system that contains only 302 neurons. The positions of these neurons and their processes have been mapped and are highly stereotyped between individuals (White et al., 1986; Varshney et al., 2011). GFP fusions have been used to identify neuron-specific gene expression and protein localization in the transparent *C. elegans* (Chalfie et al., 1994; Nonet, 1999). Although electrophysiological techniques have been used to study the activity of specific neurons (Goodman et al., 1998; Lockery and Goodman, 1998; Richmond and Jorgensen, 1999), genetically encoded calcium indicators, such as Cameleon (Miyawaki et al., 1997; Kerr et al., 2000) and more recently, GCaMP (Nakai et al., 2001; Chronis et al., 2007; Tian et al., 2009), have allowed the measurement of neuronal activity in live, behaving worms, and the advent of microfluidic techniques has allowed the assessment of neuronal activity in response to stimuli (Suzuki et al., 2003; Chalasani et al., 2007; Chronis et al., 2007).

C. elegans has the ability to sense certain stimuli using only a single neuron or a subset of neurons. These sensory neurons communicate through interneurons and command interneurons to regulate motor neuron output and motor response to stimuli (reviewed in Hobert, 2003). Many neurons required to detect sensory stimuli have been identified, including those involved in odortaxis (AWA, AWB, AWC, ASH, and ADL), chemotaxis (ASE, ASK, ADF, ASG, and ASI), touch response (ALM, AVM, PVM, IL1, and OLQ) as well as many others involved in mechanosensory response to stimuli (reviewed in Bargmann and Kaplan, 1998) and thermotaxis (AFD, AWC, and ASI) (Mori and Ohshima, 1995; Biron et al., 2008; Kuhara et al., 2008; Beverly et al., 2011). The involvement of interneurons to mediate sensory output and integration is not as well understood, although circuits for thermotaxis, touch response, and chemotaxis that include interneurons such as AIA and AIY have been characterized using the original White et al. (1986) wiring diagram coupled with neuron ablation (Chalfie et al., 1985; Mori and Ohshima, 1995; Bargmann and Kaplan, 1998; Zheng et al., 1999; Tsalik and Hobert, 2003; Gray et al., 2005). Cell-specific genetic rescue (Mello et al., 1991), *in vivo* calcium imaging (Kerr et al., 2000; Suzuki et al., 2003; Chronis et al., 2007; Tian et al., 2009), electrophysiology (Goodman et al., 1998; Lockery and Goodman, 1998; Richmond and Jorgensen, 1999; Richmond et al., 1999), and genomic techniques (Wenick and Hobert, 2004) have been used to verify and refine these circuit models, which can then be used as a starting point when testing the neurons involved in specific behaviors.

THE AGING NEURON

Until recently, it was thought that *C. elegans* neurons did not show age-related morphological decline at either a cellular or subcellular level, because while other tissues, such as skin and muscle, deteriorate with age (Garigan et al., 2002), neurons remained surprisingly intact (Herndon et al., 2002). These data seem counterintuitive, considering multiple sensory behaviors as well as motility decline with age in *C. elegans* (Glenn et al., 2004; Murakami et al., 2005; Hsu et al., 2009; Kauffman et al., 2010; Guo et al., 2012) and changes in dendritic spines and synapse number with age have been observed in other organisms, including non-human primates and rats (reviewed in Burke and Barnes, 2006; Morrison and Baxter, 2012). Due to this incongruity, recent work has again tested the integrity of neurons and found that while neuronal cell bodies stay intact, neuronal processes, subcellular structures (Pan et al., 2011; Tank et al., 2011; Toth et al., 2012), and neuronal activity (Chokshi et al., 2010; Mulcahy et al., 2012) all show age-dependent changes.

Neuronal aging is associated with morphological changes that include ectopic neurite branching from the soma and processes, GFP beading within the process, and blebbing that results in a "wavy" process (Pan et al., 2011; Tank et al., 2011; Toth et al., 2012). Blebbing may be the precursor to neurite formation (Pan et al., 2011). The extent and type of abnormal cellular structures in aged animals is highly variable across neurons (Pan et al., 2011; Tank et al., 2011; Toth et al., 2012). Excess ectopic neurites are correlated with a decrease in gentle touch response and mobility (Tank et al., 2011), and neurite branching occurs as early as day 8 (Pan et al., 2011). While these experiments analyzed touch neurons and motor neurons that run along the *C. elegans* mid-section, Toth et al. (2012) found through analysis of EM images that synapses in the nerve ring and ventral ganglion are depleted of vesicles in day 15 animals. Older *daf-2* worms have many fewer neuronal abnormalities with age than do similarly aged wild-type worms (Pan et al., 2011; Tank et al., 2011; Toth et al., 2012). The *daf-2* slowed morphology change phenotype is dependent on *daf-16* (Pan et al., 2011; Toth et al., 2012), and *daf-16* mutants have increased neurite branching with age compared to wild-type (Pan et al., 2011; Tank et al., 2011). Tank et al. (2011) found that neuron-specific rescue of *daf-16* in *daf-16;daf-2* double mutants restored the *daf-2* phenotype in mechanosensory neurons. Although Pan et al. (2011) found excessive neurite branching in *daf-16* mutants, they could not rescue this phenotype with neuron-specific *daf-16* expression. Thus, it is unclear whether DAF-16 acts cell autonomously or non-autonomously to regulate neurite morphology with age.

The heat-shock transcription factor HSF-1 is required for *daf-2*-mediated longevity, and functions with *daf-16* to promote proteostasis and mediate longevity (Garigan et al., 2002; Hsu et al., 2003; Morley and Morimoto, 2004; Cohen et al., 2006). *hsf-1* Mutants have significant increases in all age-related morphological changes in neurons (Pan et al., 2011; Toth et al., 2012). In addition to an excess of normal age-related changes, *hsf-1* mutants also have breaks in their neuronal processes (Toth et al., 2012). *hsf-1;daf-16* Double mutants do not have more neuronal defects than single mutants and therefore, probably act in the same pathway to regulate neuronal aging (Pan et al., 2011).

Unlike insulin signaling mutants, *eat-2* mutants have a long lifespan, but have normal rates of neurite branching with age (Tank et al., 2011). *clk-1* Mutants have a phenotype that is similar to *daf-2* mutants, suppressing neurite outgrowth in older worms (Tank et al., 2011). Together, these data show that neuron morphology

does change with age and is regulated by specific longevity pathways. Given that morphological defects such as abnormal branch formation are regulated during development and also arise with age, it would be interesting to test if pathways required for neuron development have an additional role in the regulation of neuron maintenance with age (Benard and Hobert, 2009).

Morphological changes in sensory neurons have not yet been reported. However, using microfluidics, Chokshi et al. (2010) found that the ASH sensory neuron's calcium response to glycerol changed with age. Specifically, day 1 adults had a lower peak response to glycerol than did days 3 or 4 adults, perhaps correlating with the peak reproductive period, but day 5 adults had a much smaller peak response than days 1–4. Chokshi et al. (2010) also identified oscillations in the calcium response to glycerol exposure of day 1 adult worms that were not present in days 3, 4, and 5 adult worms. The calcium responses of older *C. elegans* and/or other sensory neurons have not yet been investigated. Interestingly, mutants with defects in sensory cilia and worms with specific sensory neurons ablated are long-lived, suggesting that *C. elegans* lifespan is regulated by perception of its environment (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004). Sensory mutant lifespan can be partially rescued in a *daf-16* background, showing that extension is regulated in part by insulin signaling (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004).

LEARNING AND MEMORY PARADIGMS

Associative learning and memory are acquired with training that pairs a conditioned stimulus with an unconditioned stimulus, known as "classical conditioning." First made famous by Pavlov's (1927) original experiment training dogs to associate food with a ringing bell, classical conditioning has been tested in organisms from *Drosophila* to mice, rats, and humans, and more recently, *C. elegans*. Many different classical conditioning paradigms have been used in human experiments, ranging from the original controversial Little Albert experiment in which a baby was trained to associate a white rat with a loud noise (Watson and Raynor, 1920), to more recent experiments associating neutral tones with harsh white noise (Hensman et al., 1991). Paradigms in model systems include training *Drosophila*, mice, or rats to associate neutral olfactory, auditory, or spatial cues with electric shock (Tully and Quinn, 1985).

Many forms of memory decline with age (reviewed in Morrison and Baxter, 2012), including associative memory decline in *Drosophila* (Tamura et al., 2003). *C. elegans* is able to perform a conditioned response after training in which food or starvation is associated with a conditioned stimulus (Hedgecock and Russell, 1975; Saeki et al., 2001; Nuttley et al., 2002; Torayama et al., 2007; Kauffman et al., 2010). Understanding the effects of aging and age-related genetic pathways on associative memory in *C. elegans* can lead to a greater understanding of mechanisms that may regulate associative memory in higher organisms. Age-related experimental data using these paradigms are reviewed in detail below.

THERMOTAXIS

C. elegans can be conditioned to positively associate a training temperature with the presence of food (Hedgecock and Russell, 1975). After training for four or more hours, worms move to their training temperature in search of food and navigate within this temperature for several hours, a behavior termed *isothermal tracking* (IT) (Mori and Ohshima, 1995; Mohri et al., 2005). Thermotactic ability is assessed using a single-worm assay in which worms are placed on a plate with a radial gradient of temperatures from 17 to 25˚C for 90 min, and worm tracks are analyzed to study IT (Mori and Ohshima, 1995; Gomez et al., 2001). Conversely, after starvation on a conditioning plate at a specific temperature, worms avoid that temperature (Mohri et al., 2005; Kodama et al., 2006) or no longer show a preference for that temperature (Chi et al., 2007). Chi et al. (2007) found that worms cultivated at 25˚C did not migrate toward 25˚C, nor did worms starved at 25˚C avoid warmer temperatures. The authors broadly interpreted their results to imply that thermotaxis is not a form of associative learning (Chi et al., 2007). However, Mohri et al. (2005) and Kodama et al. (2006) showed that worms are able to move toward 25˚C when cultivated at that temperature, and avoid 25˚C when starved at that temperature, suggesting that at least in some training paradigms, worms can form thermal food/starvations associations at 25˚C.

One mark of associative behavior is that it can be extinguished by reversing the association. Indeed, after conditioning worms overnight with food at 20˚C, Gomez et al. (2001) tested extinction of the temperature-food memory by holding the worms that had previously been cultivated at 20˚C on plates without food at 20˚C. The number of worms showing IT decreased by 50% after about 7 h had elapsed since training, and returned to pre-conditioned levels by 18 h after training (Gomez et al., 2001). Long-lasting behavioral plasticity as a result of thermotaxis conditioning is modulated by diacylglycerol kinase at the sensory level in the neuron AFD (Biron et al., 2006). Diacylglycerol kinase is also known to regulate long-term reference memory in mice (Shirai et al., 2010). Whether or not thermotaxis meets other criteria of long-term memory, such as the requirements for protein translation, gene transcription, and CREB transcriptional activity, has not yet been tested.

Among worms showing locomotion, there is a moderate but significant decline in IT by day 6 of adulthood (Murakami and Murakami, 2005). By day 12 the fraction of worms with IT after training decreases by half, and is undetectable by day 15 (Murakami and Murakami, 2005). *age-1* Mutants increase IT ability in young and old animals, and have a 210% extension in "high IT" ability (period where more than 75% of worms show IT), but only a 65% lifespan extension compared to wild-type worms (Murakami et al., 2005). Expression of *age-1* in the AIY interneurons restored IT to wild-type levels, but did not affect lifespan, showing that AGE-1 functions directly in AIY neurons to mediate IT, rather than the phenotypic extension being a byproduct of organism-wide lifespan extension (Murakami et al., 2005; Kodama et al., 2006).

daf-2 and *age-1* mutants have increased IT as compared to wild-type worm when temperature is associated with either food or starvation in young adult worms and with age (Murakami et al., 2005). At both stages, the increase in IT is dependent on *daf-16* (Murakami et al., 2005). The calcium-dependent gene *ncs-1* is essential for IT (Gomez et al., 2001). *ncs-1* Mutants have normal chemotaxis, motility, and thermal avoidance behaviors, but have

reduced IT (Gomez et al., 2001). Murakami et al. (2005) tested *ncs-1;daf-2* double mutants to determine whether or not *ncs-1* genetically interacts with the insulin signaling pathway. *ncs-1;daf-2* Mutants have an IT defect compared to wild-type animals, but similar IT to both *ncs-1* and *daf-16* single mutants (Murakami et al., 2005). Though these data suggest that *daf-2* is acting in an *ncs-1*-dependent manner in IT, Murakami et al. (2005) concede that *ncs-1* may be essential for IT in any condition.

In a screen for mutants that do not integrate food conditions with temperature, Mohri et al. (2005) isolated an allele of *ins-1*. While wild-type animals avoid a temperature when it is paired with starvation, *ins-1* mutants move toward their cultivation temperature regardless of the presence of food (Kodama et al., 2006). Kodama et al. (2006) showed that *ins-1* mutants move and respond to feeding states normally, suggesting that they are specifically defective in forming the starvation-temperature association. *daf-2* and *age-1* mutants rescue the *ins-1* phenotype (Kodama et al., 2006). To analyze how *ins-1* regulates IT, Kodama et al. (2006) analyzed calcium dynamics in the AIZ interneuron, which is essential for thermotaxis (Mori and Ohshima, 1995). In the presence of food, intracellular calcium is increased at higher temperatures and decreased at lower temperatures in wild-type animals (Kodama et al., 2006). The response of AIZ to temperature is dampened in starvation conditions in wild-type animals (Kodama et al., 2006). In *ins-1* mutants, the response of AIZ to temperature is never dampened, suggesting that *ins-1* regulates the integration of the starvation-temperature association (Kodama et al., 2006).

At both days 1 and 9 of adulthood, *eat-2* mutants increase IT when associating food, but not starvation, with a specific temperature, indicating that CR affects food-temperature association (Murakami et al., 2005). The mitochondrial mutant *clk-1* also has increased IT at days 1 and 9 of adulthood (Murakami et al., 2005). Murakami and Murakami (2005) found that mutants with lower oxidative stress (*clk-1* and *isp-1*), increased IT in young adults, while those with high levels of oxidative stress (*gas-1* and *mev-1*), decreased IT. Treating *mev-1* mutants with the antioxidant lipoic acid partially rescued IT ability (Murakami and Murakami, 2005). *C. elegans* lifespan is shorter after long-term cultivation at higher temperatures (Klass, 1977). Thermotaxis associative learning assays show that *C. elegans* respond to even short-term changes in temperature and that these responses are temperature specific and also influenced by aging pathways.

SALT CHEMOTAXIS LEARNING

C. elegans can associate salt concentration with starvation conditions (Saeki et al., 2001). Untrained worms are attracted to 100 mM salt, and this attraction is increased when worms are starved in buffer alone (Bargmann and Horvitz, 1991; Tomioka et al., 2006; Kano et al., 2008). Animals trained to associate salt with starvation remember this association for up to 60 min, forming a stable short-term memory (Tomioka et al., 2006; Kano et al., 2008). Whether salt learning and memory change with age has not yet been tested.

After starvation in the presence of salt, the long-lived insulin signaling pathway mutants *daf-2*, *age-1*, *pdk-1*, and *akt-1* are still attracted to salt (Tomioka et al., 2006), suggesting that they are defective in forming the starvation-salt association. Interestingly, loss of *daf-16* does not rescue the *daf-2* or *age-1* mutant phenotypes, suggesting that the defect of *daf-2* in salt learning is independent of *daf-16* (Tomioka et al., 2006). Similar to thermotaxis, INS-1 was identified as the insulin-like peptide that may regulate this association, as *ins-1* mutants show neither an avoidance of salt after starvation training nor an increased attraction to salt after starvation in the absence of salt (Tomioka et al., 2006). Salt learning is rescued by expression of *age-1* or *daf-2* in the ASER neuron, and by *ins-1* expression in AIA, suggesting that feedback between the AIA interneurons and the ASER sensory neuron results in associative learning that is mediated by INS-1 (Tomioka et al., 2006).

After conditioning without food in the presence of salt, the ASER neuron exhibits a sharp increase in calcium activity and a decrease in synaptic release following a down-step in salt concentration as compared to animals trained without salt (Oda et al., 2011). In the insulin signaling mutants *daf-2*, *ins-1*, and *age-1*, calcium signaling and synaptic release after salt conditioning are indistinguishable from mock-trained animals (Oda et al., 2011). Since insulin signaling in salt learning is active in ASER, this pathway may modulate activity and vesicle release specifically in ASER to reduce salt attraction. What salt response looks like with age and how insulin signaling regulates changes in calcium activity and synaptic function after salt learning remain to be tested.

POSITIVE OLFACTORY ASSOCIATIVE LEARNING AND MEMORY

Along with thermotaxis and salt learning, *C. elegans* can learn to associate an odor with food. Torayama et al. (2007) showed that after a single exposure to food and butanone, worms have a 20% increased chemotaxis index to butanone compared to naïve animals, termed "butanone enhancement" (Torayama et al., 2007). After conditioning worms for 90 min, this enhancement lasted 4 h if they were starved after conditioning, but only 1 h if they were fed afterward (Torayama et al., 2007). The susceptibility of butanone enhancement to age-related decline and in longevity mutants has not been tested. *ins-1* Mutants have normal butanone enhancement but cannot properly associate butanone with starvation (Lin et al., 2010).

In a different food/butanone training paradigm, Kauffman et al. (2010) found that worms that are briefly starved and then trained to associate butanone and food increase their chemotaxis toward butanone by 60%. Using this paradigm, Kauffman et al. (2010) designed both massed- and spaced-training paradigms that result in short-term associative memory (STAM) and long-term associative memory (LTAM), respectively. Briefly, these assays involve a short starvation in buffer, followed by conditioning with food and butanone either once (massed training) or seven times (spaced-training) (Kauffman et al., 2010). After training, worms are held on food without butanone (to allow them to forget the association) and are tested using a standard attraction chemotaxis assay (Troemel et al., 1997). STAM declines within 2 h, but LTAM lasts between 16 and 24 h after training (Kauffman et al., 2010). LTAM is dependent on transcription, translation, and CREB activity (Kauffman et al., 2010), factors that have been shown in other organisms, such as flies, *Aplysia*, and mice, to be required for long-term memory (reviewed in Silva et al., 1998).

To determine how aging affects associative memory in *C. elegans*, Kauffman et al. examined motility, chemotaxis, massed learning, spaced learning, and 16 h long-term memory for the first week of adulthood. While movement and chemotactic ability were maintained, 16 h LTAM decreased by day 2 of adulthood, and was undetectable by day 5 (Kauffman et al., 2010). Massed learning declined soon thereafter, while 7× spaced learning was undiminished at day 3, but declined by day 7 of adulthood (Kauffman et al., 2010). These cognitive declines precede age-related changes in chemotaxis, IT (Murakami and Murakami, 2005), habituation (Beck and Rankin, 1993), and motility, suggesting that 16 h LTAM and massed learning are most sensitive to age-related changes (Kauffman et al., 2010). Interestingly, this decline in cognitive function also occurs earlier than observable age-related morphological decline in neurons and muscles (Herndon et al., 2002; Pan et al., 2011; Tank et al., 2011; Toth et al., 2012).

Kauffman et al. (2010) next tested whether memory of positive olfactory conditioning in *C. elegans* is controlled by known longevity pathways, and found that (1) *daf-2* mutants remember significantly longer than do wild-type animals on the first day of adulthood; (2) *daf-2* mutant STAM lasts over three times as long as wild-type, and (3) *daf-2* LTAM lasts longer than 40 h after training. The extension of learning and memory in *daf-2* mutants requires the DAF-16 transcription factor, as *daf-16* mutants have defects in massed learning, STAM, and LTAM. *daf-2* Mutants are also able to establish a 16-h long-term association after only five training sessions as compared to seven sessions for wild-type, although their massed learning rate is similar to wild-type's. To determine whether all longevity pathways have similar effects on learning and memory, Kauffman et al. (2010) also examined these behaviors in the CR model, *eat-2*. Unlike *daf-2*, *eat-2* mutants have normal learning and short-term memory, indicating that the feeding conditions used for training are not compromised in the *eat-2* mutant, but that the STAM extension observed in *daf-2* mutants is not generalizable to all longevity mutants. Additionally, *eat-2's* long-term memory is only 60% that of wild-type worms, suggesting that CR somehow impairs formation of the memory of the association between food and butanone, although LTAM can be restored by increasing training to 10 cycles (Kauffman et al., 2010). *eat-2's* Defective LTAM phenotypes are dependent on the *pha-4* transcription factor (Kauffman et al., 2010), which is also required for *eat-2's* longevity effects (Panowski et al., 2007). Feeding *eat-2* mutants a smaller, easier to digest bacteria, *Comamonas* sp., rescues their small body size (Avery and Shtonda, 2003) and reverses *eat-2's* lifespan extension (Kauffman et al., 2010), showing that *Comamonas* sp. feeding "undoes" CR. Kauffman et al. (2010) found that feeding *eat-2* mutants *Comamonas* sp. also rescued *eat-2's* LTAM defect. Together, these results suggest that CR, rather than the acetylcholine receptor mutation that causes the defective pharyngeal pumping in *eat-2* worms, is responsible for *eat-2's* memory defects (Kauffman et al., 2010). Therefore, different longevity pathways have different effects on learning and memory early in adulthood.

To determine the effects of the IIS and CR pathways on maintenance with age, Kauffman et al. tested the worms' performance on day 4 of adulthood. While *eat-2* animals have reduced 16 h memory on the first day of adulthood compared to wild-type worms, their memory ability is maintained at least until day 4 of adulthood and this maintenance also requires *pha-4* (Kauffman et al., 2010). By contrast, *daf-2* learning is maintained better than wild-type with age, but 16 h long-term memory at day 4 of adulthood is entirely abrogated, as it is in wild-type worms (Kauffman et al., 2010).

To resolve these seemingly disparate results, Kauffman et al. (2010) tested the cAMP response element binding protein (CREB) transcription factor, which is required for long-term memory in all organisms tested (reviewed in Silva et al., 1998) including *C. elegans* (Kauffman et al., 2010). Kauffman et al. examined whether CREB expression and activated protein levels correlated with LTAM retention in young, old, *daf-2*, and *eat-2* worms. Along with an increase in LTAM, *daf-2* day 1 adults have higher levels of CREB protein than do day 1 wild-type worms (Kauffman et al., 2010). CREB levels and activity in both *daf-2* and wild-type worms decrease with age, as does long-term memory (Kauffman et al., 2010). Conversely, *eat-2* worms have lower levels of CREB and defective memory at day 1 of adulthood, but both CREB levels and day 1 adult long-term memory level are maintained with age (Kauffman et al., 2010). Therefore, the differential effects of the insulin and dietary restriction pathways with age could be attributable to their differences in CREB expression and activity levels, and CREB levels are predictive of memory performance.

These data agree with previous findings in mammals that show that along with decreased cognitive function, total CREB and CREB activity levels decline with age (Asanuma et al., 1996; Brightwell et al., 2004; Porte et al., 2008) and long-term memory can be rescued by over-expression of CREB in the hippocampus (Mouravlev et al., 2006). Therefore, the mechanisms required for CREB regulation of long-term memory in *C. elegans* may be conserved in higher organisms. Indeed, increased memory and neuronal plasticity in mice following CR requires CREB (Fusco et al., 2012); similar studies in IIS-reduced conditions in mice would be interesting to examine. Furthermore, the downstream targets of CREB that become activated upon memory training are presumably the cellular components that actually enable memory function, and therefore are important to identify. *C. elegans* presents a tractable system to identify genome-wide memory-specific transcriptional targets of CREB, which can then be compared with CREB overexpression data (Barco et al., 2002) and *Drosophila* memory studies (Dubnau et al., 2003).

OLFACTORY AVOIDANCE LEARNING

After a single exposure to an aversive concentration of benzaldehyde and starvation, *C. elegans* avoids an attractive concentration of the same odor (Nuttley et al., 2002). Worms are starved in the presence of benzaldehyde and then tested for learning using an attraction chemotaxis assay (Troemel et al., 1997; Nuttley et al., 2002). This behavior is referred to as benzaldehyde-starvation associative plasticity. Whether this behavior is maintained or changes with age has not been reported. As in salt learning, *ins-1*, *daf-2*, and *age-1* mutants lack the ability to fully associate benzaldehyde and starvation (Lin et al., 2010). Rescuing *ins-1* only in adulthood or in AIA and ASI, two sets of interneurons, rescues the learning defect in these mutants (Lin et al., 2010). Rescuing *age-1*

specifically in AWC, the neurons that sense benzaldehyde, rescues the learning defect.

Whether *daf-2* primarily regulates memory recall or formation in massed benzaldehyde-starvation associative plasticity was addressed next (Lin et al., 2010). The *daf-2(e1370)* allele has temperature-sensitive learning phenotypes in both salt learning and olfactory avoidance learning (Tomioka et al., 2006; Lin et al., 2010). When conditioned and tested at 15˚C, *daf-2's* olfactory avoidance learning is normal, but when conditioned and tested at 23˚C, its learning is completely abrogated (Lin et al., 2010). Lin et al. (2010) found that training *daf-2* mutants at the restrictive temperature (23˚C) resulted in memory formation if testing was done at the permissive temperature (15˚C). Conversely, training at 15˚C then testing at the 23˚C resulted in a lack of benzaldehyde-starvation associative learning (Lin et al., 2010). Since testing *daf-2* at the restrictive temperature after benzaldehyde-starvation training at either temperature shows a total loss of olfactory avoidance learning, but testing at the permissive temperature after training at the restrictive temperature shows only a small loss, *daf-2* may function primarily in recall of olfactory avoidance learning. This agrees with the Kauffman et al. (2010) finding that *daf-2* worms learn a butanone-food association at the same rate as wild-type, but retain a short-term memory of this association longer, and thus primarily affects recall, at least in a massed training paradigm.

NON-ASSOCIATIVE LEARNING AND MEMORY

Age-related decline and the effects of longevity pathways have been studied in some forms of associative learning and memory as reviewed above. However, less is known about the effect of aging on non-associative forms of memory. *C. elegans* can adapt to, be sensitized to, habituated to, or dishabituated to stimuli (Rankin et al., 1990; Colbert and Bargmann, 1995) as can higher organisms such as *Drosophila*, rats, and humans (Engen et al., 1963; Thompson and Spencer, 1966; Fox, 1979). Naïve response to tap and habituation change with age (Rankin et al., 1990; Beck and Rankin, 1993). Day 9 adult worms respond to tap with smaller reversals, and recover from habituation more slowly than do days 1 or 4 adult worms (Beck and Rankin, 1993). Like LTAM (Kauffman et al., 2010), early adulthood long-term memory of habituation requires CREB (Timbers and Rankin, 2011). It remains to be seen whether or not these age-related changes are regulated by longevity pathways.

C. elegans can also be trained to disregard an inherently attractive odor by long-term exposure to that odor, a form of olfactory adaptation (Colbert and Bargmann, 1995). Currently, age-related adaptation has not been tested. Murakami et al. (2005) found that *daf-2* mutants have increased adaptation to benzaldehyde. However, since worms are starved in the presence of benzaldehyde in this assay and learning is blocked by conditioning with food, they may in fact be forming a negative associative memory instead of adapting (Nuttley et al., 2002; Pereira and van der Kooy, 2012). Chalasani et al. (2010) found that *ins-1(nr2091)* mutants cannot adapt to isoamyl alcohol when starved. By contrast, Pereira and van der Kooy (2012) found that both *daf-2* and *ins-1(nj32)* adapt normally after conditioning with isoamyl alcohol in the presence or absence of food.

DISCUSSION

LEARNING AND MEMORY DECLINE WITH AGE

Multiple forms of learning and memory decline with age in *C. elegans* as they do in *Drosophila*, mice, and humans (Bach et al., 1999; Tamura et al., 2003; Murakami and Murakami, 2005; Doty, 2009; Kauffman et al., 2010). In *C. elegans* cognitive decline occurs as early as day 2 of adulthood, when 16 h LTAM of an odor/food pairing is already significantly decreased (Kauffman et al., 2010). **Figure 1** illustrates age-related declines in learning and memory (**Figure 1A**) as well as morphological changes in neurons with age (**Figure 1B**). The decline in IT could be explained by increased neuronal outgrowths and decreased synaptic vesicle density, but several types of learning and memory decline far before neurons exhibit these gross morphological changes. While changes in sensory neuron and interneuron subcellular structures may be responsible for early behavioral declines, there may be an earlier decline in neuronal signaling, due to changes in learning and memory gene expression levels. Indeed, Kauffman et al. (2010) found that reduction in CREB expression levels and activity with age correlate with reduced LTAM ability and occur far before structural and signaling changes are observed. Further analysis of neuronal activity using calcium indicators in multiple neurons could determine the full extent and timing of sensory decline with age.

LONGEVITY PATHWAYS, LEARNING AND MEMORY

Mutants in three longevity pathways have been tested in multiple learning paradigms. Phenotypes of different mutants are listed in **Table 1**. *eat-2* CR mutants have enhanced associative thermotaxis (Murakami et al., 2005) and a defect in LTAM, but have stable long-term memory in the first 4 days of adulthood, unlike wild-type worms (Murakami et al., 2005; Kauffman et al., 2010). *eat-2* Mutants have normal neuronal morphology with age (Tank et al., 2011). However, *eat-2* mutants have lower but stable CREB protein levels with age instead of decreasing levels as in wild-type worms, which may contribute to the stable LTAM phenotype (Kauffman et al., 2010). How CR enhances the thermotaxis/food association or affects CREB levels in this paradigm is unknown. Interestingly, short-term CR in older people was recently shown to improve verbal memory (recall of a word list after 30 min) compared to controls (Witte et al., 2009). Increased insulin in humans through intranasal injection also improves verbal memory (Benedict et al., 2007).

The *clk-1* electron transport chain mutant has enhanced cellular integrity with age and enhanced positive thermotaxis (Murakami and Murakami, 2005; Tank et al., 2011). The increased thermotaxis is likely due to *clk-1*'s slower metabolism, as *isp-1* also has increased thermotaxis, but *mev-1* and *gas-1*, which have increased mitochondrial metabolic rates, display decreased thermotaxis (Murakami and Murakami, 2005; Murakami et al., 2005). While these data support the theory that ROS levels regulate age-related decline, whether or not mitochondrial metabolism or ROS is directly involved in learning and memory, or if the learning phenotype is instead a byproduct of the effects of slowed aging, is currently unknown.

The requirement of insulin signaling has been tested in multiple associative learning and memory paradigms. *daf-2* Mutants

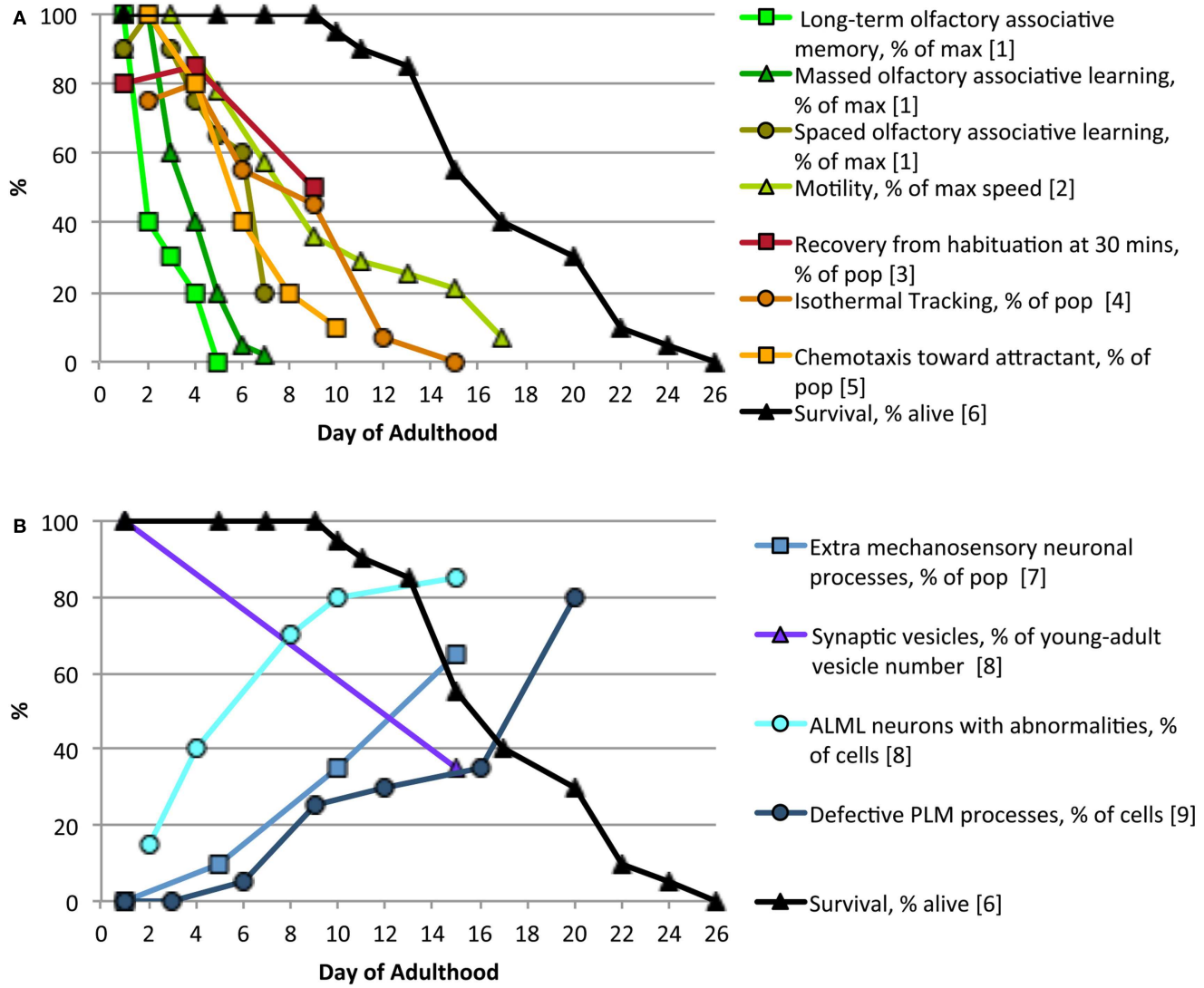

FIGURE 1 | Age-related changes in neuron morphology and behavior. Behaviors based on sensory perception **(A)** decline rapidly with age, preceding accumulation of most identified neuronal defects **(B)**, long before worms start to die [solid black line in **(A,B)**]. Complex sensory behaviors, such as long-term associative memory, decline prior to mechanosensory behaviors, such as locomotion. References: (1) Kauffman et al., 2010, (2) Hsu et al., 2009, (3) Beck and Rankin, 1993, (4) Murakami and Murakami, 2005, (5) Glenn et al., 2004, (6) Luo et al., 2009, (7) Tank et al., 2011, (8) Toth et al., 2012, (9) Pan et al., 2011.

appear to differentially regulate different learning paradigms, as they have enhanced thermotaxis (Murakami et al., 2005), long-term and short-term positive associative learning and memory (Kauffman et al., 2010), and improved neuron integrity (Pan et al., 2011; Tank et al., 2011; Toth et al., 2012), but are deficient in salt learning (Tomioka et al., 2006) and olfactory avoidance learning (Lin et al., 2010). The inability of *daf-2* mutants to perform the two latter forms of learning, which rely on negative associations, perhaps indicates that *daf-2* mutants are unable to form negative associations as readily as wild-type. Since *daf-2* mutants are more resistant to stresses such as heat, paraquat, and starvation in low bacteria concentrations (Houthoofd et al., 2003), they may need increased conditioning time or more extreme stress conditions to form negative associations.

Unlike mitochondrial metabolism, it is clear that insulin signaling regulates learning separately from longevity, since neuron-specific rescue of *daf-2*, *age-1*, or *daf-16* can rescue learning phenotypes without restoring normal lifespan (Murakami et al., 2005; Kodama et al., 2006; Tomioka et al., 2006; Lin et al., 2010). This may simply be a result of the cell autonomous nature of neuronal phenotypes, as opposed to the system-wide nature of longevity and dauer regulation (Apfeld and Kenyon, 1998). *daf-2* Mutants have higher CREB levels and increased long-term associative

Table 1 | Longevity pathway mutant phenotypes compared to wild-type worms.

Phenotype with respect to wild-type	*ins-1*	*daf-2*	*age-1*	*daf-16*	*eat-2*	*clk-1*
Neuron integrity (Pan et al., 2011; Tank et al., 2011)	n/d	+	n/d	–	No change	+
Positive associative thermotaxis (Murakami and Murakami, 2005; Murakami et al., 2005)	No change	+	+	–	+	+
Negative associative thermotaxis (Murakami and Murakami, 2005; Murakami et al., 2005)	–	+	+	–	No change	No change
Salt learning (Tomioka et al., 2006)	–	–	–	–	Naïve defect	n/d
Olfactory adaptation (Chalasani et al., 2010, Pereira and van der Kooy, 2012)	–	+	n/d	n/d	n/d	n/d
Massed positive olfactory learning (Kauffman et al., 2010; Lin et al., 2010)	No change	No change	n/d	–	No change	n/d
Short-term associative memory (Kauffman et al., 2010)	n/d	+	n/d	–	No change	n/d
Spaced olfactory learning (Kauffman et al., 2010)	n/d	No change	n/d	–	No change	n/d
Long-term associative memory (Kauffman et al., 2010)	n/d	+	n/d	–	–	n/d
Olfactory avoidance learning (Lin et al., 2010)	–	–	–	n/d	n/d	n/d

Mutants listed have been tested in at least two learning and memory paradigms reviewed here, or for neuron aging phenotypes and one learning and memory paradigm reviewed here.

(+), Aqua: increased compared to wild-type. (–), Yellow: defective compared to wild-type. (no change) The same as wild-type. (n/d) Not determined. (naïve defect) Chemotaxis defect before training.

olfactory memory as well as extended learning and STAM with age (Kauffman et al., 2010). For thermotaxis and positive associative olfactory learning and memory, it may be interesting to test downstream targets of *daf-16* for learning and memory effects. However, *daf-2* mutant response to salt learning is independent of *daf-16* and may rely on different *daf-2* signaling output. *daf-2* Mutants (as compared to *daf-16;daf-2* mutants) expressed lower levels of the guanylyl cyclases *gcy-18* and *gcy-6* (Murphy et al., 2003). As these genes are normally expressed in neurons required for thermotaxis (AFD) and salt learning (ASE), respectively, some of *daf-2's* defects may be due to altered signaling within those neurons.

ins-1 May be the cue for integration of a starvation-stimulus association, but not a food-stimulus association. *ins-1* Mutants are still attracted to odors, salt, and temperature even after starvation (Mohri et al., 2005; Tomioka et al., 2006; Lin et al., 2010). Rescuing *ins-1* expression in the AIA interneuron restores both salt and olfactory avoidance learning (Tomioka et al., 2006; Lin et al., 2010). This is especially intriguing since rescue of *daf-2* or *age-1* in the sensory neurons ASER or AWC restores normal behavior in olfactory avoidance learning and salt learning, respectively (Tomioka et al., 2006; Lin et al., 2010). These data inform a model in which INS-1 is secreted by AIA after association of starvation with a stimulus, and acts in a feedback loop to regulate insulin signaling in the sensory cells (Chalasani et al., 2010). The mechanism of INS-1 secretion in AIA and the downstream effects on insulin signaling in the sensory neurons are unknown, though Chalasani et al. (2010) found that *ins-1* does not require *daf-2* for regulation of turning behavior, suggesting that INS-1 can act through a different and as yet unidentified receptor. This would be an interesting alternative to the "40 insulins/one insulin receptor" model of insulin signaling in *C. elegans*. Because *daf-2*, *age-1*, and *ins-1* mutants have defective AIZ interneuron responses to starvation and thermotaxis, *ins-1* may also regulate insulin signaling in downstream interneurons (Kodama et al., 2006).

CONCLUSION

C. elegans is an established organism in the field of aging and longevity, as well as a model for complex behaviors, such as learning and memory. While many organisms show age-related declines in learning and memory, few molecular mechanisms that regulate these processes have been identified. Since longevity pathways actively regulate learning and memory declines and morphological changes in neurons, *C. elegans* will be an ideal system to identify molecular mechanisms that regulate such declines. Our understanding of both normal aging and neurodegenerative disease-related decreases in learning and memory at a cellular, synaptic, and molecular level will be aided by further *C. elegans* investigations at the intersection of aging, longevity, and neurobiology.

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Age-by-disease biological interactions: implications for late-life depression

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Onset of depressive symptoms after the age of 65, or late-life depression (LLD), is common and poses a significant burden on affected individuals, caretakers, and society. Evidence suggests a unique biological basis for LLD, but current hypotheses do not account for its pathophysiological complexity. Here we propose a novel etiological framework for LLD, the age-by-disease biological interaction hypothesis, based on the observations that the subset of genes that undergoes lifelong progressive changes in expression is restricted to a specific set of biological processes, and that a disproportionate number of these age-dependent genes have been previously and similarly implicated in neurodegenerative and neuropsychiatric disorders, including depression. The age-by-disease biological interaction hypothesis posits that age-dependent biological processes (i) are "pushed" in LLD-promoting directions by changes in gene expression naturally occurring during brain aging, which (ii) directly contribute to pathophysiological mechanisms of LLD, and (iii) that individual variability in rates of age-dependent changes determines risk or resiliency to develop age-related disorders, including LLD. We review observations supporting this hypothesis, including consistent and specific age-dependent changes in brain gene expression and their overlap with neuropsychiatric and neurodegenerative disease pathways. We then review preliminary reports supporting the genetic component of this hypothesis. Other potential biological mediators of age-dependent gene changes are proposed. We speculate that studies examining the relative contribution of these mechanisms to age-dependent changes and related disease mechanisms will not only provide critical information on the biology of normal aging of the human brain, but will inform our understanding of age-dependent diseases, in time fostering the development of new interventions for prevention and treatment of age-dependent diseases, including LLD.

Keywords: late-life depression, depression, molecular aging, gene expression, telomere, oxidative stress, epigenetic modifications

INTRODUCTION

Among elderly individuals, depressive symptoms are common and burdensome. Approximately 1% of individuals over the age of 65 meet criteria for major depressive disorder (MDD), as defined by the diagnostic and statistical manual of mental disorders, fourth edition, text revision (DSMIV-TR; American Psychiatric Association, 2000), a prevalence lower than that in younger adults (Kessler et al., 2003). Another 15–25%, however, experience depressive symptoms that, while not meeting criteria for MDD, do cause significant distress and interfere with daily functioning (Koenig and Blazer, 1992). In this article, the term late-life depression (LLD) will be used to refer to individuals over the age of 65 who for the first time in their lives meet criteria for MDD or display clinically significant depressive symptoms. Individuals with LLD experience greater functional disability (Dombrovski et al., 2007) and cognitive decline (Lenze et al., 2005) than those without. Further, they are at increased risk of morbidity and mortality from medical illness (Ganguli et al., 2002). LLD also appears to contribute to increased rates of suicide among older individuals (Van Orden and Conwell, 2011).

The biological substrates of LLD are being characterized and several hypotheses for the etiology and pathophysiology of LLD have been proposed, including the vascular hypothesis (Alexopoulos et al., 1997), inflammation hypothesis (Alexopoulos and Morimoto, 2011), and dementia prodrome hypothesis (Byers and Yaffe, 2011; reviewed in McKinney and Sibille, 2012). Here, we propose an alternative and complementary hypothesis, which we termed the age-by-disease biological interaction hypothesis of LLD. Central to this hypothesis is the concept of molecular aging of the human brain. An earlier version of this hypothesis has been described elsewhere (McKinney and Sibille, 2012).

MOLECULAR AGING OF THE HUMAN BRAIN

Despite its critical importance to a population that is growing older, "normal" brain aging is understudied. This may be due to the often expressed, but false belief held by many that aging is inescapable, broad-ranging and non-specific. Studies that have investigated biological aging have revealed specific changes and thus avenues for intervention. At the cellular level in the human brain, morphological and stereological studies reveal a decrease

in neuron volumes, a small loss or no change in cell numbers (Morrison and Hof, 1997; Pakkenberg and Gundersen, 1997), and a progressive thinning of cortical thickness, affecting both gray and white matter (Resnick et al., 2003; Sowell et al., 2003). Similar structural changes with age have been demonstrated in the brains of animal models (Jucker and Ingram, 1997; Peters, 2002). At the molecular level in animal models, less than 10% of brain-expressed genes exhibit age-related changes in gene expression (Lee et al., 1999, 2000; Jiang et al., 2001; Blalock et al., 2003; Sibille et al., 2007). Similar numbers have been reported in studies of human tissue (Lu et al., 2004; Avramopoulos et al., 2011). In one such study of human tissue from the prefrontal cortices of subjects aged 13–79, our group used gene microarray technology to investigate age-related changes in gene expression and reported that approximately 7.5% of genes changed significantly with aging (Erraji-Benchekroun et al., 2005). Other studies have confirmed these results, identifying a maximum of ∼10% of all detected genes, depending on sample size and analytical power of the respective studies (Yankner et al., 2008; Glorioso et al., 2011). Of note, not only is the identity of the genes and gene classes that are affected with aging consistent among studies, but so are the directions of change.

Interestingly, the identity of the genes whose expression changes with age suggests that specific cellular populations and biological processes are selectively affected by the aging process. For instance, expression of genes playing a role in glial-mediated inflammation, oxidative stress responses, mitochondrial function, synaptic function and plasticity, and calcium regulation and neuropeptide signaling have consistently been shown across multiple studies to be affected by aging, while numerous other neuronal and glial genes remain apparently unchanged (Yankner et al., 2008; Glorioso and Sibille, 2011). Overall, age-upregulated genes are mostly of glial origin and related to inflammation and cellular defenses, while downregulated genes display mostly neuron-enriched transcripts relating to cellular communication and signaling (Erraji-Benchekroun et al., 2005).

Using the expression levels of the age-dependent genes and their expected trajectories with age, we have generated predicted ages of individual subjects from which the brain tissue was sampled, and demonstrated that this predicted age is highly correlated with the chronological age of that individual (Erraji-Benchekroun et al., 2005; Glorioso and Sibille, 2011; Glorioso et al., 2011). We have termed this predicted age the "molecular age". These findings suggest that gene expression changes with age can be used as biomarkers for brain aging.

MOLECULAR AGING AND BRAIN-RELATED DISEASE PATHWAYS

This correlation between molecular and chronological ages is robust in individuals without neurodegenerative and neuropsychiatric disorders (Erraji-Benchekroun et al., 2005), but investigations of individual genes suggest that the molecular age or individual gene trajectory can deviate from chronological age in individuals with these disorders. To illustrate this phenomenon, one can look at somatostatin (SST), a signaling neuropeptide that is expressed in a subpopulation of gamma-aminobutyric acid (GABA)-positive inhibitory interneurons (Viollet et al., 2008). We have demonstrated that expression of SST decreases progressively with age in individuals without neurodegenerative and neuropsychiatric disorders such that expression levels at 70 years of age are approximately 40–50% of those at 20 years of age (Erraji-Benchekroun et al., 2005; Tripp et al., 2011; **Figure 1A**). SST is also downregulated in individuals with MDD. Interestingly, the magnitude and direction of change in SST expression with age is similar to that of control individuals, but the absolute values of SST expression are lower at most ages in individuals with MDD compared to those without (Sibille et al., 2011; Tripp et al., 2011; **Figure 1A**, top panel). Similar findings have been observed in subjects with schizophrenia (Morris et al., 2008; **Figure 1A**, bottom panel).

The relationship between gene expression of a disorder-associated gene and aging is not limited to SST. In fact, genome-wide investigations have reported that up to a third of age-regulated genes in the human brain have been at some point associated in the literature with neurodegenerative (Alzheimer's, Parkinson's, and Huntington's diseases, amyotrophic lateral sclerosis) and neuropsychiatric disorders (bipolar depression, major depression, and schizophrenia; see Figure 3 in Glorioso et al., 2011 for details). Not only do the genes relevant to these brain disorders show age-related changes, but the direction of the changes that occur with age is almost always in the direction thought to cause or promote diseases. See Table 1 in Glorioso et al. (2011) for a list of approximately 40 top candidate disease genes that exhibit agreement between age- and disease-related changes. Studies of gene expression in Alzheimer's disease (AD) and normal aging find that most of the changes in gene expression that occur in normal aging also occur in AD, only in greater magnitude (Miller et al., 2008; Avramopoulos et al., 2011). The difference in the magnitude of change in gene expression between individuals with brain disease and those without during aging is attributable to the disease. Our hypothesis posits that it is these changes that drive the disease process; however, it cannot be ruled out that these additional changes in magnitude are the result of the disease process rather than the cause. That said, there are certainly genes for which it is known that the age-related direction of change in gene expression directly causes, rather than is a consequence of, the disease, for example, the Parkinson's disease genes $PINK_1$ and DJ_1/$PARK_7$. The expression of these genes progressively declines with age and it is known that individuals with loss of expression/function mutations in these genes develop Parkinson's disease (Schapira, 2008). In contrast, relatively fewer of the larger pool of genes that do not display age-dependent changes (<5%), are associated with neurodegenerative and neuropsychiatric diseases.

Recently, we have further investigated the relationship between age, gene changes, and neuropsychiatric disorders, specifically in the context of MDD (Douillard-Guilloux et al., 2012). Results demonstrated that most MDD-related genes were frequently age-regulated in both MDD and in control subjects, and that the effects of MDD and age were positively correlated. Moreover, most genes that are age-dependent in control subjects displayed greater age effects in MDD subjects, and the overall increased prevalence of age effects on genes in MDD subjects corresponded to similar trends in controls, rather than representing *de novo* age

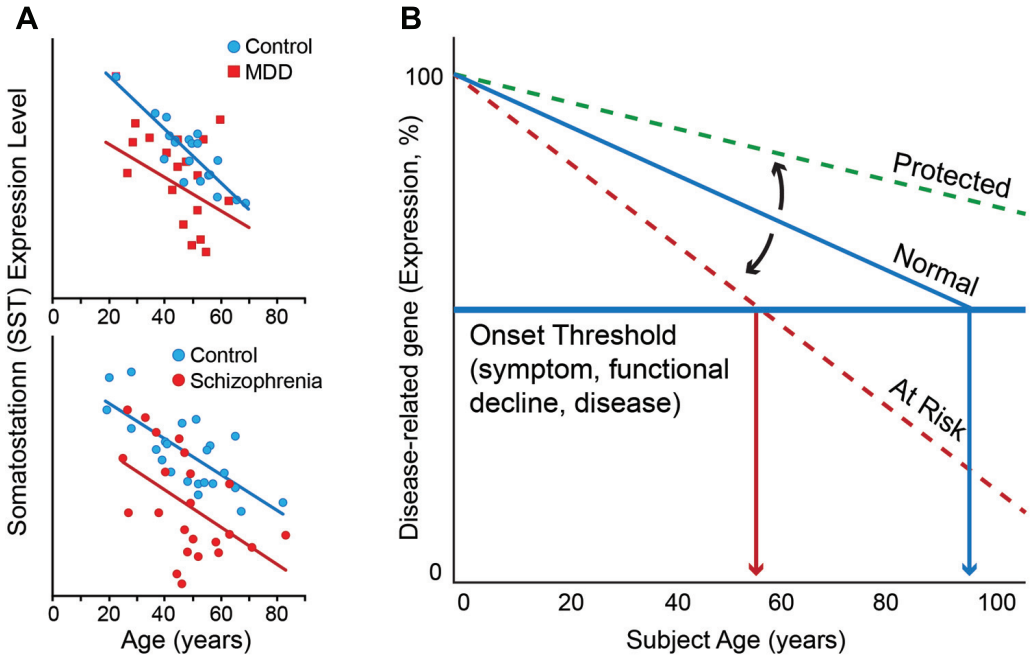

FIGURE 1 | (A) Somatostatin expression decreases with age in control subjects. Individuals with MDD (top panel) and schizophrenia (bottom panel) display lower levels of expression than control subjects at most ages, together prompting the hypothesis that decreased expression of somatostatin in depression and schizophrenia may represent early brain age-related molecular phenotypes in these individuals, which would render these subjects more vulnerable to developing psychiatric diseases. **(B)** Based on similar age and disease observations for numerous additional genes, we have proposed a model of "age-by-disease biological interaction" (Glorioso et al., 2011). In this model, change in expression of disease-related genes (a decrease is shown) across a threshold (horizontal blue line) marks the onset of disease symptoms. Changes in the trajectory of age-related changes in expression of disease-related genes (Y-axis) determine at what age (X-axis), or even if, an individual develops disease symptoms (vertical red arrows). Modulators (black arrows) may thus place an individual on "at risk" or "protected" trajectory. Per this model, individuals with LLD may have modulators, genetic or environmental, that place them on an "at risk" trajectory for developing mood symptoms. Figures are adapted from Morris et al. (2008), Tripp et al. (2011), Glorioso and Sibille (2011), and McKinney and Sibille (2012).

effects. This systematic correlation between age-dependent and depression-related changes, with greater effects in depressed subjects, further suggests that normal brain aging is a risk factor for biological changes observed in MDD subjects.

One interpretation of these observations is that age-dependent changes (i.e., molecular aging) are on an earlier trajectory in individuals who develop MDD and potentially other neuropsychiatric disorders. However, it is important to note that these studies are cross-sectional and do not follow the longitudinal progression of gene changes within individuals, so it is not known whether age-dependent changes are on an earlier trajectory, or whether changes occurred at earlier time points and were fixed at lower levels, for instance in the case of SST. So while we hypothesize that age may be pushing the expression of genes in disorder-causing directions, an alternate scenario is that of earlier and fixed changes, which then act as latent vulnerability factors that are revealed with advancing age, resulting in increased vulnerability to develop neurodegenerative and psychiatric disorders, including LLD.

AGE-RELATED CHANGES IN GENE EXPRESSION APPEAR TO BE, IN PART, GENETICALLY MODULATED

While molecular and chronological ages are highly correlated, we have also reported individual deviations from predicted ages (Erraji-Benchekroun et al., 2005; Glorioso et al., 2011). The fact that molecular age can deviate from its chronological age suggests that modulating factors exist and may contribute to one's vulnerability to brain aging and to developing late-life brain disorders, such as LLD. In the age-by-disease biological interaction hypothesis we have proposed that those individuals with older predicted molecular ages compared to their chronological age may not only display greater biological brain aging, but may also be at greater risk of age-gated brain diseases, because gene expression of disease-related genes would have proceeded further in disease-promoting directions. Conversely, subjects with younger age-dependent gene trajectories and lower predicted molecular ages would be at lower risk, and may in fact display resiliency against LLD and other late-life disorders (**Figure 1B**). Environmental and genetic factors represent obvious candidates to modulate the trajectory of biological aging.

In a proof-of-principle study, our laboratory sought to demonstrate a genetic role in modulating the aging process. The above-described "molecular age" assay was used to characterize the brain tissue of individuals carrying different polymorphisms of the sirtuin genes (Glorioso et al., 2011), a family of genes previously demonstrated to modulate age and longevity in nematodes, insects, and rodents. We focused on SIRT5, due to prior report of altered expression for that gene in a rodent model of anticipated

brain aging (Sibille et al., 2007). This study found that subjects carrying a low-expressing polymorphism of the SIRT5 gene had molecular ages that were older than actual chronological age, as measured in the three different areas of the cerebral cortex (i.e., BA9, BA24, and BA47) of human postmortem samples (Glorioso et al., 2011). Interestingly, this effect was not demonstrated in the amygdala, a brain area in which the low-expressing polymorphism of the SIRT5 gene does not appear to affect expression levels of SIRT5 as it does in the cerebral cortex. These findings at the molecular level are consistent with findings from studies of brain structure with age that show robust decreases in gray matter in the cerebral cortex but more variable decreases in the amygdala (Good et al., 2001). The effect of the low-expressing SIRT5 polymorphism on molecular age was accompanied by expression changes for a set of genes whose products are localized to the mitochondria, including PINK-1 and DJ-1, two Parkinson's disease-associated genes, in ways that would promote mitochondrial dysfunction-related diseases, including Parkinson's disease. This (correlative) proof-of-principle study suggests that factors that affect brain aging can potentially place an individual at higher risk for disease, through a mechanism by which it accelerates brain biological aging, and thus promotes changes in expression of disorder-relevant genes in disease-causing directions. With respect to **Figure 1B**, the low-expressing polymorphism of the SIRT5 gene can be thought of a modulator that puts one on the "at risk" trajectory. The converse of this model is that factors delaying age trajectories of gene changes may lead to younger brain molecular aging and potential resiliency toward developing functional declines and age-related disorders, including LLD (**Figure 1B**).

PUTATIVE MECHANISMS FOR AGE-RELATED CHANGES IN GENE EXPRESSION

The mechanisms by which age-related changes in gene expression occur are unknown. Candidate mediators include among others, loss of telomere integrity, increased oxidative stress, and epigenetic modifications.

LOSS OF TELOMERE INTEGRITY

Telomeres are regions of repetitive nucleotide sequences at each end of a chromosome. One of the hypothesized functions of telomeres is to deter the degradation of genes near the ends of chromosomes by instead allowing repetitive telomeres to shorten, a necessary part of chromosome replication. Telomeres are highly susceptible to oxidative stress because of their high content of guanines. As both chromosome replication and oxidative stress increase with age, one would expect telomeres to shorten with increased age. Indeed, in peripheral tissues, it has been consistently demonstrated that telomeres become shorter as one ages and once telomeres reach a critical length, irreversible arrest of cell division or apoptosis is triggered (Hayflick and Moorhead, 1961; Chin et al., 1999; Sharpless and DePinho, 2004; Flores and Blasco, 2009; Sahin and DePinho, 2012). Although telomere shortening has not yet been observed in the human brain, studies suggest that peripheral telomere length is a biomarker for aging. Leukocyte telomere shortening is positively correlated with the chronicity of stress and depression (Epel et al., 2004; Wolkowitz et al., 2011a) and is associated with incidence of age-related diseases such as myocardial infarction, stroke, and shorter lifespan (reviewed in Wolkowitz et al., 2011b). It is not known whether telomere shortening increases the risk of LLD, however, given the common pathway of aging and depression, the possibility cannot be excluded.

Recent animal studies suggest that the putative link between telomere integrity and depression-like behaviors extends to the brain, and that this link may be mediated by telomerase activity (Zhou et al., 2011). In that study, the expression of telomerase was decreased in the hippocampi of mice subjected to chronic mild stress, and hippocampal infusion of a telomerase inhibitor induced depressive-like behaviors that did not respond to antidepressant treatment (Zhou et al., 2011). Given that neurogenesis has been implicated in antidepressant responses in mice (Santarelli et al., 2003) and that the proliferation capacity of neural stem cells highly depend on telomerase activity (Ferron et al., 2009), the authors suggested telomerase activity may play a role in linking mechanisms of aging and depression (Zhou et al., 2011); although the translation of these observations to humans is contentious, due to very low rates of neurogenesis in adult human subjects (Bhardwaj et al., 2006).

The anti-apoptotic role of telomerase is thought to reflect its capacity of maintaining DNA integrity, however, recent studies have reported other putative functions (reviewed in Saretzki, 2009). Overexpression of telomerase reverse transcriptase (TERT), protects mouse neurons from excitotoxicity by improving basal mitochondrial membrane potential and Ca^{2+} uptake into mitochondria (Kang et al., 2004) and decreases cellular reactive oxygen species (ROS; Ahmed et al., 2008). Furthermore, TERT mediates the neurotrophic action of BDNF (Fu et al., 2002) and affects the global pattern of gene expression in the direction of cell survival, including upregulation of growth promoting genes (FGF5, EGFR, and etc.) and downregulation of cell-growth inhibitors such as IGFBPs (Smith et al., 2003; Choi et al., 2008). Changes in telomerase activity with age and MDD have yet to be explored in the human brain. However, given the fact that neurotrophic growth signaling, including reduced BDNF, is decreased in MDD (Sen et al., 2008; Guilloux et al., 2011; Tripp et al., 2011), we cannot exclude the possibility that telomeres and telomerase activity may significantly contribute to the mediation of stress and brain aging.

INCREASED OXIDATIVE STRESS

Oxidative stress is the damage caused to cells as a result of an imbalance between the production of ROS and the ability of the cells to reduce the ROS or repair the resulting damage. The degree of oxidative stress to cellular components, including DNA, correlates positively with age (Joseph et al., 2005; Epel, 2009; Wolkowitz et al., 2011b). Because of their high demand for energy and postmitotic status, neurons appear to be particularly sensitive to oxidative stress and thus aging.

One way in which oxidative stress may contribute to age-related changes in gene expression is via its direct effect on DNA. Lu et al. (2004) showed that age-related decrease in gene expression is related to the accumulation of oxidative DNA damage. The underlying mechanism was suggested that promoter regions

with high GC contents are specifically vulnerable to oxidative damage. Oxidated promoter regions may potentially adopt different conformation and lose affinity for transcription factors (Lu et al., 2004). Damage on mitochondrial DNA (mtDNA), which is considered extremely vulnerable to oxidation due to its proximity to the site of ROS production, respiratory chain, and the absence of protective histone (Lee and Wei, 2007), results in downregulation of genes related to respiratory chain and further, energy metabolism impairment (Lin et al., 2002). Several studies showed that psychiatric diseases including MDD, bipolar disorder, and schizophrenia are associated with mitochondrial dysfunction (Rezin et al., 2009) and that accumulation of mtDNA damage induces mood disorder-like phenotypes as well as premature aging in mice (Trifunovic et al., 2004; Kasahara et al., 2006). These results support the idea that oxidative stress plays a role as a link between aging and depression.

Another direct way in which oxidative stress may contribute to age-related changes in gene expression is via its effect on transcription factors. For example, ROS are able to activate nuclear factor kappa B (NF-κB) by decreasing binding affinity of the inhibitory subunit, Iκ-B, to NF-κB, an observation made relevant by the fact that NF-κB activity has been demonstrated to increase with aging and depression (Toliver-Kinsky et al., 1997; Koo et al., 2010). Also, transcription factors containing the zinc-finger DNA binding motif appear to be especially susceptible to damage from oxidative stress due to their high cysteine residue content. As intracellular ROS accumulate, oxidation of the thiol residues in cysteine occurs and binding affinity for DNA is lost. One of the zinc-finger transcription factors, Sp1, an ubiquitous transcription factor for housekeeping genes and enzymes involved in glucose metabolism and DNA synthesis, has been demonstrated to have decreased DNA binding affinity with advancing age (Ammendola et al., 1992, 1994; Wu et al., 1996). Interestingly, genes with lower affinity binding sites to Sp1 are more influenced by oxidative stress than those containing high-affinity sites (Ammendola et al., 1992, 1994; Wu et al., 1996). Furthermore, ROS decreases telomerase activity. Antioxidant treatment normalizes catalytic activity of TERT and delays cellular senescence (Haendeler et al., 2004).

In addition to the conformational change of gene and transcription factors, ROS can act on various cellular signaling pathways to control gene expression. For example, ROS increase p53 signaling, which has been implicated in various neurodegenerative diseases and thought to mediate its effect by increasing expression of genes related to cell cycle arrest, DNA repair, and apoptosis in response to cellular stressors such as DNA damage and hypoxia (Lundberg et al., 2000). Another example is illustrated by p38 MAP Kinase (MAPK). When p38 MAPK is activated by oxidative stress, it promotes lamin B1 accumulation and expression of several transcription factors related to cellular senescence and apoptosis such as p53, CREB, C/EBPβ, and ATF2 (Wagner and Nebreda, 2009; Barascu et al., 2012). Interestingly, selective p38 MAPK deletion in serotonergic neurons produces stress resilience in an animal model of depression by inhibiting serotonin transporter translocation to plasma membrane (Bruchas et al., 2011).

In summary, the main effect of oxidative stress on aging has been thought to be the accumulation of toxic, inactive molecules produced randomly by ROS. However, oxidative stress may also have an active role in aging and related diseases, through direct modification of DNA and transcription factor integrity and through indirect pathways regulating upstream modulators. These observations suggest that antioxidants may contribute to preventing biological changes and/or associated symptoms of depression, in addition to their potential anti-aging effects.

EPIGENETIC MODIFICATIONS

Epigenetic modifications, including DNA methylation and histone modification, regulate gene expression without changing the primary DNA sequence. Though classically viewed as a permanent event, recent data indicates that such modifications are influenced by genetic and environmental factors in adult organisms, including changes in methylation patterns across the lifespan (Numata et al., 2012). At the genome level, DNA methylation decreases with age. In contrast, CpG islands of many specific promoter regions that are typically not methylated become methylated with aging, including in promoters of tumor suppressor genes, estrogen receptor (ER), and insulin-like growth factor 2 (IGF2; Issa et al., 1994, 1996; So et al., 2006; Numata et al., 2012). Changes in DNA methylation at the glucocorticoid receptor, potentially due to early-life stress, was also correlated with altered vulnerability to psychiatric disorders and death by suicide in adults (McGowan et al., 2009).

Similarly, histone modifications such as acetylation, phosphorylation, symoylation, and methylation change with age. It was recently demonstrated that the aging-related deficit of long-term synaptic plasticity in the rodent hippocampus is related to decreased BDNF expression secondary to decreased acetylation of histones residing at the BDNF promoter region (Zeng et al., 2011). This observation fits well with human studies demonstrating reduced BDNF function in aging and depression (Webster et al., 2002; Guilloux et al., 2011). Also, changes in histone modifications in rodent systems may be protective against the effects of stress (Covington et al., 2011; Uchida et al., 2011). In support of a specific role for histone acetylation in age-related changes in gene expression, sirtuins (NAD-dependent histone deacetylases) have been implicated in longevity in yeast and invertebrates (Longo and Kennedy, 2006). A recent rodent study directly links SIRT1 to the risk of anxiety-like behaviors through its activity on monoamine oxidase A and serotonin levels (Libert et al., 2011). In the same study, the authors reported association between a single nucleotide polymorphism in the human SIRT1 gene and the risk of various psychiatric disorders such as anxiety disorder, panic disorder, and major depression. Furthermore, overexpression of SIRT6 in mice has been reported to increase lifespan and protect from diet-induced physiological damage (Kanfi et al., 2010, 2012) and SIRT6 knockout mice exhibit accelerated aging. Interestingly, decreased expression of SIRT6 has been observed during MDD (Abe et al., 2011). How SIRT6 mediates its effect on aging or is involved in MDD is not clear, but its functions as an HDAC (Michishita et al., 2008; Kawahara et al., 2009; Tennen et al., 2011) and in DNA repair (Mao et al., 2011) suggest that it may contribute to protecting

against aging and psychiatric illness by maintaining telomere integrity or protecting against and repairing the effects of oxidative stress.

Together, the occurrence of epigenetic modifications during aging and in the context of neuropsychiatric disorders may thus provide mechanistic underpinnings for the proposed age-by-disease biological interaction hypothesis, through the dual role of longevity and other age-associated genes.

SUMMARY AND IMPLICATIONS

We propose a novel framework for investigating the development of late-life brain disorders, including LLD, which we term the age-by-disease biological interaction hypothesis. This paper expands upon an earlier version described elsewhere (McKinney and Sibille, 2012). This hypothesis posits that symptoms of LLD and other late-life brain disorders are the emerging properties of underlying biological changes, which in turn are supported by normal changes in the expression of multiple genes with age, including disease-related genes changing in disease-causing directions. Here, in addition to presenting the gene expression data on which the hypothesis is based, we discussed molecular mechanisms that may account for age-dependent gene expression changes, including loss of telomere integrity, increased oxidative stress, and epigenetic modifications. Importantly, this hypothesis complements existing hypotheses, including the vascular, inflammatory, and dementia prodrome hypotheses of LLD, but it differs in that it positions age-dependent gene expression changes as the mechanism potentially driving dysfunctions in multiple biological pathways, including vascular, inflammatory, and neurotrophic functions. A potential sequence of events is summarized in **Figure 2**. The purpose of this paper was to discuss the general framework. Examples of gene changes at the intersection of depression and aging were provided (e.g., SST and BDNF), but the exact nature and complexity of changes in multiple genes and pathways and their relevance to disease pathways will be described in details elsewhere.

The implications of this hypothesis for the prevention and treatment of LLD and other late-life brain disorders are exciting. Understanding the mechanisms mediating age-related changes in gene expression is expected to provide insight into pathophysiological mechanisms and potential targets for intervention into these disorders. Identifying key upstream hub genes mediating patterns of altered age-dependent changes would provide novel targets for further investigations. Although sirtuins and BDNF may represent obvious candidates, the large set of age-dependent genes (~10% of all genes; Erraji-Benchekroun et al., 2005) and its overlap with genes previously implicated in brain disorders (Glorioso et al., 2011) should be viewed as an enriched pool of candidate genes. Targeting such upstream factors (transcription or function) should represent productive research avenues. Early candidate interventions may include known interventions such as antidepressant medications, psychotherapy, exercise, and others. Investigating how these known interventions affect age-dependent changes in the function of critical genes may help in optimizing their implementation with respect to timing and duration of intervention for age-dependent disorders. Further, identifying genetic and environmental factors that slow or accelerate age-related changes in gene function may lead to individualized strategies aimed at promoting resilience and successful aging. Other entry points and targets for intervention are likely to arise out of understanding the mechanisms by which gene expression changes with age, including determining the role of telomere integrity, oxidative stress, and epigenetic modifications. Finally, for the broader fields of aging and gerontology, the implication of this hypothesis is that it brings together research on normal aging more closely with the investigation of neuropsychiatric and neurodegenerative diseases. Indeed, our data firmly support the assertion that they may in fact be related facets of similar biological processes, and also provide the basis for a putative mechanism of age-by-disease biological interactions.

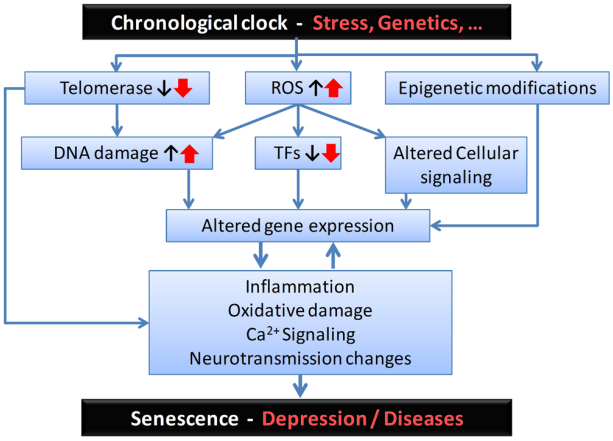

FIGURE 2 | Proposed sequence of biological events and putative mediators for the age-by-disease biological interaction hypothesis. *From the top*: Although its biological substrates are unknown, a chronological clock drives age-related changes in gene expression. These changes can be exacerbated by psychophysiological stress and/or genetic variants, and placed on accelerated age trajectories. In this model, age-related changes in telomeres, oxidative load and epigenetic landscape, among other putative mechanisms, may represent a first level of biological events, which in turn affect basic cellular processes involved in regulating gene expression (i.e., including DNA damage, altered structure and function of transcription factors (TFs), and associated local cellular signaling). The resulting changes in the global pattern of age-dependent gene expression mediate the next set of deleterious biological events, exemplified here by increased inflammation, oxidative damage-related signaling, and changes in neurotransmission. These two levels of changes are likely to reciprocally interact. At the neural network and brain levels, the emerging properties of those specific cellular events are expressed as senescence in normal aging subjects and, in subjects at risk, as age-related symptom dimensions and diseases, including depression. Notably, the degree of individual vulnerability is thought to be under genetic and environmental control, so decreased vulnerability may mediate resiliency through the same pathways.

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The developing, aging neocortex: how genetics and epigenetics influence early developmental patterning and age-related change

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A hallmark of mammalian development is the generation of functional subdivisions within the nervous system. In humans, this regionalization creates a complex system that regulates behavior, cognition, memory, and emotion. During development, specification of neocortical tissue that leads to functional sensory and motor regions results from an interplay between cortically intrinsic, molecular processes, such as gene expression, and extrinsic processes regulated by sensory input. Cortical specification in mice occurs pre- and perinatally, when gene expression is robust and various anatomical distinctions are observed alongside an emergence of physiological function. After patterning, gene expression continues to shift and axonal connections mature into an adult form. The function of adult cortical gene expression may be to maintain neocortical subdivisions that were established during early patterning. As some changes in neocortical gene expression have been observed past early development into late adulthood, gene expression may also play a role in the altered neocortical function observed in age-related cognitive decline and brain dysfunction. This review provides a discussion of how neocortical gene expression and specific patterns of neocortical sensori-motor axonal connections develop and change throughout the lifespan of the animal. We posit that a role of neocortical gene expression in neocortex is to regulate plasticity mechanisms that impact critical periods for sensory and motor plasticity in aging. We describe results from several studies in aging brain that detail changes in gene expression that may relate to microstructural changes observed in brain anatomy. We discuss the role of altered glucocorticoid signaling in age-related cognitive and functional decline, as well as how aging in the brain may result from immune system activation. We describe how caloric restriction or reduction of oxidative stress may ameliorate effects of aging on the brain.

Keywords: cortical gene expression, intra-neocortical connections, brain anatomy, caloric restriction, aging

INTRODUCTION

The human neocortex is the part of the brain that makes us uniquely different from other non-human mammals. Throughout mammalian evolution, the neocortex is the part of the brain that has increased disproportionately in size and complexity, affording our species enhanced abilities including higher-order cognition and reasoning, language, advanced motor skills, and social-emotional behavior. The precise profile of neocortical function results from a series of complicated developmental processes, wherein genes interact with *in utero* and neonatal environmental factors to pattern the structure into a network of functionally and architectonically distinct sensory, motor, and association areas. Developmental neuroscience has made great strides in furthering our understanding of early cortical patterning, however, much less is known about how this patterning is maintained throughout the lifespan of the animal, and even less is known about how the cortex changes throughout aging and senescence.

Scientists who specifically study the biology of aging are developmental biologists and what we learn from studies of early developmental patterning can be applied to the study of the senescent state. This review described age-related change in brain that spans from embryogenesis to the aging adult, and presents the notion that neurological plasticity mechanisms, regulated by both nature and nurture (genetics and epigenetics) are responsible for changes during both early development and late aging. Thus, aging is presented here as an extension of early development, another development time period that occurs late in the animal's life, but one that relies on similar molecular mechanisms as early development and, like early development can be impacted significantly by both stochastic and epigenetic events.

This review presents research on the developing and aging neocortex, describing how intra-neocortical connections (INCs), which lay the foundation for proper cortical network function, develop in the prenatal and post-natal period. This report investigates the relationship between INC development and cortical gene expression and describes how aging influences gene expression and hence, cortical function. We propose novel concepts surrounding the relationship between cortical gene expression and critical period plasticity and discuss how age-related changes in cortical organization may potentially impact behavior. We review research

on immune system contributions to the aging phenotype as well as caloric restriction (CR) and its unique ability to thwart the aging processes that occur in mammalian brain.

Studying of the aging brain, particularly the aging neocortex, represents a tremendously complex and ambitious task. Several approaches must be taken to begin to understand the mechanisms underlying age-related phenotypes in brain anatomy, physiology, and behavior. This review, then, presents research in genetic contributions to aging across lifespan, including early development, specifically highlighting age-related gene expression in the neocortex, but also describes how stochastic, non-programmed events and experience can alter the aging trajectory. How the neocortex is built, maintained, and changed throughout aging is a fundamental issue in neuroscience that deserves great attention. A focus on ways in which experience and epigenetics can ameliorate some of the negative aspects of brain aging is of paramount importance not only to researchers in the field, but also to humans as a species.

THEORETICAL MODELS OF EARLY NEOCORTICAL PATTERNING

All mammalian behavior is generated and regulated by the nervous system. In humans, neocortex is responsible for complex integration of information, the ability to utilize language, decision-making, motivation, and other high-level emotive-cognitive processes and behaviors. The complexity of neocortex emerges during development through a process called arealization, when specific sensory and motor functional areas are formed and connected to one another and to sub-cortical nuclei through a vast and complex network of intra- and extra-neocortical connections. Research on the developmental mechanisms that drive arealization has been influenced by two alternative hypotheses. Rakic (1988) famously detailed his Protomap hypothesis, suggesting that the fate of different neocortical regions were pre-specified in early development by yet-to-be characterized molecules within the proliferative zone, independent of input from the sensory systems (**Figure 1**, left). The notion that developing neocortex is patterned early in development, regardless of driven sensory input, with differential expression of genes during arealization is highly supported (Rakic, 1988; Miyashita-Lin et al., 1999; Nakagawa et al., 1999; Rubenstein et al., 1999; Bishop et al., 2000; Liu et al., 2000; Ragsdale and Grove, 2001; Zhou et al., 2001; Cecchi, 2002; Nakagawa and O'Leary, 2003; Funatsu et al., 2004; Sansom et al., 2005; Mallamaci and Stoykova, 2006; O'Leary and Sahara, 2008; Rakic et al., 2009; Bedogni et al., 2010). The alternate model, coined the Protocortex Hypothesis, emphasized the role of neural activity, via neocortically extrinsic thalamic sensory input, in determining neocortical areal fate (O'Leary, 1989; **Figure 1**, right). Based on our experimental finding in the neocortex of a blind mouse bilaterally enucleated at birth, we posit that both cortically intrinsic mechanisms, such as gene expression, and extrinsic mechanisms that involve input from the sensory organs via the dorsal thalamus interact to form the cortical map (Dye et al., 2012).

GENE EXPRESSION AND EARLY NEOCORTICAL PATTERNING

Consistent with the general idea Rakic first proposed, recent results have shown that the developing neocortex is "patterned" early in development, with differential expression of genes during arealization (Donoghue and Rakic, 1999; Miyashita-Lin et al., 1999; Nakagawa et al., 1999; Bishop et al., 2000; Liu et al., 2000; Zhou et al., 2001; Fukuchi-Shimogori and Grove, 2003; Yun et al., 2003; Abu-Khalil et al., 2004; Funatsu et al., 2004; Hamasaki et al., 2004; Shimogori et al., 2004; Sansom et al., 2005; for review see Rubenstein et al., 1999; Ragsdale and Grove, 2001; Ruiz i Altaba et al., 2001; Cecchi, 2002). This patterning is thought to occur independently of sensory input reaching the cortex via thalamocortical afferents, as cortical gene expression patterns are unperturbed in mutant mice lacking these thalamocortical inputs (Miyashita-Lin et al., 1999; Nakagawa et al., 1999). It has been postulated that patterning centers in the midline of the developing telencephalon have a primary role in regulating neocortical regionalization (Rubenstein et al., 1999; Crossley et al., 2001; Fukuchi-Shimogori and Grove, 2001, 2003; Huffman et al., 2004; Sansom et al., 2005). For example, a dorsal patterning center expresses high levels of Bmp and Wnt genes. Mutations that affect Wnt signaling lead to defects in the most medial cortical regions (e.g., the hippocampal complex; Grove et al., 1998; Lee et al., 2000b; Shimogori et al., 2004). Mutations affecting BMP-signaling lead to dorsal-midline patterning defects (Furuta et al., 1997). Additionally, the rostrodorsal midline of the telencephalon expresses high levels of Fgf8; this region is derived from the anterior neural ridge and is known as the commissural plate. Fgf8 has been postulated to

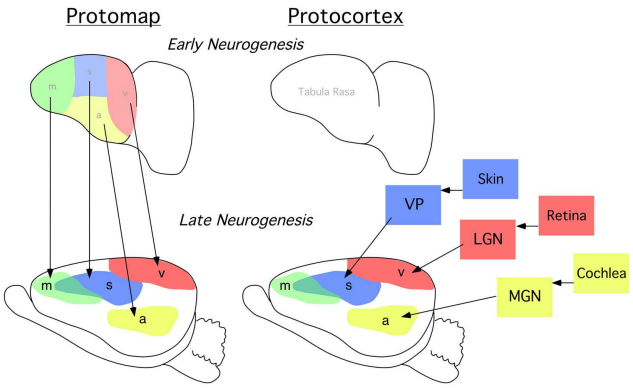

FIGURE 1 | Classic models of cortical patterning. Lateral view of cortical primordium at an early stage of neurogenesis (*top row*), and a later stage after thalamocortical axons have begun to enter the cortical plate when putative cortical sensory and motor areas are forming (*bottom row*). (*Left*) simplified protomap model. Early neocortex is patterned by predetermined molecular gradients across the developing cortex. Areal distinctions arise as specified by molecular determinants and these cortically intrinsic factors impart positional areal information. This is an activity-independent process. (*Right*) simplified protocortex model. Early neocortex is considered a blank slate, i.e., "*tabula rasa*." Area differences arise based on sensory input from axons originating from the dorsal thalamus. Cortical cells and regions are "assigned" sensory and motor territories based on thalamic input. This is an activity-dependent process. m, putative motor cortex; s, putative somatosensory cortex, v, putative visual cortex; a, putative auditory cortex; VP, represents the ventral posterior nucleus of the dorsal thalamus which receives somatosensory input from receptors in the skin via brainstem nuclei; LGN, represents the lateral geniculate nucleus of the dorsal thalamus which receives visual input from the retina; MGN, represents the medial geniculate nucleus which receives auditory input from the cochlea.

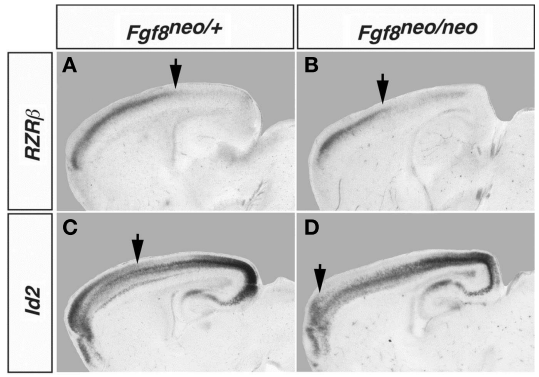

FIGURE 2 | Shift in *RZR*β and *Id2* expression in the Fgf8$^{neo/neo}$ mutant cortex. Matched sagittal sections from *in situ* hybridization experiments in control Fgf8^{neo} **(A,C)** and Fgf8$^{neo/neo}$ **(B,D)** E18.5 mouse brain are shown. There is a rostral shift in the caudal boundary of high *RZR*β expression in the mutant and a rostral shift in the caudal boundary of the superficial domain of *Id2* expression (arrows). Figure adapted from Huffman et al. (2004).

regulate aspects of rostral patterning of the telencephalon and its constituents, including the cerebral cortex (Rubenstein et al., 1999).

Mice with altered FGF function in the brain have disrupted cortical arealization, further supporting the Protomap hypothesis (Fukuchi-Shimogori and Grove, 2003; Garel et al., 2003; Huffman et al., 2004; Cholfin and Rubenstein, 2008, Iwata and Hevner, 2009). Cortical gene expression patterns are disrupted in mutant mice with reduced FGF8 signaling. Specifically, a reduction in *Fgf8* expression at the rostral pole of the neocortex leads to a rostral shift of both *RZR*β and *Id2* expression (**Figure 2**, arrows). This disruption in normal genetic patterning is correlated with ectopic ipsilateral sensory INCs in the mutant (**Figure 3**). Caudal neurons send projections to far rostral locations, perhaps following the shift in gene gradients (**Figures 2** and **3**; Garel et al., 2003; Huffman et al., 2004). Results from these studies and others have demonstrated that Fgf8 plays a regulatory role in the development of intra-neocortical connectivity and led our laboratory to further investigate gene expression-INC relationships (Fukuchi-Shimogori and Grove, 2001, 2003; Garel et al., 2003; Huffman et al., 2004; Shimogori and Grove, 2005; Dye et al., 2011a,b). We have examined the gene expression patterns of seven regulatory genes that are expressed in specific regions or gradients across the cortical sheet in early development (Miyashita-Lin et al., 1999; Garel et al., 2003; Huffman et al., 2004; Sur and Rubenstein, 2005) from the embryonic period to adulthood in mouse and studied their relationship to INC development. These genes, which are showcased in two recent reports (Dye et al., 2011a,b), are believed to be involved in the process of area and areal boundary formation as expression patterns often correlate with emergence of area borders in development (Dye et al., 2011a). The seven genes included in the analyses were *COUP-TFI*, *Id2*, *RZR*β, *Cadherin 8*, *Ephrin A5*, *Eph A7*, and *Lhx2* (Dye et al., 2011a,b), some of which are shown in this review.

CORTICAL AREAS AND THEIR CONNECTIONS: INCS AND GENE EXPRESSION FROM EMBRYOGENESIS THROUGH ADULTHOOD

The earliest sensory INCs documented in mice were found in the caudal cortex, in a location corresponding to developing visual cortex on embryonic (E) day 13.5 (Dye et al., 2011a, **Figure 4**). This growth of projections was correlated with *Lhx2* expression and flanked by expression of *Id2* and *COUP-TF1* (**Figure 4**, top and **Figure 5**, top row). The areal patterning period (APP), or the time in which features of neocortical sensory and motor areas are established, was described and defined as the period from embryonic day (E) 16.5 to post-natal day (P) 3 in mice (Dye et al., 2011a, **Figure 4**). This APP occurs before eye opening and active whisking and represents a period of time where the distribution of INCs matures into an adult-like pattern, present as early as P3, where the borders of cortical sensory and motor areas are distinct and similar to what is observed in adults (**Figure 4**). Interestingly, although INC patterns remain fairly stable from P3 to P50 (early adulthood, **Figure 4**), gene expression patterns do not (**Figures 5** and **6**). Specifically, most expression patterns of the seven genes tested (see above list) in Dye et al. (2011a,b) either lose the location specificity of expression as the mouse ages, or decreases significantly over time (**Figure 6**). For example, *Lhx2* expression correlated with developing visual and auditory cortical areas from E13.5-P20, after which expression becomes undetectable in cortical tissue (**Figures 5** and **6**).Of the seven genes we tested, four were expressed into adulthood but showed decreased levels of expression (*COUP-TFI*, *Id2*, *Cad8*, and *RZR*β) across the cortex. Also, the expression of these four genes in the barrel field differed dramatically from the onset of barrel field formation to the adult barrel pattern (**Figure 7**). For example at P40, *COUP-TF1* expression is dense in the barrel septa, where it was not present at P6 (**Figure 7**). This directed our hypothesis that genes expressed in cortex during adulthood may have switched their functional role from developmental to maintenance of cortical area borders or features.

Our study of gene expression and INCs in the *Fgf8* mutant and the normal wild-type mouse spanning from embryogenesis to adulthood led to the idea that gene expression not only regulates INC position but that the decline of cortical gene expression throughout life correlated with period closures of sensory critical periods. If, indeed, gene expression regulates critical period closure, we posit that sensory deprivation, which is known to extend the critical period for plasticity in cortex, would also extend the decline of gene expression. In a P72 mouse bilaterally enucleated at birth, we observed increased expression of *COUP-TFI* present in the caudal neocortex when compared to levels of expression in control mice (Huffman et al., 2010; **Figure 8**). This extension of normal gene expression in a mouse with long term visual deprivation supports our hypothesis that natural reduction of gene expression in cortex with age plays a role in closure of critical periods for plasticity and that sensory deprivation may extend critical periods via extension of cortical gene expression.

The studies described above in the developing mouse highlight the Protomap model and speak to the importance of gene expression in early neocortical patterning. However, based in our work in an enucleated mouse, where bilateral enucleation at birth not

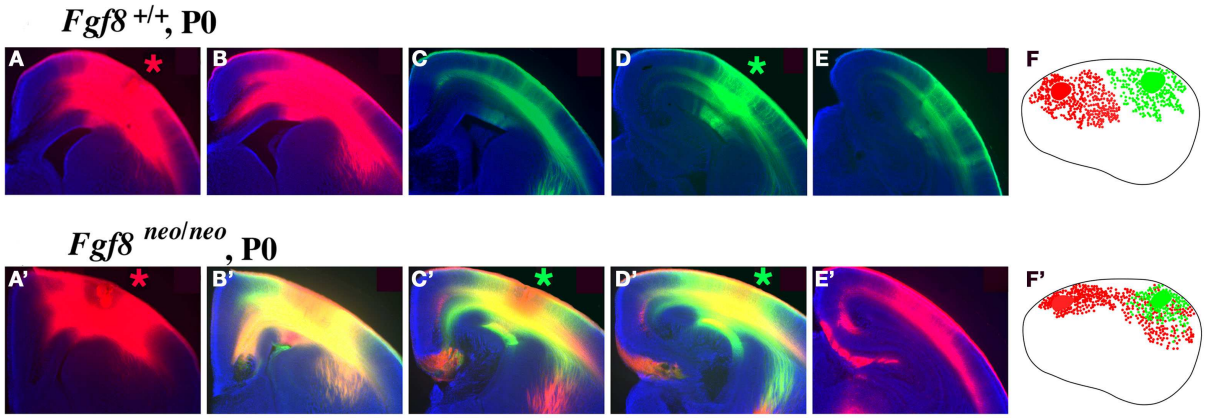

FIGURE 3 | Intra-neocortical projection patterns in P0 mice with rostral patterning defects (Fgf8$^{neo/neo}$ mutants) compared with P0 control littermates (Fgf8$^{+/+}$). Hundred micrometer coronal sections presented in rostral to caudal series of brain hemispheres following DiI [red asterisk, **(A,A′)**] or DiA [green asterisk, **(D,D′)**] crystal placement the rostral and caudal neocortex (putative somatosensory and visual cortex, respectively), oriented with dorsal up and lateral to the right. Sections were analyzed for the distributions of retrogradely labeled cell bodies, with lateral view reconstructions shown in **(F,F′)**. Hemi-sections from control mice **(A–E)** demonstrate no overlap of retrograde label from dye placements in putative somatosensory **(A)** or visual **(D)** cortex, as red and green label remain segregated. However, Fgf8$^{neo/neo}$ mutants showed a robust phenotype, indicated by red–green overlap [yellow label, **(B′–D′)**] and red label caudal to this overlap **(E′)** reflecting ectopic caudal projections to rostral somatosensory cortical locations. The ectopic intra-neocortical connections are easily observed in the reconstructions where caudal locations aberrantly project to rostral fields in the mutant **(F′)** but not in the control **(F)**. **(F,F′)**-Rostral is left, dorsal up. Figure adapted from Huffman et al. (2004).

only altered the pattern of the INCs and the neocortical network, but also generated a shift in gene expression (Dye et al., 2012); it becomes clear that the role of epigenetics and experience cannot be ignored. Although epigenetic change is not heritable, the plasticity mechanisms that allow for the change are, and we believe that these genetically mediated plasticity mechanisms, which are poorly understood at this point, serve as a building block for age-related change.

NEOCORTICAL AGING AND GENE EXPRESSION

We have demonstrated that the precise development of at least one aspect of cortical anatomy, the INCs, is regulated by gene expression (Huffman et al., 2004; **Figures 2** and **3**). Furthermore, through a comprehensive developmental analysis from embryogenesis through adulthood, we have correlated changing patterns of gene expression in neocortex as changes in functional anatomy emerge and are maintained (Dye et al., 2011a,b; **Figures 4–7**). These studies only assayed a small number of genes that were previously thought to be involved with the establishment of cortical areas in development, and did not extend into late adulthood. In an attempt to determine molecules or sets of molecules that may be involved in aging of specific tissues at specific locations, several laboratories have used microarray technology to survey great numbers of genes in neocortex and other brain regions of aging and control mice. The first published study on this topic cast a global analysis of age-related changes in mouse brain gene expression using oligonucleotide arrays analyzing 6,347 genes. Researchers found changes in cortical mRNA expression in 63 (about 1%) of the genes studied. Interestingly, 13 of these genes (20%) were related to an immune response in the neocortex (Lee et al., 2000a). Since then, several groups have used microarray in mouse model to look for up- or down-regulation of genes within the aging brain, with several studies showing most changes present in the prefrontal cortex and many correlating with immune system response (Jiang et al., 2001; Prolla, 2002; Zahn et al., 2007; Chen et al., 2010; Kedmi and Orr-Urtreger, 2011). Recently, researchers have found upregulation of microRNAs during aging in the mouse brain and have suggested that microRNA upregulation begins in mid-life and that extreme longevity is correlated with more stability and less upregulation of those microRNAs in later life (Li et al., 2011). Others have found developmental changes in microRNA expression in brain throughout the life of the animal, indicating the potential role of microRNAs in cell proliferation and brain growth, as well as in mechanisms related to brain aging (Eda et al., 2011). This provides some support for the notion that similar mechanism involved in early brain growth and patterning may also be involved in age-related change.

Although the vast majority of genetic studies of the aging brain have been done using microarray technology in the mouse model, some groups have used this and other techniques to study age-related changes in both human and non-human primate brains. Most genetic studies conducted in human brain direct their focus on the prefrontal cortex. Researchers have reported that several genes involved in synaptic and mitochondrial function are down-regulated after about age 40 in human prefrontal cortex, which is, in turn, followed by an induction of an immune response not dissimilar to that described in mouse models of brain aging (Lu et al., 2004). DNA damage has been observed in the promoters of genes with reduced age-related expression, and it has been suggested that the promoter damage resulted from oxidative stress in the cells, a theory that has recently become popular among aging researchers (see below; Lu et al., 2004).

Another study in human prefrontal cortex noted microarray gene changes of 540/22,000 genes studied in the aging brain

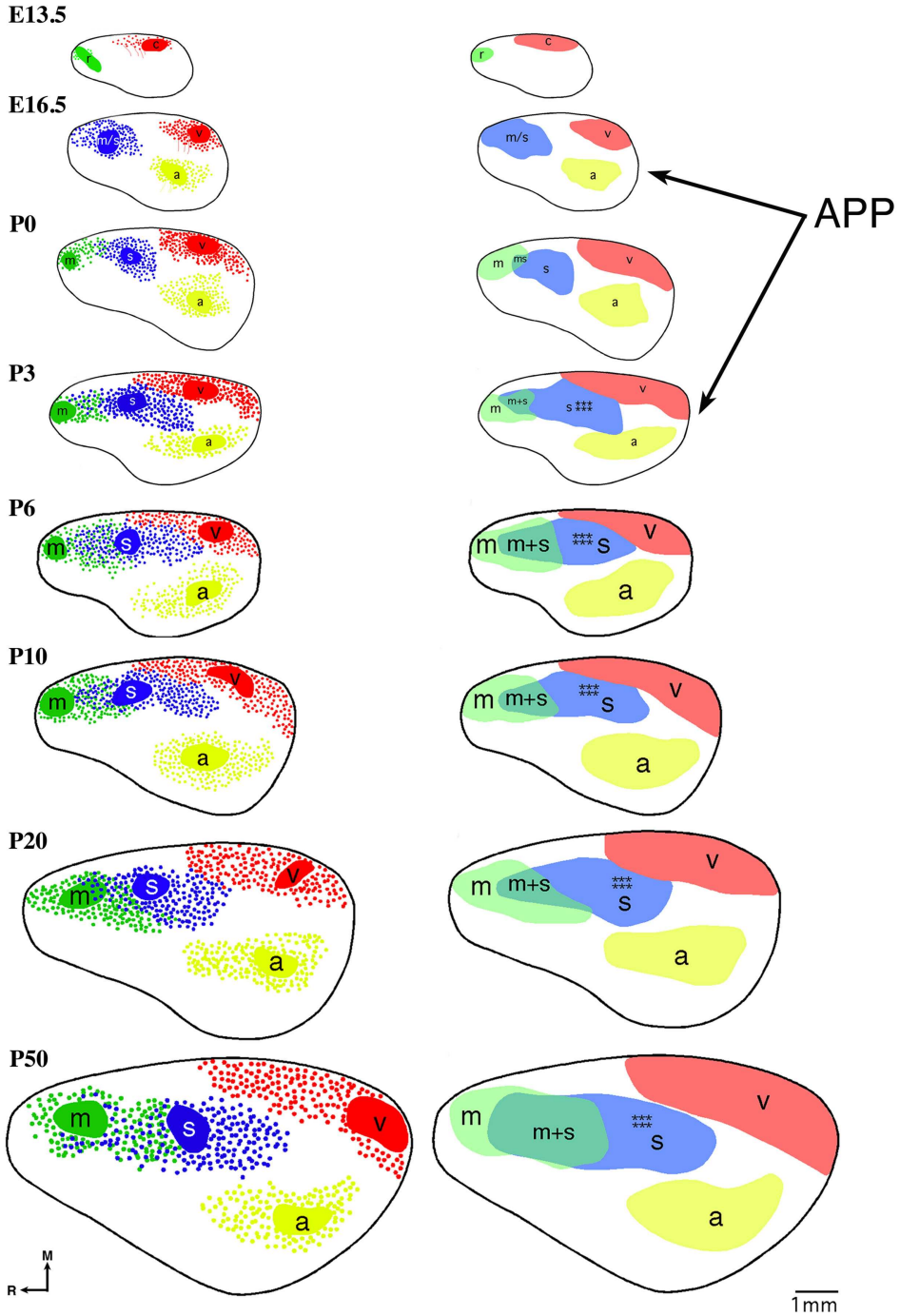

FIGURE 4 | Reconstruction of areal boundaries through analysis of intra-neocortical connections in E13.5-P50 wild-type mice. All panels represent a lateral view of one hemisphere. Left column: dye placement locations and organization of retrogradely labeled cells (colored patches, dye placement, and dye spread; red filled circles, retrogradely labeled cells in putative caudal/visual cortex; blue filled circles: retrogradely labeled cells in putative somatosensory cortex; green filled circles, retrogradely labeled cells in putative rostral/motor cortex; yellow filled circles, retrogradely labeled cells in putative auditory cortex; thick black line, hemisphere outline). Right column: lateral view reconstructions of putative areal boundaries as determined by INC analyses (colored areas, putative cortical areas as labeled; r, putative rostral area; c, putative caudal area; m, putative motor cortex; m + s, putative sensory-motor amalgam; m/s or s, putative motor/somatosensory or somatosensory cortex; a, putative auditory cortex; v, putative visual cortex). Areal patterning period (APP) is from E16.5-P3. Stars indicate location of putative barrel field. Oriented medial (M) up and rostral (R) to the left. Scale bar = 1 mm. Adapted from Dye et al. (2011a,b).

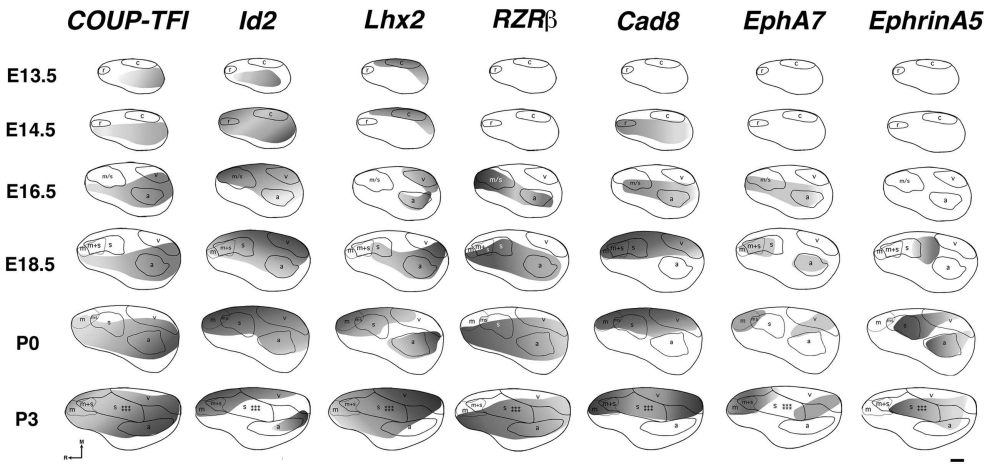

FIGURE 5 | Lateral view reconstructions of *COUP-TFI, Id2, Lhx2, RZR*β, *Cad8, EphA7,* and *EphrinA5* gene expression gradients or gene maps (gray shaded areas) coregistered with areal reconstructions of E13.5-P3 brain hemispheres. R, putative rostral area; c, putative caudal area; m, putative motor cortex; m + s, m/s, putative sensory-motor amalgam; s, putative somatosensory cortex; a, putative auditory cortex; v, putative visual cortex. Stars indicate location of barrel field. Oriented medial (M) up and rostral (R) to the left. Scale bar = 500 μm. Adapted from Dye et al. (2011a).

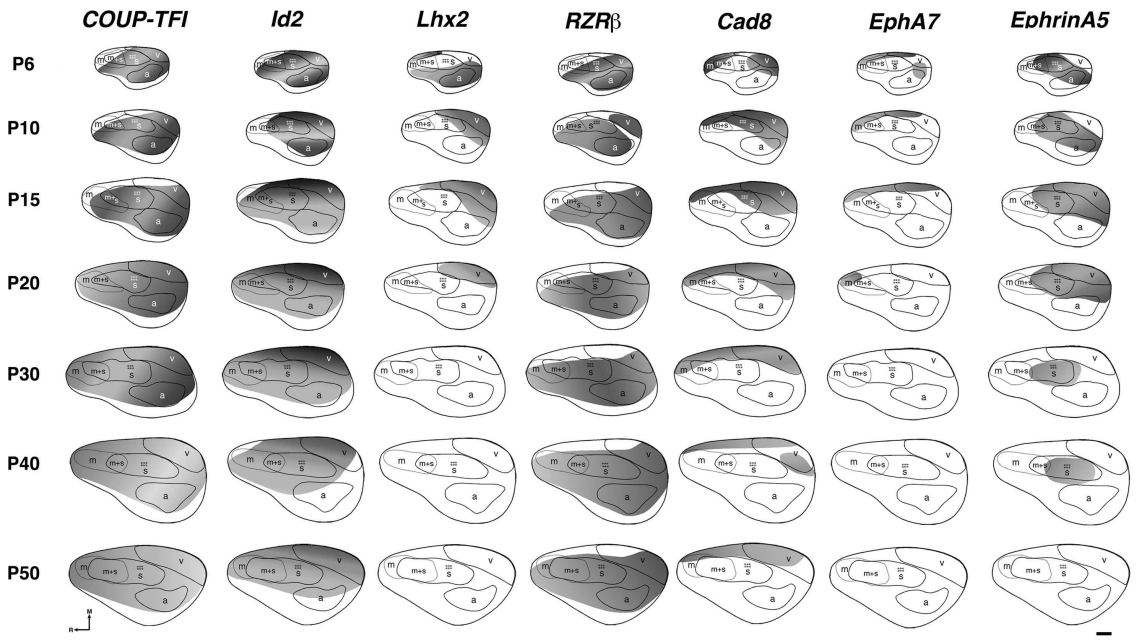

FIGURE 6 | Lateral view reconstructions of *COUP-TFI, Id2, Lhx2, RZR*β, *Cad8, EphA7,* and *EphrinA5* gene expression gradients or gene maps (gray shaded areas) coregistered with areal reconstructions of P6-P50 brain hemispheres. R, putative rostral area; c, putative caudal area; m, putative motor cortex; m + s, m/s, putative sensory-motor amalgam; s, putative somatosensory cortex; a, putative auditory cortex; v, putative visual cortex. Stars indicate location of barrel field. Oriented medial (M) up and rostral (R) to the left. Scale bar = 500 μm. Adapted from Dye et al. (2011b).

(Erraji-Benchekroun et al., 2005). Most downregulated genes showed neuron-enriched transcripts that are most likely involved in cell–cell communication and circuitry, whereas upregulated genes were potentially linked to the immune response.

Studies in non-human primates and those that included human and animal models have been very informative, providing much needed comparative genetic analyses. Fraser et al. (2005) found that human and monkey brains aged differently. Specifically, the human and chimpanzee cortex showed different age-related changes in molecular profiles, showing great dissimilarity between the two primate species. However, in a study of humans and macaques, a subset of gene changes were found to be conserved between the species including a significant age-related upregulation of the neuroprotective gene apolipoprotein D (APOD)

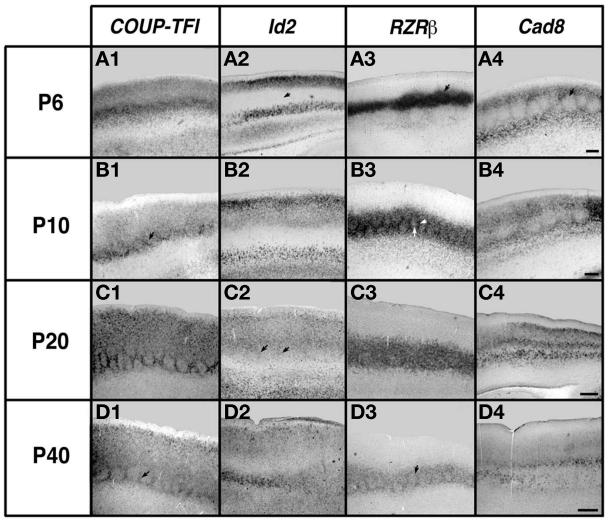

FIGURE 7 | Analysis of neocortical gene expression in the barrel field of somatosensory cortex. One hundred micrometers sagittal sections of brain hemispheres after *in situ* RNA hybridization with probes against *COUP-TFI*, *Id2*, *RZR*β, and *Cad8*. Ages examined for each gene: P6, P10, P20, and P40. Oriented with dorsal up and rostral to the left. *COUP-TFI* exhibits robust expression the barrels at P6, and at later ages expression is mostly restricted to barrel septa (outlines of barrels, arrows in B1, D1). *Id2* expression in the layer 4 barrel field is absent at P6 (arrow in A2), but some light expression in septa can be seen at P20 (arrows in C2). *RZR*β exhibits robust expression in the barrel septa and hollows at P6 (arrow in A3), and although expression continues in both septa and hollows at later ages, the septal expression become relatively stronger (arrows in B3, D3). *Cad8* displays limited expression in the barrel hollows but strong expression is seen in the septa at P6 and P10 (arrow in A4). Specific barrel expression is lost at P20 and P40 (C4, D4). Scale bar = 100 μm. Adapted from Dye et al. (2011b).

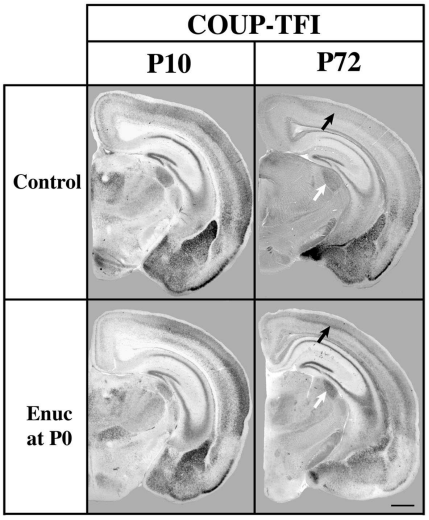

FIGURE 8 | Analysis of *COUP-TFI* gene expression in P10 and P72 control mice and mice bilaterally enucleated at birth. One hundred micrometers coronal sections of P10 and P72 brain hemispheres following *in situ* hybridization with a probe against *COUP-TFI*. At P10 strong *COUP-TFI* expression is seen in layer 4 of the caudo/lateral cortex in both control and enucleated animals. Layer 4 *COUP-TFI* expression at P72 is maintained in the enucleated animal, but is notably reduced in control animals (black arrows).Additionally, expression of *COUP-TFI*, although present in a smaller domain due to the decreased sized of the nucleus after enucleation, shows increased expression in the LGN of mice enucleated at birth as compared to controls at P72 (white arrows). Normal developmental time limits of *COUP-TFI* expression in mouse brain are extended by removal of visual activity, perhaps representing an extension of the critical for plasticity. Oriented dorsal up and lateral to the right. (Data from a published abstract, Society for Neuroscience conference, Huffman et al., 2010).

and down-regulation of the synaptic cAMP signaling gene calcium/calmodulin dependent protein kinase IV (CAMK4; Loerch et al., 2008). This change in APOD was also reported as a frequent finding across many studies in a recent meta-analysis of age-related change in gene expression in studies of rodent and human brain (de Magalhães et al., 2009). Although Loerch et al. (2008) demonstrate significant differences between primate and mice species in their analysis of age-related changes in brain gene expression, human and primate comparative microarray data suggest that neuronal genes tend to be downregulated with age; a finding consistent with our findings in the maturing CD-1 mouse where most transcripts reduce in specificity with age (Loerch et al., 2008; Dye et al., 2011b; **Figures 5** and **6**).

Although changes in gene expression are present in the aging brain, it does not prove that these age-related alterations are from a cell program dictating fate, as is supported in early developmental patterning of brain tissue. There is evidence that stochastic, non-deterministic changes in the post-natal brain may play a significant role in the aging brain. Although changes in global gene expression have been observed, variation among individual cells has also been described (Bahar et al., 2006) which is most likely from genomic instability that produces the non-systematic alteration in gene expression level. Bahar and colleagues have reported cell mutations that accumulate with age at an organ- and tissue-specific rate. They suggest that stochastic genomic instability plays a critical role in the aging process through the impact of stochastic mutations that alter normal gene expression, producing cellular degeneration, and death (Vijg et al., 2005). Given the observed age-related changes in gene expression in the mammalian brain, it is quite plausible that stochastic deregulation of gene expression directly leads to cellular degeneration and death that adversely impacts normal cognitive function (Bahar et al., 2006). The ability for stochastic deregulation of gene expression to occur could quite possible represent a heritable plasticity mechanism that allows for both age-related and evolutionary change in neocortical structure and function. As tends to be true in most nature vs. nurture debates, the most likely explanation is that both age-related changes in genetic program and stochastic changes in genomic stability together influence the aging cellular structure and function.

AGING AND CORTICAL NEURON MORPHOLOGY AND WIRING

Although our published studies of INC development in the adult mouse, described in a previous section, ended in early adulthood, we have recently analyzed the patterns of INCs of somatosensory, visual, and motor cortex in 18-month-old mice and did not find any phenotypic differences in the overall number of projections from the cortical areas (Abbott et al., 2012). Our data in mouse are

consistent with studies in primates that have not shown a decrease in the number of neurons in the hippocampus or neocortex related to aging (Peters et al., 1998; Peters and Rosene, 2003). However, aging does appear to significantly impact myelinated nerve fibers as well as some microstructural changes in neocortical anatomy. Specifically, Peters and colleagues have published several reports on structural changes, such as increased lipofuscin within cells and loss of dendritic spines in the cerebral cortex of rhesus macaque monkeys (Peters et al., 1998; Page et al., 2002; Peters and Rosene, 2003; Peters, 2007, 2009; Peters and Kemper, 2012). The cell type within neocortex that seems to demonstrate the greatest age-related alteration is neuroglia (Peters, 2007). Although some structures such as the anterior commissure (Sandell and Peters, 2003), and layer 1 in cortical areas 17 and 46 (Peters and Sethares, 2002) do not show a significant change in neuroglial numbers with age; there is a 45% age-related increase in the number of oligodendrocytes in area 46 (Peters and Kemper, 2012), a 20% increase in fornix (Sandell and Peters, 2002), and a 50% increase in area 17 (Peters and Kemper, 2012). These age-related changes in glia appear to be area-specific, could be generated from an integration of genetic and epigenetic processes and may be relate to functional changes in neocortex that lead to cognitive decline in aging.

The integrity of the intra-neocortical circuit is critical for proper cognitive function. Natural age-related demyelination is observed in the primate model and may represent damage to the intra-neocortical circuitry, which, in turn, could lead to cognitive decline (Peters, 2009). Peters and Kemper (2012) posit that age-related cognitive decline correlates with and possibly results from problems with myelination, altered intra-neocortical connectivity, and abnormal synaptic and dendritic function.

Patrick Hof's group has also made significant inroads in understanding age-related anatomical changes in the primate brain. Specifically, they reported that in Alzheimer's disease, pyramidal cells and their projections were particularly vulnerable to cell death (Morrison and Hof, 2002). In normal aging, although the cells did not appear vulnerable to death, their function was greatly impacted by other anatomical changes such spine loss, and a decrease in NMDA receptor signaling in hippocampal tissue. Thus, they posit that these sub-lethal changes in neurons and their circuits could play a role in normal age-related cognitive decline. In a study of short- and long-range INCs in non-human-primates, Hof's group found that levels of GluR2 and NMDAR1 glutamate receptor subunit protein immunoreactivity were significantly decreased in a proportion of projection neurons in cortex (Hof et al., 2002). They found that both types of projection neurons showed age-related neurochemical changes. Furthermore, a study in aging Patas monkeys examined the INCs between temporal and prefrontal cortex (Page et al., 2002). In this primate species, projection neurons demonstrated a loss of dendritic spines at all levels of their dendritic trees. In a follow up study, Hof and colleagues describe differences in dendritic spine loss in different positions on the dendrites of projection neurons in macaque cortex (Duan et al., 2003). They found the biggest spine reduction in the proximal portion of apical dendrites, with an overall reduction observed throughout. On the basal dendrites, the greatest reduction of spines was located on the distal branches. Despite an overwhelming amount of data suggesting that neuron loss is not associated with normal aging (Peters et al., 1998; Morrison and Hof, 2002; Peters and Rosene, 2003), the alterations in the dendritic branches of important projection neurons in aging monkey cortex described above suggest that subtle changes in brain anatomy can have significant impact on brain function, and thus, cognition (Duan et al., 2003; for review, see Morrison and Hof, 2007). Recently, researchers from Croatia were able to reverse these age-related effects on projection neuron dendrites through the use of environmental enrichment highlighting the impact of epigenetics on the aging brain. In their study in a rodent model, the age-related loss of dendritic spines on projection neurons was ameliorated in a group of aging rats exposed to an enriched housing environment (Rasin et al., 2011). As in early development, this implicates the role of sensory input in shaping and maintaining structural aspects of the brain.

Although tract tracing data from our laboratory in 18-month-old aging mice show no significant changes in area-to-area global projections in neocortex, it is highly possible that changes in myelin and cortical microstructure including dendritic spine number and morphology are affected in a murine model of aging (Abbott et al., 2012). This warrants further study at the microstructural level.

Studies utilizing standard anatomical techniques in monkeys have been replicated in both humans and primates using diffusion tensor imaging (DTI), also called diffusion tensor magnetic resonance imaging (DT-MRI). For example, DTI studies in aging macaque monkeys showed a loss of myelinated axons within the neocortex without any obvious loss of gray matter thickness. Most significant changes were found in the major fiber tracts of the frontal lobe (within the anterior corpus callosum) where myelination was reduced (Makris et al., 2007). These findings corroborate theories implicating the role of frontal lobe function in age-related human cognitive decline. Although these findings suggest a greater phenotype in more rostral regions, other studies have shown more global effects of age-related changes in myelination (Moy et al., 2011). For example, DTI analyses revealed white-matter axonal changes not only in frontal areas but in multiple regions throughout neocortex as well. These white-matter changes were related specifically to impaired reaction time in a simple reaction time task (Moy et al., 2011). Another human DTI study demonstrated microstructural changes in the white matter of multiple cortical regions in older people diagnosed with mild cognitive impairment (Cho et al., 2008).

The neocortex is a living, complex structure that continues to change molecularly, structurally, and physiologically throughout the lifespan of the animal. The structural components of the brain are first patterned by interplay of genetic and epigenetic influences, are maintained through both gene expression and neural activity, then continue to develop and change later in life. The degradation of myelin structures decreases function and slows processing, which has a profound effect on the intra-neocortical circuitry that is critical for normal cognitive function. Although neuronal cell death does not appear to be a major issue in the non-diseased aging brain (Peters et al., 1998; Morrison and Hof, 2002; Peters and Rosene, 2003), the ability of neurons to function properly is greatly impacted by changes in microstructure, including loss of dendritic spines and myelination. As we have suggested previously

that neocortical gene expression plays a role in the development and maintenance of cortical structure, and that patterns of gene expression within neocortex change throughout the life of the animal, it follows that gene expression may be involved in age-related change in the brain, specifically age-related changes that may impact the brain's structure and neuronal microstructure, and hence, cognitive function. However, given the experience-related effects on brain anatomy and even gene expression, epigenetics must interact with gene expression to create the aging phenotype.

THE IMMUNE SYSTEM AND THE AGING BRAIN

As mentioned previously, some of the observed anatomical change in dendrites or myelinated axons in the aging brain may be related to immune mechanisms. A recent paper has documented some very interesting changes in immune gene regulation in the neocortex of aging mice. Specifically, researchers have correlated age-related impairments in motor behaviors, cognition, and motivation with upregulation of serum cytokine levels in the medial prefrontal cortex (Bordner et al., 2011). The medial prefrontal cortex is a part of the neocortex that has been frequently discussed as a potential key player in mild cognitive impairment observed in aging humans (Allard et al., 2012; Caetano et al., 2012). Finally, glucocorticoids are molecules that have been shown to alter cerebral cortex development in clinical and animal studies of perinatal exposure (Antonow-Schlorke et al., 2003; Mutsaers and Tofighi, 2012; Zuloaga et al., 2012). Glucocorticoids have also been implicated in age-related degradation of brain anatomy and thus age-related decline of cognitive function (Holmes et al., 2010; Soontornniyomkij et al., 2010). Exactly how and to what extent glucocorticoid exposure can impact cortical microstructure throughout the lifespan of the animal is an area that warrants further study.

Another possible immune system related mechanism is related to epigenetic effects, not unlike the impact of stochastic changes in the system that perturb gene expression patterns of individual cells. It has been reported recently that senescent cells secrete factors that impact the surrounding tissue (Rodier et al., 2009). Specifically, DNA damage resulting from DNA double-strand breaks in aging initiates the secretion of inflammatory cytokines such as interleukin-6. This release of cytokine was specifically related to cellular senescence and may significantly impact the cellular phenotype. It is quite plausible that groups of these senescent cells that secrete cytokines from DNA damage could greatly impact patterns of gene expression in aging brain. Subsequent change in gene expression, could thus impact features of brain anatomy, as is observed in early developmental time periods. Neocortical anatomy, which is most often related to cognitive function in humans, may be adversely affected by this stochastic immune response.

CALORIC RESTRICTION AND THE AGING BRAIN

After first documenting many age-related changes in multiple tissues including brain gene expression from microarray studies in aging mice, Prolla's research group has advanced our knowledge of this research area substantially by investigating the impact of CR on age-related changes in gene expression throughout the body. First, in 1999, it was reported that age-related changes (up- or down-regulation) in gene expression in mouse skeletal muscle were partially or completely prevented through a reduction in dietary caloric intake (Lee et al., 1999). This was a landmark study that presented the now popularized and widely accepted notion that CR in mammals retards the aging process. Subsequently, Prolla presented the hypothesis that the prevention of age-related changes in gene expression with CR potentially resulted from the decrease in oxidative stress that occurs during CR. They suggested that CR provides a neuroprotective effect on the degrading, aging brain, particularity the cerebral cortex (Prolla and Mattson, 2001; Weindruch et al., 2001). More recently, several laboratories have demonstrated the robust effect of CR on the attenuation of aging in the brain, particularly demonstrating phenotypic prevention of gene up- or down-regulation effects in measures of mRNA and microRNAs in brain tissue (Khanna et al., 2011). Genes associated with apoptosis in aging are downregulated and the age-related decrease of SIRT1 expression, a longevity factor that appears to play a neuroprotective role in neuropathological disease, in cerebral cortex that has been reported by several laboratories has been shown to be prevented in late-onset CR (Chen et al., 2008; Khanna et al., 2011; Quintas et al., 2012). Age-related increases in 5-hmC, thought to be involved in regulation of gene expression in brain, and age-related changes in Nnmt-3a, a molecule that catalyzes DNA methylation, are prevented by CR (Chouliaras et al., 2011, 2012).

CONCLUSION

There is a vast amount of data demonstrating the relationship between gene expression and anatomical features in early brain development, including neocortical circuit development, as well as late-stage changes in gene expression and circuitry in the aging animal. Based on our research that spans the lifespan from the embryonic period to adulthood as well as data from those studying the aging animal model, we suggest that similar genetic and epigenetic mechanisms continue to impact the structure and function of the brain throughout life. Early on, both genetics and experience guide neocortical and brain patterning, and these mechanisms continue to impact the maintenance of cortical areas and their boundaries as well as physiological area function throughout adulthood. Late in life, similar genetic mechanisms may be involved in the breakdown of brain microstructure, and changes in experience, as in early development, can either advance or ameliorate the deleterious effects of aging. Data from multiple laboratories around the world suggest that CR and environmental enrichment can impact gene expression in aging brain, which most likely affects microstructural aspects of cortical architecture. This research represents an exciting direction that will greatly advance our knowledge of the aging process in mammals.

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