



From healthy aging to frailty: in search for the underlying mechanisms

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Keywords: neuroplasticity, epigenetic, neuroinflammation, HPA axis, stress, aging

For Review Only

Abstract

Population aging is accelerating rapidly worldwide, from 461 million people older than 65 years in 2004 to an estimated 2 billion people by 2050, leading to critical implications for the planning and delivery of health and social care.

The most problematic expression of population aging is the clinical condition of frailty, which is a state of increased vulnerability that develops as a consequence of the accumulation of microscopic damages in many physiological systems that leads to a striking and disproportionate change in health state, even after an apparently small insult.

Since little is known about the biology of frailty, an important perspective to understand this phenomenon is to establish how the alterations that physiologically occurs during a condition of healthy aging may instead promote cumulative decline with a subsequent depletion of homoeostatic reserve, and increased vulnerability also after minor stressor events.

In this context, the present review aims to provide a description of the molecular mechanisms that, by having a critical impact on behavior and neuronal function in aging, might be relevant for the development of frailty. Moreover, since these biological systems are also involved in the coping strategies set in motion to respond to environmental challenges, we propose a role for lifestyle stress as important player to drive frailty in aging.

1. From healthy aging to frailty

Life expectancy in the 20th century has greatly increased in the Western Countries. However, middle-aged and elderly people currently do not experience a parallel increase in health span due to the development of age-related pathologies. The most problematic expression of population aging is the clinical condition of frailty, a state of increased vulnerability to poor resolution of homeostasis after a stressor event, which increases the risk of adverse outcome [1]. The high prevalence of chronic disabling conditions impact both on the quality of life of the individual as well as on the economic burden. On these bases it is crucial to understand the molecular mechanisms underlying frailty. It would be particularly important to identify the risk factors leading to frailty instead of healthy aging and their impact at molecular level.

Aging is associated with structural and physiological changes in the brain, the majority of which involve the synapses, the most important structures in neuronal network for maintaining accurate neuronal activity and normal brain functions. The maintenance of synaptic functionality requires preservation of proper synaptic structure, coordination of synaptic vesicle release and membrane excitability, and integration of retrograde signals from the postsynaptic terminal [2]. During aging these processes undergo a physiological decline [3] and structural changes in neurons and spines as well as alterations in neurotransmitters receptors expression and changes in electrophysiological properties occur [4-9]. Specifically, several morphological changes have been reported during aging, such as reduction in synaptic density, pruning of the dendritic tree, loss of dendritic spines, structural changes in the presynaptic active zone and alterations of receptors expression and trafficking [10]. Moreover, in both in human and rodents, loss of synapses in aging has been associated with cognitive impairment [11-13].

Spines represent key element of glutamatergic synapses, whose morphological and functional changes may affect synaptic events and excitatory synaptic signaling in aged neurons. Indeed, several lines of evidence indicate that aging is associated with a reduction of NMDA (N-methyl D-Aspartate) subunit expression and receptor activity in brain regions involved in higher brain function including synaptic plasticity, learning and memory [14-21].

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3 Age-related changes can be detected in both excitatory and inhibitory synapses with a shift of the
4
5 excitatory/inhibitory balance that can cause cognitive impairments [22], both in humans and in rodents[11-
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7 13]. GABAergic transmission is markedly impaired during aging, causing an increase in cortical excitability
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9 [23-25]. Indeed, many studies showed a reduction of GABA (γ -aminobutyric acid) synthesis [26] probably
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11 due also to the downregulation of the two isoforms of the enzyme responsible of GABA synthesis, namely
12
13 GAD65 (Glutamic Acid Decarboxylase) and GAD67 [27] in different brain areas. Furthermore also the
14
15 composition of GABA_{A,B} receptors changes during aging, contributing to changes in learning and memory
16
17 processes [28]. For example, age related with an up-regulation of $\alpha 1$ subunits of GABA_AR in the rat
18
19 hippocampus [29] and a reduction of GABA_BR1 in the same brain area is associated with with spatial
20
21 learning impairment [28, 30].
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25 It is not surprising that all these alterations in turn may impact the functions of other systems playing a
26
27 critical role in the maintenance of synaptic function, as presented in the next section.
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31 **2. Molecular mechanisms altered in the aged brain**

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33 Based on the definition of frailty, this condition may be considered the expression of a loss in resilience –
34
35 the ability to cope with adverse conditions- that may be the result of a progressive impairment in systems
36
37 and mechanisms crucial for the proper synaptic function within the brain. Alterations of synaptic structure
38
39 and/or function are in fact considered a potential risk factor or even a critical determinant for several
40
41 neurodegenerative disorders associated with aging e.g. Alzheimer and Parkinson' diseases as well as for
42
43 cognitive decline. These pathological conditions, which are indeed characterized by reductions in synapse
44
45 numbers and alterations of genes encoding proteins involved in synapse formation and function can be
46
47 considered as “synaptopathies”, in which the dysfunction of the synapse is the central element [31].
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51 Deficits of such systems and mechanisms would lead to increased vulnerability of the synapse to the impact
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53 of internal or external environment as well as to an enhanced risk to develop pathological conditions that
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55 characterize unhealthy aging.
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58 Although we are aware that multiple molecular systems may contribute to alteration of synaptic function
59
60 during aging, in this mini-review we will specifically focus on deficits of neuronal plasticity, dysfunction of

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3 hypothalamic–pituitary–adrenal (HPA) axis, alterations of immune/inflammatory system as well as
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5 epigenetic modifications.
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9 2.1 Deficits in neuronal plasticity and neurotrophic mechanisms

10
11 Among the mechanism that may contribute to age-related impairment, deficits of neuronal plasticity may
12
13 play a crucial role. This term was used for the first time by Santiago Ramon y Cajal (1852-1934) [32] and
14
15 now refers to “the ability to make adaptive changes related to the structure and function of the nervous
16
17 system” [33]. According to this idea, even though brain plasticity is essentially a physical process, the term
18
19 “neuronal plasticity” can stand not only for morphological modifications – for instance of the gray matter
20
21 that can increase or narrow [34] - or for alteration in the connections between neurons that can be formed,
22
23 shaped, modified or impaired. Indeed, it’s important to consider that neuroplasticity is inherently
24
25 connected and works together with the generation of new neurons (neurogenesis) and specific
26
27 neurobiochemical changes in order to improve the brain’s ability to adapt to its environment.
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29

30
31 Historically, being plasticity essentially a physical and morphological phenomenon, it was thought that
32
33 primary contributions to the decline of this brain feature were massive cell loss [35] as well as deterioration
34
35 of dendritic branching [36]. However, we now know that the brain’s modifications occurring during normal
36
37 and successful aging are more subtle and selective than was once believed. Indeed, the plastic changes in
38
39 structure and function, such as modifications in the neuronal morphology and alterations in the tissue
40
41 density, that the brain goes through do not follow a homogenous and universal pattern within the different
42
43 brain areas but are specific and peculiar [10]. Moreover, dendritic and spine densities and dynamics and
44
45 functional interactions among different neurotransmitters are known to change differently in specific areas
46
47 of the brain [37].
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51 Among the brain regions affected by aging, the prefrontal cortex (PFC) and the hippocampus seem to be
52
53 particularly vulnerable, but even within and between these regions the impact of aging on neuronal
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55 function can differ [10]. For instance, the morphology of neurons in the PFC is more susceptible to age-
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57 related change, as these cells show a decrease in dendritic branching in rats [38] and humans [39], while in
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59 the hippocampus, subregion-specific changes in the expression of genes involved in the regulation of
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3 neuroplasticity have been observed [40]. Neurogenesis in the hippocampal dentate gyrus also decreases
4
5 with age, which may contribute to the changes in brain's plasticity over the lifespan [41]. Moreover,
6
7 induction and maintenance of long term potentiation, alongside alterations in calcium homeostasis,
8
9 changes with age (Lister and Barnes, 2009). These alterations could be related to oxidation of proteins that
10
11 are involved in cellular ion homeostasis. The resulting elevated calcium concentrations can cause neuronal
12
13 degeneration and cell death, initiating several age-related impairments such as learning and memory
14
15 deficits [42].

16
17
18 Among all the molecular regulators of neuronal plasticity, neurotrophic factors (NTFs), and in particular the
19
20 neurotrophin family, may be considered key players. Indeed, besides their well-established activity in
21
22 supporting the survival, differentiation and maintenance of neuronal functions, NTFs finely modulate all the
23
24 crucial steps of network construction, from neuronal migration to experience-dependent refinement of
25
26 local connections [43]. It is now well-known that NTFs are important mediators of neuronal plasticity not
27
28 only during the development of the brain but also in adulthood where they continue to support and
29
30 modulate axonal and dendritic growth, membrane receptor trafficking, neurotransmitter release, spine
31
32 morphogenesis and neurogenesis [44, 45].

33
34
35 The neurotrophin family comprises of NGF, Brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-
36
37 3) and neurotrophin-4 (NT-4) that have all evolved from a common neurotrophin ancestor gene. Their
38
39 actions are dependent on binding to transmembrane receptor systems – the tropomyosin receptor tyrosine
40
41 kinase family and the p75 neurotrophin receptor [46]. Neurotrophins have preferential binding for specific
42
43 receptors: NGF binds to TrkA, BDNF and NT-4 to TrkB, and NT-3 to TrkC, although there are also a number
44
45 of promiscuous interactions [47]. All four neurotrophins can bind to the p75 receptor and the association of
46
47 p75 with Trk receptors can regulate the affinity of Trk receptors for each respective neurotrophin, allowing
48
49 for greater control of ligand–receptor interactions within this system [48].

50
51
52 BDNF is a key mediator of activity-dependent processes in the brain that have a major impact on neuronal
53
54 development and plasticity. A central function of BDNF is to help neurons to adapt, survive and to speed up
55
56 the brain's ability to make new connections.
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1
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3 Neurotrophic factors seem to be very much relevant in the aging brain, indeed, a negative correlation
4 between BDNF serum levels and age has been reported in a large cohort of healthy elderly individuals [49].
5
6 In preclinical studies, the gene expression of the total form of BDNF was reduced in the hippocampus and
7
8 in the prefrontal cortex of 18-month-old aged rats and this reduction was paralleled by a significant
9
10 decrease in the protein levels of the neurotrophin and of its receptor TrkB [50]. Moreover, in CA1 and CA3
11
12 sub-region of the hippocampus, aging caused reduction in total BDNF mRNA, in Exon IV-specific transcripts,
13
14 and in transcripts with the long 3' UTRs, an effect that may be related to the cognitive decline observed in
15
16 these animals [51]. In line with this idea, a clinical study on a cohort of 142 participants between 59 and 81
17
18 years of age has established a correlation between the reduction of serum BDNF levels and the shrinkage of
19
20 the hippocampal volume with the decline in the spatial memory performance [52].
21
22

23
24 Interestingly, it has been shown that aging is also associated with an impaired responsiveness of BDNF
25
26 expression and signaling to environmental stimuli [53]. For example, the abilities of challenges such as
27
28 contextual learning [54], physical exercise [55] and brain lesion [56] to upregulate BDNF signaling are
29
30 impaired in aged rodents.
31

32
33 Supporting the involvement of BDNF in age-related variations of brain plasticity, it was shown that the
34
35 administration of exogenous BDNF in the frontal cortex of aged rats produced a marked increase of multi-
36
37 threshold neurons of the locus coeruleus (LC), a major noradrenergic cell group with a remarkable capacity
38
39 of remodeling, accompanied with a decrease in threshold current, suggesting that BDNF may contribute to
40
41 functional changes in the presynaptic axon terminals of LC neurons in the aging brain [57].
42
43

44 Moreover, a clinical study involving 116 adults ranged in age from 20-93 investigated the effects of BDNF
45
46 polymorphism on multiple indices of memory (prospective, associative, subjective complaints and item).
47
48 The results suggest that genetic predisposition to the BDNF val66met polymorphism exerts an influence on
49
50 multiple indices of episodic memory exacerbating age-related differences in memory across the lifespan
51
52 [58]. This polymorphism caused by a valine (Val) to methionine (Met) base change at position 66 in the
53
54 BDNF pro-domain [59] results in a decrease of regulated BDNF secretion and is known to contribute to the
55
56 development of psychiatric disorders such as anxiety-related behavior [60] and depressive-like behavior
57
58 [61]. Furthermore, this polymorphism also plays a role in age-related neurodegenerative diseases, such as
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3 Alzheimer's disease and Parkinson's disease. Specifically, decreased expression of BDNF mRNA and protein
4
5 has been reported in the hippocampus, neocortex and Meynert *nucleus basalis* of patients with AD [62-64],
6
7 while a reduced BDNF level due to decreased *BDNF* gene transcription has been found in the *substantia*
8
9 *nigra pars compacta* of patients with PD [65].
10

11 12 13 2.2 HPA axis dysfunctions

14
15 The hypothalamic–pituitary–adrenal (HPA) axis is an auto-regulating system that controls the circulating
16
17 levels of glucocorticoids (cortisol in humans and corticosterone in rodents) (GCs).
18

19
20 In physiological condition, the paraventricular nucleus of the hypothalamus secretes corticotrophin
21
22 releasing hormone (CRH) and vasopressin (VP), which are transported to the anterior pituitary where
23
24 adrenocorticotrophic hormone (ACTH) is released. ACTH then stimulates the adrenal cortex to secrete GCs.
25

26
27 The activity of the HPA axis is regulated by a negative feedback mechanism: the increased levels of GCs in
28
29 the blood turn off their own secretion by down-regulating the release of the CRH and of ACTH.
30

31
32 In the brain GCs bind two types of receptors: the mineralocorticoid (MRs) and the glucocorticoid (GRs). GRs
33
34 are highly ubiquitous and expressed in most brain regions, whereas MRs are predominantly expressed in
35
36 limbic regions. MR receptors have a high affinity for corticosteroids, so they are mostly occupied in basal
37
38 conditions when circulating corticosteroid levels are low, while GRs have tenfold lower affinity and are
39
40 more occupied as corticosteroid levels increase [66].
41

42
43 GCs can rapidly modulate neuronal activity through a non-genomic pathway (activation of membrane
44
45 receptors that cause the release of neurotransmitters in the synaptic cleft) or a genomic pathway. In the
46
47 latest, corticosteroid receptors translocate into the nucleus, where by binding the glucocorticoid
48
49 responsive elements (GRE) can affect the transcriptional activity of target genes [67, 68].
50

51
52 The HPA axis exerts its effect following a U-shaped dose-response curve [69-72]. Indeed, low or high dose
53
54 of hormone may have short term or long-term adverse consequences for the individuals, while the optimal
55
56 response is achieved at intermediate doses.

57
58 For example, the exposure to an acute challenge increase the release of GCs in terms of minutes to hours
59
60 with the enhancement of synaptic transmission, LTP, learning for self-preservation, while chronic stress

1
2
3 exposure, lead to high levels of circulating GCs from months to years causing the suppression of synaptic
4
5 functions and adaptive plasticity mechanisms leading to detrimental effect [71].

6
7 Several lines of clinical and preclinical evidence demonstrated that hyperactivity of the HPA axis contributes
8
9 to neuronal and peripheral deterioration associated with aging [73-75] with a deregulation of basal HPA
10
11 axis function and increased stress responsiveness [76].

12
13 In physiological conditions, cortisol is secreted over a 24-h period according to daily fluctuations [77]. In
14
15 general, we can say that it rises early in the morning and decrease throughout the rest of the day [78] even
16
17 if this profile may be also influenced by the wake-sleep, the light-dark cycles and the endogenous circadian
18
19 rhythm [79]. In human, studies showing the correlation between changes in diurnal cortisol release and
20
21 aging are variable: indeed, results are contradictory with some researcher showing a decline, others an
22
23 increase probably due to individual's variability [80] and methodological flaws.

24
25 In old adults, altered morning-cortisol levels or a flatter morning-to-evening diurnal cortisol slope lead to
26
27 HPA dysregulation and impaired neuroendocrine regulation, with increased vulnerability to stressors [78,
28
29 80-89]. These alterations may be involved in the clinical presentation observed in frail older subjects.
30
31 Indeed, greater diurnal cortisol secretion and smaller decline in cortisol during morning hours have been
32
33 associated with frailty [90, 91], whereas lower diurnal cortisol is correlated with longevity [92]. These
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35 increases occur both in male [93] and in female [94] even if the age-related changes in cortisol appear to be
36
37 more clear for man, as compared to woman [86, 88].

38
39 HPA axis alterations during aging, both in humans and in rodents, are related to modification in
40
41 glucocorticoid circadian rhythm [81, 95], with mild increases in morning levels and a failure to increase in
42
43 the evening [81] and with changes in GC sensitivity that could lead to an impairment of the negative
44
45 feedback, the so-called "glucocorticoid cascade" [96]. The impaired function of the glucocorticoid negative-
46
47 feedback is associated with higher levels of circulating GCs that lead to the reduction of GR and altered GR
48
49 signaling in the forebrain [97-101]. Dysfunction of negative feedback can produce higher stress-induced
50
51 peaks in cortisol and prolonged stress response [102]. Moreover, aging-related elevation of glucocorticoids
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53 levels appears to threaten hippocampal neurons and may contribute to the loss of dendrite complexity in
54
55 the rat hippocampus [103].
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3 Aging is also associated with alteration of CRH and VP that may in turn account for the aberrant activity of
4 the HPA axis. While some studies show progressive decreases of CRH expression [104, 105], the majority of
5 the studies found increases of CRH production with advancing age [75, 83, 106-108]. Moreover, increased
6 CRH levels during aging can be detrimental and may contribute to the development of age-related
7 pathologies, such as depression, anxiety, neurodegeneration, immune and metabolic disorders[109]. On the
8 contrary, VP increases in parvocellular neurons during aging, while a decrease has been shown to occur in
9 the suprachiasmatic nucleus [75].

10
11
12 Furthermore, the 11- beta hydroxyl steroid dehydrogenases (11 β HSD-1), the enzyme responsible of
13 restoring active GCs is significantly increased in the hippocampus of aged mice, thus amplifying the steroid
14 action and contributing to the aging process [110, 111].

15
16
17 GR down-regulation in hippocampus leads to memory impairment and HPA axis dysregulation, as well as
18 deficits in the activation of GR-dependent transcription [99, 101, 112, 113]. Moreover, plasma
19 glucocorticoids levels correlate with deficits in spatial learning and hippocampal degeneration in aged rats
20 [114, 115] and with dysfunction of hippocampus-dependent memory tasks and reduced hippocampal
21 volume in aged humans [82], while high salivary cortisol concentration predict declining performance on
22 declarative memory tests [116]. Despite this, many older individuals have cognitive performance similar to
23 younger ones [97, 117-120] and this can be due to the accumulation of a lifetime of experience that could
24 alter the progression of brain aging, a phenomenon named "allostatic load" [121, 122].

25 2.3 Alterations of Immune/inflammatory

26
27
28 Another system altered in aging is the immune/inflammatory system. Historically, the brain was believed to
29 be an immunologically privileged space, functioning fully isolated from the immune system. However, it is
30 now accepted that there is a wide and constant bi-directional communication between the immune system
31 and the CNS, across the relatively impenetrable blood- brain barrier (BBB) [123, 124]. As a consequence of
32 this connection, neural activity is dramatically affected during and following an immune challenge that
33 occurs in the periphery [125, 126], leading to the production of cytokine -signaling molecules possessing
34 unique immunomodulatory functions- within the brain tissue, with activated microglia -resident immune
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3 cells of the CNS- driving the response [127]. Moreover, the discovery of the meningeal lymphatic system's
4 capability of carrying fluids, immune cells, and macromolecules from the CNS to the draining lymph nodes
5 [128, 129] corroborates the relevance of the signaling between the brain and the peripheral immune
6 system.
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11 As we age, our immune system undergoes several modifications of the inflammatory status with a decline
12 of the adaptive immunity, called immunosenescence, coupled with a concomitant activation of the innate
13 immune system, so-called "inflamm-aging", which may lead to a low-grade, chronic, systemic inflammation
14 [130-132]. Immunosenescence of the acquired system has received a great attention in recent years. After
15 birth, thymic productivity decreases with age, resulting in reduced T-cell repertoire in parallel with an
16 increased growth of memory and effector-memory cells [133]. This imbalance culminates in a reduced
17 ability to clear novel pathogens, prolonging infection duration as well as an increase in functionally distinct
18 T-cell populations, which have an amplified pro-inflammatory phenotype [134]. Increases in numbers of
19 antigen-specific cells with age are associated with an increment in the number of terminally differentiated
20 'senescent' cells, which occupy a large proportion of immune space. These cells, particularly CD8⁺, are
21 extremely potent producers of inflammatory cytokines and cultures of senescent CD8⁺ T cells show
22 resistance to apoptosis, permanent loss of CD28 expression, altered cytokine profiles, reduced ability to
23 respond to stress and various functional changes [135]. Moreover, with age these antigen-specific cells do
24 not proliferate properly and after mitogenic/viral stimulation produce more IL-6 and TNF than their
25 younger counterparts [136]. Therefore, T-cell immunosenescence is characterized by an increased
26 proinflammatory phenotype and most probably contributes to inflamm-aging in a manner dependent on
27 previous exposure to antigenic challenges and exhaustion of T-cell repertoire [137].
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48 Inflamm-aging is thought to be a result of a cumulative lifetime exposure to antigenic stimuli caused by
49 infections as well as exposure to non-infective antigens [138]. The consequent immune response, tissue
50 injury and production of reactive oxygen species (ROS) that cause oxidative damage also triggers the
51 release of additional cytokines, primarily from cells of the innate immune system [139]. This results in a
52 vicious cycle, where the release of cytokines provokes a further damage, immune activation and
53 remodeling of the tissue.
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3 Within the innate immune system, microglia, as part of the myelomonocytic lineage, constitute the
4 predominant cells and serve many functions including immune-surveillance of the microenvironment for
5 pathogen invasion, danger signals, cellular debris, apoptotic cells, and alterations in neuronal phenotype
6 [140]. In the young adult brain, normally quiescent microglia become activated in response to a threat
7 [141] and undergo morphological changes, proliferation and production of pro-inflammatory cytokines
8 [140]. Once the threat is resolved, alternatively activated microglia produce anti-inflammatory cytokines in
9 addition to other signaling molecules that facilitate a return to resting state [141]. For example,
10 alternatively activated microglial cells secrete growth factors such as BDNF, which promotes the growth,
11 survival, differentiation of neurons and synapse formation [142]. In contrast, during normal aging, microglia
12 undergo a process of senescence. Senescent microglia, which has been detected in aged rodents, exhibit
13 telomere shortening when cultured [143], dissimilar brain distribution and decreased motility and
14 phagocytic activity [144, 145], smaller dendritic arbors that look more variable in size, less circularly
15 symmetric, and more elongated in shape [146, 147]. Moreover, histo-pathological evidences of aged
16 human brains depict an increase in microglia expression of IL-1 β and CD11b [148] and increased mRNA
17 levels of IL-1 β were found in microglia of aged mice following inflammatory lipopolysaccharide stimulation,
18 suggesting that an exaggerated inflammatory response occurs in healthy aging [149].

19
20 These age-related abnormalities in structure, ramification and therefore function are called “microglia
21 dystrophy” and are indicative of a senescent phenotype [150]. Recently, Bisht et al. coined the name “dark
22 microglia” to identify a new cells phenotype rarely present in the adult brain, marked by condensation of
23 cytoplasm and nucleoplasm, accompanied by endoplasmic reticulum dilation -the most well-characterized
24 sign of oxidative stress- engulfed dendritic spines and axon terminals and nuclear chromatin remodeling
25 [151, 152]. This phenotype becomes abundant not only during pathologic state, such as chronic stress or
26 neurodegenerative diseases, but it was also observed during normal aging [151].

27
28 In summary, even during healthy aging individuals show a progressive increase in neuroinflammation
29 characterized by increased glial activation and elevated steady-state levels of inflammatory cytokines. This
30 altered inflammatory status may contribute to the cognitive decline typical of elderly people as well as to
31 other pathological conditions as well as to a state of enhanced vulnerability.

2.4. Epigenetic modifications

During aging, insults or damages that normally accumulate with age (oxidation, advanced age glycation products, DNA damage, amyloid accumulation etc.) can interfere with normal epigenetic control.

Epigenetics refers to the heritable but reversible modifications on DNA that regulate the transcription of genes. These processes are modulated, throughout the lifespan, by environmental cues that can be “remembered” due to changes in the epigenome [153]. Epigenetic mechanisms include histone modifications, DNA methylation and microRNA (miRNA).

Recently, it has been shown that DNA methylation represents a critical biomarker for aging that may predict the age of human cell type and tissue [154-157]. In particular, DNA methylation status is close to zero in embryonic and iPS and also reflects cell passage number, supporting a strict link between epigenetic and aging process [154]. Moreover, DNA methylation pattern is significantly different between centenarians and newborn [157], with a more homogeneous profile in the latest. In particular, centenarians show loss of DNA methylation in promoters poor of CpG, while hypermethylation was observed in CpG island promoters [157].

Age-associated methylation occurs in a site and tissue-specific manner, with specific hypermethylation for example in human colon or liver [158, 159].

Furthermore, age is also associated with altered expression of DNMT1 and DNMT3, two key enzymes involved in DNA methylation, with a decreased expression of DNMT1 [160-162] and an increase in DNMT3 expression [160]. Regarding histone modifications, the changes in histone methylation and acetylation observed during aging [163] is associated with an unbalance between histone acetyltransferases (HATs) and histone deacetylase (HDACs) with an increase in HDACs, negative regulators of memory acquisition and maintenance [164]. Accordingly, sirtuins (SIRT), which regulates histone acetylation, affect the aging process. For example, in yeast Sir2 regulates longevity and its abundance decline with aging [165] and SIRT-6 deficient mice have premature aging traits [166]. DNA methylation and histone modifications are strictly related since age-related hypomethylated regions are associated with various histone modifications, including H3K27ac, H3K4m1, H3K4m2, H3K4m3 and H3K9ac [167].

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3 Taking together these results suggest that the lasting changes in DNA methylation may represent a form of
4
5 molecular memory, even if it is currently unclear if age-associated changes in epigenetic pattern are merely
6
7 an epiphenomenon or play a casual role in the development of age-related disease.
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11 **3. Role of stressful events**

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13 As previously described, alterations of different mechanisms contributing to synaptic function may occur
14
15 during normal aging. However, when and how the impairment of these mechanisms leads to frailty and its
16
17 pathological consequences is still unknown. We propose that, beside the physiological alteration of these
18
19 molecular mechanisms, a critical impact on the switch from healthy aging to a pathological state may
20
21 derive from the inability to cope with stressful and challenging events.
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25 It is well known that stress represents a critical risk factor for the development of several pathologies,
26
27 including those associated with aging. However, another important issue to consider is that the stress
28
29 impact may have a different grade of severity with respect to the time in which this experience takes place.
30
31 Accordingly, we can speculate that while the stress response in young, healthy individuals is adaptive,
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33 without a health burden, if such stimuli occur in old age or in unhealthy subjects, the outcome may be
34
35 different, resulting in a number of alterations that might favor the insurgence of a pathological condition.
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37
38 In line with this idea, we have previously demonstrated that the expression and function of the
39
40 neurotrophin BDNF are reduced in animals exposed to chronic stress at different stages of life, an effect
41
42 that is associated with pathological phenotype [168-171]. Since BDNF plays a critical role in resilience and a
43
44 reduction of its expression is important for stress-coping strategies [172], the changes observed in aged
45
46 rats may impair resilience thus contributing to functional deficits associated with aging.
47

48
49 Moreover, it is known that the HPA axis is the primary stress system in the body and it is sensitive to both
50
51 acute and chronic stresses thus influencing aging process [173], an effect probably mediated by
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53 glucocorticoids circulating levels [174]. During aging, acute stress can prolong physiological recovery with a
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55 higher peak in cortisol in humans [175], but in rodents a contradictory effects were reported with
56
57 corticosterone response reduced, exaggerated or prolonged in old compared to young rats [81]. On the
58
59 contrary, chronic stress exposure impaired HPA sensitivity, probably by reducing GR concentration in
60

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2
3 hippocampus and hypothalamus [98] thus leading to less functional feedback and high levels of GCs
4
5 circulating [176, 177]. Nevertheless, many older adults exhibit resilience to the HPA axis dysregulation
6
7 [178], by compensating and adapting to the physiological deficits that accompany aging.

8
9 In elderly humans, the higher cortisol level is also associated with increase cognitive decline [116, 173, 179,
10
11 180]. Accordingly, a critical risk factor for the poor cognitive function associated with aging is life-long
12
13 exposure to stressful events [71, 181] and both sustained GC basal levels and stress can drive hippocampal
14
15 neuron loss and associated cognitive decline [182]. Moreover, environmental factors seem to contribute to
16
17 age associated changes in DNA methylation [154, 183-185].

18
19 Indeed, cumulative lifetime stress correlates with accelerated aging brain in humans [186]. In addition, a
20
21 high number of the genes interested by changed in methylations during aging contain a functional
22
23 glucocorticoid responsive elements supporting the possibility that stress-induced acceleration of epigenetic
24
25 aging is mediated by glucocorticoid signaling [186, 187].

26
27 Aging may also affect the inflammatory response to stress exposure. Indeed, the overall increase of pro-
28
29 inflammatory mediators and the general activation of microglia observed after an adverse stimuli, are
30
31 exacerbated when the adverse event occur in old age[188, 189]. For example, it has been reported that the
32
33 activation of peripheral innate immune system induced by lipopolysaccharide injection in 24-month old aged
34
35 mice results in an exaggerated neuroinflammatory response, with a significant up-regulation of the cerebral
36
37 expression of the pro-inflammatory cytokines IL-1 β and IL-6, as compared to 3-6 month old mice [190].
38
39 Interestingly, these changes were associated with behavioral alterations and with a more severe sickness
40
41 behavior syndrome in aged mice [190]. A similar result was found in aged mice following a peripheral
42
43 immune-challenge with *E. coli*, where the inflammatory response was not only exacerbated but also
44
45 prolonged [191]. In addition, it has been demonstrated an over-reactivity of aged microglia when exposed
46
47 *ex-vivo* to lipopolysaccharide[192] as well as *in vivo* after an experimental injury [193], thus leading to
48
49 extended chronic inflammation.
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55 All in all, these observations clearly indicate that the response to stress exposure is affected during aging,
56
57 which may lead to an exacerbated cellular and functional response that may contribute to the switch from
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59 healthy to frailty aging (Fig.1).
60

4. Conclusions

There is no doubt that everyone experiences adverse events during life that impact both health and quality of life. However, trajectories of health in aging can vary significantly depending on the individual.

According to our hypothesis, a reduction of resilience may underlie an increased risk to develop pathologic conditions during aging. In line with this idea, an elder fragility would be the consequence of reduced function and responsiveness of biological systems crucial for coping with stressful events.

It is therefore fundamental to identify and characterize the mechanisms that may contribute to the resilience or the vulnerability to stressful events, since such mechanisms may be significantly altered in the aged brain. However, a major gap in this field of research is that the studies are limited to assess molecular alterations under basal or resting conditions, without information on if and how these functional impairments result in lack of stress responsiveness.

The investigation of these mechanisms will be critical to identify genes and pathways whose changes may contribute to achieve a better understanding of the risk architecture for age-related vulnerability and may represent potential targets for the development of novel pharmacological intervention.

Legend

Figure 1: Normal brain aging is characterized by alterations of neuroplasticity, epigenetic mechanisms, neuroinflammation and HPA axis function. Stress by acting on the same systems, may drive the switch between health and frailty.

List of abbreviation

NMDA (N-methyl D-Aspartate); GABA (γ -aminobutyric acid); GAD65 (Glutamic Acid Decarboxylase); hypothalamic–pituitary–adrenal (HPA); PFC (prefrontal cortex); NTFs (neurotrophic factors); BDNF (Brain-derived neurotrophic factor); NT-3 (neurotrophin-3); NT-4 (neurotrophin-4); LC (locus coeruleus); Val (valine); Met (methionine); GCs (glucocorticoids); CRH (corticotrophin releasing hormone); VP (vasopressin); ACTH (adrenocorticotrophic hormone); MR (mineralocorticoid); GR (glucocorticoid); GRE (glucocorticoid responsive elements); 11 β HSD (11- beta hydroxyl steroid dehydrogenases); BBB (blood-brain barrier); CNS (central nervous system); ROS (reactive oxygen species); HATs (histone acetyltransferases); HDACs (histone deacetylase); SIRT (sirtuins)

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