Brain ageing in the new millennium

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Objective: This paper examines the current literature pertaining to brain ageing. The objective of this review is to provide an overview of the effects of ageing on brain structure and function and to examine possible mediators of these changes.
Methods: A MEDLINE search was conducted for each area of interest. A selective review was undertaken of relevant articles.
Results: Although fundamental changes in fluid intellectual abilities occur with age, global cognitive decline is not a hallmark of the ageing process. Decline in fluid intellectual ability is paralleled by regionally specific age related changes apparent from both structural and functional neuroimaging studies. The histopathological mediators of these changes do not appear to be reduction in neuronal number, which, with the exception of selected hippocampal regions, remain relatively stable across age. At the molecular level, several mechanisms of age related change have been postulated. Such theoretical models await refinement and may eventually provide a basis for therapy designed to reduce effects of the ageing process. The role of possible protective factors such as 'brain reserve', neuroprotective agents and hormonal factors in modifying individual vulnerability to the ageing process has been the focus of a limited number of studies.
Conclusion: Our understanding of the functional and structural changes associated with both healthy and pathological ageing is rapidly gaining in sophistication and complexity. An awareness of the fundamental biological substrates underpinning the ageing process will allow improved insights into vulnerability to neuropsychiatric disease associated with advancing age.
Key words: brain ageing, cognition, dementia, neuroimaging.

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The ageing brain undergoes biochemical, molecular, structural and functional changes, rendering it vulnerable to a range of neuropsychiatric disorders. Until recently, little has been understood regarding the nature of these changes, the factors promoting them and their relationship to mental health. With the rapid growth in the proportion of old and very old in our community, the need for a comprehensive understanding of brain ageing has taken on a sense of urgency. Recent advances in the neurosciences have allowed some insights into this ageing process, together with research into age associated diseases such as Alzheimer's disease (AD). Although the changes associated with brain ageing are complex and multifaceted, a clearer understanding of this process is now possible. It is hoped that further advancement in our knowledge of the mechanisms of brain ageing may lead to interventions designed to slow the ageing process, thus reducing the burden of neuropsychiatric disorder in our old and very old populations. Our review aims to summarize the literature pertaining to brain ageing from the molecular to the functional levels.

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Cognitive ageing

Almost half a century ago, Cattel [1] and Horn [2] introduced an influential distinction in intelligence theory, that between fluid and crystallised factors. Crystallised intelligence refers to our accumulated knowledge base through exposure to education, culture and information. Fluid ability was originally envisaged to be a type of 'intellectual potential', dependent upon the growth and integrity of the central nervous system. It is now defined more functionally, that is, those cognitive faculties involved in the solution of any type of new problem [3]. These factors change in markedly different ways with advancing age. Cross-sectional and longitudinal studies have found that fluid ability begins to decline by the age of 50 [4]. Speed of information processing, working memory and complex attention are particularly affected [5]. Crystallised intellectual performance, in contrast, is either stable or shows only minimal decline even up to the eighth decade of life [4,6–8].

Cognitive decline beyond the normative pattern discussed above has been the subject of much interest for its possible relationship to incipient AD. One of the first such syndromes to be defined was age associated memory impairment [9,10], which emphasizes subjective memory complaints in otherwise healthy elderly. In practice this has had the problem of little predictive specificity. Mild cognitive impairment (MCI), a more recently introduced concept, is characterized by memory complaints in a nondemented and otherwise healthy older person and corroborated by a knowledgeable informant. Memory should be affected beyond that of age- and education-matched controls, but not so far as to interfere with normal activities of daily living [11]. Mild cognitive impairment progresses to AD in 10–15% of individuals per annum compared with 1–2% in appropriate controls [12].

While it is important to appreciate the limitations in cognitive processing that are implicated in human ageing, it is equally pertinent to remember that older people have access to a unique repository of cultural, historical and personal information. Baltes *et al*. [13] operationalized a definition of 'wisdom', combining ratings of flexibility of perspective, compassion, decision-making under conditions of uncertainty and life-span knowledge. The Berlin model of wisdom was then transformed into a scenario-based questionnaire and administered to a wide sample of the community. Older individuals did not diminish in 'wise' cognitive performance compared with younger people, and in fact were significantly superior in some areas.

Structural brain changes in ageing

Examination of the structural brain alterations that occur with age may assist in understanding age-related functional changes and also propensity to neurodegeneration. A potential confounding issue, however, is identification of 'healthy' aged subjects, as there is a risk of inclusion of those with preclinical stages of diseases such as AD. This can be minimized by careful entry criteria, but of course not entirely prevented. This remains an issue of ongoing relevance to brain ageing research. Age-related structural brain changes from volumetric magnetic resonance imaging (MRI) studies are summarized in Table 1.

In vivo imaging

Atrophy

Reviews of computed tomography [14] and MRI [15] changes with age have been recently published. The field is conspicuous for its paucity of longitudinal imaging studies in normal individuals. Whole brain analysis has revealed an increase in lateral ventricular size (central atrophy) and an increase in sulcal volume (cortical atrophy). These two types of atrophic changes are relatively independent [16]. Volumetric MRI studies concur on an approximate correlation between age and cerebral volume of $r = -0.40$ [17]. Interpretation is complicated by methodological issues such as controlling for cranial size, sociodemographic background and cohort effects. The pathological basis for these atrophic changes is now the subject of a lively debate. This may account for the inconclusive attempts to link whole-brain size and intelligence in the normal elderly when appropriate corrections have been made [18].

Regional brain changes

Neocortical region: The finding of preferential shrinkage of the frontal lobe in normal individuals with age has been well replicated [19,20]. Studies have also shown that this loss of volume is greater in the grey matter ($r = -0.57$) than white matter ($r = -0.31$) [17]. Prefrontal atrophy is about twice that found in the temporal or parietal neocortex [21]. A phylo- and onto-genetic hypothesis has been proposed in which those brain structures that are evolutionarily and developmentally younger are more susceptible to age-related change [17]. It is tempting to speculate that such selective prefrontal volumetric change may underlie the generalized decline in higher-order cognitive abilities noted earlier. However, attempts to link the two have been disappointing [22,23]. This

Table 1. Average Pearson's product-moment statistic (r) for volumes of different brain regions on MRI, showing correlation with age in cross-sectional studies of normal elderly

Brain Region	Correlation	Consistent findings?
Whole Brain Atrophy	–0.40	Y
Limbic		
Hippocampus	–0.31	N
Entorhinal cortex	–0.09	Y
Amygdala	–0.26	N
Striata		
Globus pallidus	–0.14	Y
Putamen	–0.44	Y
Caudate	–0.47	Y
Thalamus	–0.17	Y
Neocortical		
Prefrontal	–0.47	Y
Temporal	–0.27	Y
Parietal	–0.29	Y
Mid-Brain	–0.42	Y
Brainstem	–0.07	Y
Cerebellum	–0.29	N
White Matter Hyperintensities	0.37	Y

Values are adjusted for head size. Data adapted from Raz's (2000) [17] substantive review of the field.

result is also of interest because longitudinal studies suggest that accelerated temporal lobe volume decline is a better predictor of early AD than prefrontal atrophy [24,25]. The dissociation between physiological brain shrinkage in the frontal lobe and accelerated pathological change in the temporal lobe is likely to be a focus of study over the next few years.

Limbic areas: The hippocampus has been studied in more than 16 studies comparing young adults with older individuals [17]. Estimates of volumetric shrinkage with age vary with correlations of r = –0.12 to r = –0.63 between age and hippocampal volume when adjusting for skull size. These results parallel the wide variance in results from neuropathological studies (see below). This variability may in part be attributed to difficulty in accurate separation of the hippocampus from other surrounding structures [26]. ApoE4 effects on hippocampal volume in normal older persons are currently unclear [27,28]. Nevertheless, hippocampal degeneration is certain to be implicated in age-related decline in declarative memory, new learning and spatial navigation skills [29–31]. Other limbic structures show an ad hoc pattern of age-related change. For example, whereas the entorhinal cortex is the first to show volumetric change in MCI [11] and AD [24,32], studies of normal ageing show little if any change [33]. The amygdala may be affected by ageing as indicated from results in the few studies to date [34,35].

Striatum areas: Age effects have been reliably measured as being moderate on both the caudate and putamen nuclei (r = –0.46 [36]). Dopaminergic regulation of motor skills [37] and stimulus salience [38] may suffer in line with age-related neostriatal volume decline. The globus pallidus and thalamus are only minimally affected [17].

Primitive structures: The cerebellum has been studied in a series of focused studies. These point to a moderate decline in volume (r = –0.29 [17]), althoughthe pattern of alcohol usage is likely to be a confounding factor [17]. Midbrain structures such as locus coeruleus and substantia nigra show marked sensitivity to age-related volume losses (r = –0.42 [39,40]). The volume of the caudal brainstem is unaffected by age [41].

Subcortical white matter: The advent of T2-weighted magnetic resonance imaging techniques that highlight areas of increased water-molecule motility, such as Fluid Attenuated Inversion Recovery (FLAIR) [42], has allowed *in vivo* measurement of white matter change. On MRI these changes show as white matter hyperintensities (WMHs). The pathological basis for these changes is multifarious and still being investigated [43]. Up to 30% of normal elderly demonstrate significant areas of WMH [44], most often in the subcortical frontal regions such as periventricular area and internal capsule. Correlations between extent of WMH in the frontal lobe and age are moderate [45]. Our own research has shown that changes to these myelinated fibres are inversely correlated to performance in executive tasks (r = –0.68 [46]), corroborating earlier findings [47,48]. Speed of information processing seems to be particularly affected in individuals with high levels of WMHs [48].

Post-mortem studies

Neuropathological studies have for over 100 years confirmed the modest but reliable diminution of the brain with advancing age (for review see Kemper [49]). These studies reveal an approximate 5% reduction in brain weight and volume per decade after 40 years of age. A pattern has emerged of invulnerable areas that contrast with pathology-susceptible brain regions and circuits.

Demylineation

Post-mortem analyses highlight a greater loss of white than grey matter [50,51]. Granular degeneration of myelinated axons is observed regularly by the age of 40 years and is claimed to be present in all examined brains of older individuals [52]. This process is at least in part related to WMHs seen on MRI [43] and is likely to reduce axonal conductance speed. This process may potentially contribute to motor and cognitive slowing [53] that has been suggested as fundamental to age-related cognitive decline [54].

Senile plaques

Senile plaques (SPs) are spherical, multicellular lesions that show considerable heterogeneity under the microscope (for a review see Dickson [52]). These plaques are composed of extra-cellular fibrillar and non-fibrillar β-amyloid protein (Aβ). Aβ deposits are typically located surrounding, and surrounded by, degenerating axons and dendrites. Neural cell death from Aβ invasion is thought to be an excito-toxic event mediated by immunological activation of microglia that also inhabit the SP region [55–57]. When SPs do occur in the normal aged, they tend to accumulate in the association cortices, are moderately dense in the visual and auditory cortex and seem to spare the primary sensory and motor cortices [49,58].

The pathogenesis of SP formation in AD is an active area of research [55,59], yet its relationship to the SPs found in normal ageing is less clear. The existence of two significant types of SPs has been proposed [52]: dense SPs that are the hallmark of AD [60], and pale diffuse plaques which can be abundant in older people showing no signs of dementia. Diffuse plaques are thought to be generated by a separate biological process involving cleavage of amyloid precursor protein into shorter amyloid subunits resulting in deposits that are less neurotoxic than dense SPs [61].

The prevalence of SPs (whether diffuse or dense) in the normal older brain is still controversial. Some studies suggest a relative infrequency in brains of older people who died without signs of dementia [51,61,62]. Others support the notion that SPs increase with age [63], and studies of the very old suggest that these changes may be inevitable if one lives to be old enough [64]. Further investigations of this sort are required, particularly in the very old, that distinguish between short-length amyloid deposits and dense Aβ plaques. Complicating matters is a subset of aged individuals whose brains demonstrate extensive cerebral amyloidosis, often exceeding criteria for AD, but whose cognitive performance equates to that of individuals without any SPs whatsoever [65–67]. The relationship between this form of 'pathological ageing' to amyloid typology and preclinical AD status is as yet largely unstudied.

Neurofibrillary tangles

Neurofibrillary tangles (NFTs) are intraneuronal inclusions composed mainly of aggregates of paired helical filaments (PHFs). Biochemical studies of PHFs have shown that they are primarily made up of the tau protein. Tau promotes stabilization of cellular microtubles, an ability that decreases when it is phosphorylated. Hyperphosphorylated tau spontaneously self assembles to form PHFs (for a review of see [68]).

In the normal elderly, NFTs are generally restricted to only a few select brain regions, where they are almost always found [52]. These regions are in the entorhinal cortex, basal nucleus of Meynert and locus ceruleus [62,69]. Hippocampal involvement is unpredictable [52]. In nondemented people, NFTs do not progress to inhabit cortical neurones. By contrast, NFTs and abnormally phosphorylated tau are widespread throughout almost all cortical areas in AD individuals [52]. At the cellular level, NFTs are often found in the neural processes (neuropil threads) in AD but almost never in the normal elderly [61].

Cerebrovascular change

Neurones are one of the most energy and nutrient hungry cells in the human body [70]. Capillaries are more densely packed in brain areas with higher processing demands [71] and their density tends to fall in most parts of the brain with age [72]. It is therefore of vital interest to understand the changes that occur in the brain's vascular system with normal ageing.

For each decade of life, beginning at 50 years of age, the degree and number of microvessel deformities increase [73]. Kinking and spiraling of small vessels has been known to be more prevalent in demented brains than in age-matched controls [74]. At the ultrastructural level, tortuosity is related to increased basement membrane

thickening in capillaries, which can leave vessels susceptible to endothelial and luminal compression, brain-barrier leakage and endothelial-mitochondrial depletion [75]. Neuronal dysfunction may therefore occur because facilitative transport of glucose and passive diffusion of oxygen across the capillary wall is disrupted [76].

Cerebroarterial change begins mostly in the intima. In the fourth decade, about 50% of vessels show intimal thickening and by the eighth decade it is seen in about 80% of all vessels studied [77]. The middle cerebral artery territories seem particularly affected and cerebellar and brainstem arteries relatively spared [78]. These changes are often the precursors to arteriosclerosis which increases vascular resistance and decreases perfusion pressure, thereby compromising neurocognitive function. Careful treatment of the classic cardiac risk factors such as physical inactivity, diabetes, hypertension, hyperlipidemia, hyperhomocysteinemia and cigarette smoking can reduce the likelihood that benign arterial damage develops into a cerebrovascular event (see [79]).

In general, ageing seems to move towards a gradual decrease in caloric (glucose) and substrate (oxygen) supply. Ischaemia may therefore be the predominant reason for white matter hyperintensities on MRI [43], although other processes are no doubt involved. A critical question to ask is: does interruption of blood supply lead to neural loss or does neural dysfunction cause decreased vascular demand and so vessel attrition? Kuchinsky and Paulson [80] found that degeneration of neurones is never followed by capillary loss supplying these neurones. Experimental occlusion of a single capillary, in contrast, caused neurones in close proximity to degenerate, and those supplied by patent capillaries showed no change [81,82]. In addition, nondemented hypertensive individuals have been found to have higher levels of NFTs than nonhypertensive matched controls [83]. The possibility of a synergistic relationship between vascular and neuronal factors in ageing and AD is an exciting area of research (for a review see [84]).

Neural depletion?

Given the growing list of structural changes witnessed in the ageing brain, it may seem reasonable to presume a result of neural loss. This idea has for many years underpinned theories of cognitive and neurological age-related decline and is a critical assumption in brain ageing hypotheses that invoke neuronal apoptosis [85–87]. Surprisingly, modern stereological methods of neurone counting [88,89]; which avoid biases inherent in earlier techniques, suggest that these assumptions may have been quite inaccurate [90].

An excellent review by Long *et al*. [91] of stereological studies of neuronal cell number is summarized in Table 2. Three points are immediately appreciable. First, there is a paucity of nonbiased assessments of neural numbers in the human. Second, with the exception of the human hippocampus, studies so far indicate no clear evidence of age-related neuronal loss. Third, in the limited number of human hippocampus studies, there appears to be regional selectivity. Some studies have even shown an absence of neuronal depletion in those ageing rats with the worst cognitive profiles [92]. Further studies are obviously needed, particularly in cognitively characterized individuals, but initial results point towards a conservation of neural numbers with age [93].

While the studies of neuronal numbers are numerous, fewer studies have examined the morphology and integrity of the ageing neurones. There is suggestion of reduced synaptic density [94] with age, but little is known about the effects on axons [95] and glial cells [96]. The demonstration of neurogenesis in the mouse hippocampus in response to cognitive stimulation in both young [97] and old [98] animals has challenged the long-held views of an absence of new nerve cell genesis in mature animals. This capacity of hippocampal neurones to replicate has been shown in humans as well [99]. The age of individual neurones may well be as important as their presence or absence.

Functional neuroimaging in ageing

Dementia and 'at risk' groups

Functional imaging techniques such as single photon computed emission tomography (SPECT) and positron emission tomography (PET) have been used extensively to evaluate AD patients. A distinctive pattern of blood flow and metabolic alterations have been noted in AD, consisting of bilateral or unilateral temporoparietal deficits early in the disease with later involvement of frontal regions [100–104]. A number of studies have established an association between areas of hypoperfusion and performance on neuropsychological tests reflective of local dysfunction [105–107]. Furthermore, these SPECT and PET abnormalities have been shown to correlate with rates of subsequent cognitive decline [108,109]. Deficits in relative cerebral metabolic rate of glucose utilization (rCMRgl) that are similar but less severe than those seen in AD have been observed in cognitively intact individuals from familial AD pedigrees [110]. Similar findings have been demonstrated in cognitively normal subjects with a family history of AD who were either heterozygous [111] or homozygous [27] for the apolipoprotein E4 genotype. Significant

Table 2. Summary of results from stereological studies of neural numbers during ageing in different mammalian species

Species	Brain Region	Number of studies showing no change with age	Number of studies showing decline with age
Human	Cortex	1	0
	Brain stem	2	0
	Hippocampus		
	CA1	1	1
	CA2/3	2	0
	Hilus	1	1
	Subiculum	0	2
Monkey	Striate	1	0
	Entorhinal Cortex	1	0
Rat	Hippocampus	2	0
Mouse	Hippocampus	2	0

Data adapted from Long *et al.* (1999) [91].

temporo-parietal abnormalities have been noted with SPECT in those with mild cognitive impairment (MCI) [112,113]. Although a large portion of such subjects may be expected to go on to develop AD, the positive predictive value of resting SPECT in MCI is at present questionable [113].

Healthy ageing

Functional imaging techniques have also been used extensively to evaluate the healthy ageing brain at rest. Early relative cerebral metabolic rate of oxygen consumption (rCMRO2) [114] and [^{18}F]-deoxyglucose (rCMRg) [115] studies suggested a generalized decrease in both cerebral blood flow and metabolism in grey matter, with preservation of blood flow in white matter. Some studies have also supported the notion of some regionally specific decrease in $rCMRO_2$ [116,117] and rCMRgl [115] in cortical association areas, particularly frontal lobes [118]. Such findings could reflect regionally specific pathological effects of the ageing process [116]. However, not all studies have demonstrated global or regional decreases in rCMO2 or rCMRglu with age [119,120]. Possible explanations for inconsistency between studies include: differences in scanning procedures; variability in definition and screening of 'healthy aged' subjects; differences in scanning equipment and image processing; and failure to take account of confounding factors such as the partial volume effects of cerebral atrophy. More recently, a magnetic resonance imaging based correction method for partial volume effects has been applied to PET data [121]. This study demonstrated that most age-associated changes in cerebral blood flow failed to reach significance after the correction process was applied. Such findings challenge the notion of a large age specific decrease in brain blood flow and metabolism, and indicate the importance of correction for possible confounding factors.

Functional imaging studies of normal cognitive function, particularly during memory tasks, indicate differences between aged and younger subjects (for a review see [122]). During retrieval tasks, for example, the aged brain appears to activate more widespread networks, with more bilateral representation of activation during recall of verbal material [123,124]. Such effects have been interpreted as reflecting an underlying inefficiency, resulting in recruitment of additional networks. This inefficiency may be reflective of the net effects of the cellular and neurotransmitter changes occurring with the ageing process.

Magnetic resonance spectroscopy

Proton magnetic spectroscopy (1H-MRS) is a tool with immense potential in the study of brain ageing. This non-invasive technique allows *in vivo* estimations of specific brain chemical profiles, which can be compared and contrasted across a variety of neuropsychiatric disorders. 1H-MRS can detect *N*-acetyl containing compounds such as *N*-acetylaspartate (NAA), which is a putative marker of neuronal viability and density. Measurement of choline containing compounds (Cho) (such as free choline, phosphocholine and glycerophosphocholine)

and creatine/phosphocreatine (Cr) allows assessment of membrane synthesis and metabolism. Other metabolites such as myoinositol (mI) are considered possible glial cell or intracellular toxin markers. It is also possible to determine spectra for neurotransmitters such as glutamate and GABA.

Application of 1H-MRS to the study of brain ageing is in its infancy. A series of 1H-MRS studies report post-adolescent biochemical profiles that provide *in vivo* evidence of a brain maturation process which is completed by young adulthood and then regresses from middle age [125,126]. This maturation process follows a chronological sequence in which the frontal lobes are last to mature and first to regress. Contrasts have been reported between young and older adult subjects including an age related decrease in NAA, Cho and Cr in a variety of brain regions [127–129]. Some studies have not replicated the decrease in NAA [127,130] or other metabolites [131] in older subjects. More recent studies have demonstrated region-specific decreases in prefrontal and sensorimotor cortex across a multichemical profile in middle-aged adults compared with young adults [132], and medial and lateral temporal lobe NAA deficits in older adults [133]. Other areas implicated in age-related changes include the hippocampus, where a decreasing NAA/Cr and NAA/Cho ratio with age has been interpreted as reflecting neuronal loss in this region [134]. A recent study of healthy older subjects reported from our group [46], demonstrated a region-specific correlation of frontal white matter NAA concentration with neuropsychological measures of executive and attentional abilities. Such a finding suggests a role for 1H-MRS as a probe for evaluating regional integrity of the brain and may allow exploration of the mechanisms responsible for the disparate effects of the ageing process. Longitudinal MRS studies of human ageing are yet to be accomplished and are of high importance.

The chemical profile in normal ageing has also been compared with AD in some preliminary studies (for a review see [135]). Findings reflecting neuronal degeneration have included reduction in NAA levels and increase in mI [9,136–138]. These findings are independent of regional atrophy and correlate with dementia severity [136]. A small study using a volume of interest selected from the temporoparietal area reported that subjects with age-associated memory impairment had NAA levels between that of healthy aged subjects and AD [9].

In summary, 1H-MRS has already shown promise as a tool in the evaluation of normal and pathological ageing. The *in vivo* monitoring of biochemical markers with 1H-MRS is likely to substantially improve our understanding of both brain ageing and age related pathological processes.

Molecular changes

In the human brain, a number of molecular mechanisms of ageing have been proposed, and a number of neurotransmitter alterations suggested. Although no single mechanism adequately explains the process of brain ageing, a number of theories have received significant experimental support and are presented below. These have been divided into molecular theories of relevance to ageing and neurotransmitter changes of ageing.

Molecular theories of ageing

Oxidation and free radicals

One of the most prominent theories asserts that brain ageing results from the generation of reactive oxygen and nitrogen species which occurs in the course of normal cellular metabolism. The production of these so called free radicals is thought to cause damage to critical lipid, protein and DNA components of cells, leading to neuronal dysfunction and eventually cell death. This theory has been used to explain cellular damage in healthy ageing and has also been integrated into models of AD [139]. In AD, free radicals associated with beta-amyloid (Aβ), the central component of senile plaques, are proposed as a key factor in disruption of neuronal membrane integrity. The resultant alteration in ion homeostasis, including calcium, has been proposed as the mechanism of accelerated cell death [139].

Calcium

The calcium hypothesis of brain ageing states that changes in calcium homeostasis occur with age, leading to sustained changes in intracellular calcium levels and cell death [140]. Calcium is known to participate in a variety of cellular functions including maintenance of membrane excitability, regulation of neuronal metabolism and neurotransmitter synthesis and release [141]. Alterations in calcium regulation may occur with age at a variety of levels (for a review see[140]). These may include: changes in calcium influx via membrane based calcium channels; dysregulation of intracellular stores via malfunction of intracellular calcium channels in the endoplasmic reticulum and alteration in intracellular cytosolic buffers; and impaired efficiency of calcium clearance as a result of impaired extrusion into extracellular space and reduced reaccumulation into endoplasmic reticulum. Indirect measurements of intracellular calcium in the aged rat neurones suggest a modest increase in resting intracellular calcium and prolonged recovery time after excitation [140].

Further suggestion of calcium's role in cellular ageing has been obtained from studies of calcium antagonists. These drugs have been associated with reduction in cell death in models of ischaemic stroke [141] and improved learning in rats and aged primates [142]. However, this strategy has only been minimally successful in AD [143]. Significant questions remain unanswered concerning the primacy of calcium dysregulation in both healthy ageing and pathological processes [140].

Gene expression

Molecular genetic studies are now investigating the role of genetic expression in mediation of the ageing process. Preliminary *in vitro* and animal evidence suggests that altered genetic expression does occur with advancing age, and that such alterations may play an important role in modulating vulnerability to oxidative stress, inflammatory responses and regulation of DNA repair. Recent studies have utilized high-density oligonucleotide array techniques to compare expression of numerous genes across age groups in mice [144,145]. Comparing adult with aged mice, Lee *et al*. [144] demonstrated both increases and decreases of gene expression occurring in neocortex and cerebellum with age. Of those genes which demonstrated increased expression in neocortex, a high proportion were genes considered to be involved in inflammatory and stress responses (20% and 24%, respectively). Of those genes showing down regulation, 11% were considered to be linked to neuronal plasticity and central nervous system development. Caloric restriction in the mice, a measure previously demonstrated to slow the ageing process [146], attenuated these age-related alterations in gene expression. Similarly, Jiang *et al*. [145] demonstrated alteration in expression of a number of genes involved in neuronal integrity and signalling in the hypothalamus and cortex of aged mice. They hypothesize that altered protein synthesis arising from these genetic changes could contribute to some aspects of brain ageing and vulnerability to age related diseases.

In addition to these general changes in protein synthesis, an awareness of specific alterations to expression of genes relevant to the ageing process is emerging. Quantification of the mRNA for proteins such as 5-Lipoxygenase (5-LOX), a key enzyme in the synthesis of leukotrienes, has suggested an age-related reduction in rats [147]. Such a finding suggests one of many possible mechanisms by which an enhanced inflammatory response could occur with age, leading to neural degeneration. Genetic controls over mediators of neural protection have also received some preliminary attention. Oxidative insults inflicted on cell culture have been shown to be reduced by expression of Bcl-2 [148], a protein known to be involved in regulation of programmed cell death. Up-regulation of this protein is also seen in AD, suggesting that this may represent a compensatory or adaptive response to enhance neuronal viability [148]. Such findings are preliminary and the relevance to understanding of the pathophysiology of ageing in humans is uncertain. However, the understanding of the molecular aspects of the ageing process rests with future research in these areas.

Mitochondrial dysfunction

Decline in mitochondrial function has been proposed as another key factor in the ageing process and neurological disease [149]. Advancing age and reduction in the mitochondrial process of oxidative phosphorylation are thought to promote mutations in the mitochondrial genome [150,151]. This process is thought to result in accelerated generation of reactive oxygen species, promoting further damage [151]. Results from studies of mitochondrial function in human tissue are beginning to be reported. A recent postmortem study of human brain tissue [152] documented reduction in mitochondrial respiratory enzyme activity associated with increasing age but noted considerable variability in this finding amongst aged subjects. The authors proposed that greater reduction in efficiency of oxidative phosphorylation in some aged individuals may predispose to central nervous system dysfunction. Similarly, there are reports of down regulation of oxidative phosphorylation in AD [153].

Glucocorticoids

Converging evidence implicates the role of adrenal secretion of glucocorticoids in promotion of vulnerability to ageing (for a review see [154]). The central focus of this theory has been the effects of glucocorticoids on the hippocampus. Hippocampal-dependent learning and memory is mediated by a process of strengthening synaptic communications, known as long-term potentiation (LTP). The effect of glucocorticoids on this process is represented by an inverted 'U', with glucocorticoid insufficiency and excess both inhibiting LTP. Studies of the effects of glucocorticoids have indicated adverse effects on memory in endogenous excess states such as Cushing's disease [155], and with administered glucocorticoids [156]. Ageing subjects with elevated and increasing basal cortisol levels over a four year period demonstrate a reduction in hippocampal volumes and memory deficits [157,158]. Another worsening study suggests at least a possibility of a reversible effect on cognitive performance. In this study, aged women with

elevated baseline urinary cortisol excretion demonstrated poorer memory function. Increases in urinary cortisol secretion over a 2.5-year follow up were associated with declines in memory performance, whereas women with decreases in urinary cortisol secretion demonstrated improvements in memory performance [159]. Although a causal association has not yet been established between cognitive decline and glucocorticoids, it is important to consider how such an effect could be mediated at a cellular level.

Possible effects of glucocorticoids on hippocampal neurones may include inhibition of neurogenesis in the dentate gyrus, atrophy of dendritic processes, reduced ability to survive neurological insults such as ischaemia or hypoxia and direct neurotoxic effects [154]. At a molecular level these effects may be mediated by glucocorticoid inhibition of glucose utilization, and impairment of the cells natural defence or buffer systems (e.g. worsening of the calcium dysregulation during cellular damage) [154]. The impairment of these molecular and cellular events by glucocorticoids have yet to be demonstrated in ageing human brains. In conclusion, the understanding of the effects of elevated glucocorticoids on human ageing and cognition is in its infancy, with initial observations supporting an association with cognitive dysfunction which could be either a primary or secondary phenomenon. There may be a role for excess glucocorticoids in enhancing cellular vulnerability to age related insults and hence cognitive change.

Homocysteine

Elevated levels of the plasma based amino acid homocysteine have been shown to be a risk factor for age associated diseases such as cardiovascular disease, atherosclerotic stroke and peripheral vascular disease. With particular relevance to brain ageing, elevated homocysteine levels have been associated with an increased risk of stroke in large prospective studies [160,161] and with precursors of stroke such as carotid artery stenosis [161] as well as intimal thickening and plaque formation [162]. As vitamins B12, B6 and folate are cofactors in homocysteine metabolic pathways, their deficiency is associated with elevated levels of homocysteine [163]. Our group has found an independent correlation between homocysteine levels and cerebral ventricular dilation in normal healthy elderly [230]. Studies of cognition and homocysteine levels in the elderly suggest an association between elevated homocysteine and reduced cognitive performance (for a review see [164]), but this finding is by no means a universal one [165]. It remains unclear whether any effect is mediated by the vitamin deficiency, elevated homocysteine levels or other factors. A relationship between AD and homocysteine levels, possibly mediated by reduced folate and B12 levels, has also been proposed and received some support [166,167]. The mechanism by which elevated homocysteine mediates such effects is unclear, but may relate to promotion of further generation of free radicals.

Neurotransmitter correlates of ageing

Acetylcholine

A neurochemical hypothesis has been proposed in which brain ageing is related to changes in cerebral neurotransmission. The initial focus in this area has been on cholinergic neurotransmission. Ancient Hindus, American Indians and Africans demonstrated the importance of the cholinergic system for higher cognitive function by the use of belladonna-containing plants. Early pharmacological experiments administering cholinergic antagonists such as atropine to rats [168,169] and scopolamine to primates [170], confirmed that they disrupt learning and memory. Of interest was the observation that young primates given scopolamine performed like old monkeys on cognitive tasks [170]. Further investigations revealed that specific muscarinic (M1) antagonists injected *in vivo* into rat hippocampus [171] and cerebral ventricles [172] resulted in impairment of memory. The importance of basal forebrain cholinergic projections was demonstrated by Flicker *et al*. [173] who found that destruction of cholinergic neurones in this region of the rat brain led to impairment of memory. In addition, Sastry *et al*. [174] documented reduced acetylcholine synthesizing capacity in brains of aged rodents, and Lippa *et al*. [175] demonstrated reduced responsiveness of aged rat hippocampal pyramidal cells to acetylcholine. This body of animal literature forms the basis for considering a prominent role for cholinergic systems in learning and memory, and suggests that disruption to the cholinergic system could be one possible mediating factor in age-related cognitive change in humans.

The relevance of the cholinergic hypothesis of human memory function has been demonstrated recently by the modest benefit obtained through the treatment of AD with cholinesterase inhibitors. Alterations to cholinergic neurotransmission occurring in AD include: decrease in choline acetyltransferase (CAT), the severity of which correlates with the severity of cognitive function and density of plaques and tangles [176]; reduced levels of acetyl cholinesterase [177]; and reduced capacity for acetylcholine synthesis [178]. Could similar but less severe changes explain reduction in cognitive performance in healthy ageing? The ageing brain is characterized by minimal change in acetylcholine synthesis, minimal

alteration to acetylcholinesterase activity, modest reduction in choline acetyltransferase, reduced acetylcholine release following neural stimulation and reduced sensitivity of autoreceptors [179]. Furthermore, PET studies using muscarinic ligands have also indicated an age associated reduction in muscarinic receptors [180,181]. Overall, the modest alteration in cholinergic neurotransmission observed with age stands in some contrast to the dramatic reduction in cholinergic integrity seen in AD. In isolation, these changes do not appear to adequately explain loss of cognitive function in normal ageing. Although MCI is often a forerunner of AD, cholinergic integrity in MCI has not been investigated. If deficits of an intermediate nature between healthy ageing and AD were found, this would have theoretical implications for the understanding of MCI and implications for possible early intervention with cholinergic drugs.

Dopamine

Although the cholinergic hypothesis is central to the AD story, alterations to neurotransmission occur in a variety of systems in the ageing brain. Dopaminergic changes have been suggested as the most reliable age-related change in the human [182]. Positron emission tomography studies have pointed to significant alteration in dopamine neurotransmission with age, including reduction in density of striatal D2 receptors [183], decreased striatal D1 receptors [184], and reductions in striatal dopamine transporters [185]. Reduction in D2 binding has been shown to correlate with age-related decrements in motor tasks (e.g. finger tapping) and neuropsychological tests of frontal lobe function [186]. A more recent study demonstrated correlations between the age-related reduction in striatal D2 binding and reduction of metabolism in frontal and anterior cingulate regions [187].

Other neurotransmitters

Ligand studies indicate a widespread reduction in the number of serotonin (5-HT2A) receptors with age over many cortical areas using PET [188]. Glutamatergic changes have also been noted (for a comprehensive review of these changes, see [182]).

In summary, the healthy ageing brain is associated with modest changes in a variety of neurotransmitter systems, which are likely to be implicated with age-related decrements in cognitive function. Ageing thus stands in contrast to the relatively selective early loss of cholinergic integrity of AD. The neurotransmitter changes in particular groups such as MCI await evaluation.

Protective factors in brain ageing

Inter-individual differences in susceptibility to brain ageing has led to the exploration of a number of protective factors. In this section we review the concept of brain reserve, the role of antioxidants and neuroprotective agents and hormonal protective factors.

Brain reserve

Brain reserve has acquired distinct meanings which stem from the observation that there is no necessarily direct correspondence between neuropathology and clinical expression [189]. Evidence for this comes from neuropathological studies of nondemented elderly, which have found that anywhere between 10 and 67% of such individuals fit Khachaturian histological criteria for AD (see [190]). If these people 'should' be demented, what is protecting them? One clue was provided by Katzman *et al*. [67] who found that these individuals had greater numbers of large neurones in the cortices and higher overall brain weight. One explanation for this is that they started off in life with a bigger brain. By extrapolation, such people should have a larger intracranial volume to the norm at any point in time. A number of studies have shown that a larger intracranial volume, or even head circumference, is an independent protective factor against expression of AD [191–194]. Increased neural redundancy has been suggested as the main mechanism of protection in this scenario [195].

Another clue provided by the same study [67] was that those with AD pathology, but no antemortem dementia, outscored completely disease-free controls in most neuropsychological tests. Rather than maximum brain size or neural number, this finding indicated that overall cognitive ability was a more relevant mediator of dementia expression. Under this proposal (sometimes called cognitive reserve), those with greater abilities succeed because of increased cognitive resources and strategies which allow for better coping at any disease load. A number of community studies have shown that higher education levels lead to a lowered risk for dementia or cognitive decline [196–200]. Longitudinal studies [65,201] and research using alternative reserve estimates such as occupation status [202], mental activity ratings [203,204] or premorbid neuropsychological instruments [205,206], also support this idea. Neuroimaging studies have found that if one controls for cognitive status, people with AD and higher cognitive reserve show more significant temporoparietal perfusion deficits than those with lower cognitive reserve [207,208]. By corollary, a history of more complex mental activity may allow elderly to minimize the effects of high disease burdens.

Another interpretation of the brain reserve idea is that it may retard or attenuate brain ageing or pathology per se [209]. Limited evidence for this idea is provided by the 'Nun study' [210], which involved older nuns whose autobiographies from early life were available (some 60 years ago) and who were followed to autopsy. Remarkably, those with poorer early life linguistic ability were much more likely to have neurofibrillary tangles in the cortical regions (87% were positive) compared with those with higher early life linguistic complexity (25%), a highly significant difference (OR = 6.0). Whereas the authors suggested that low linguistic capacity in early life was the beginning of expression of AD pathology, the alternative explanation that high linguistic capacity reduces the likelihood of developing NFTs in old age is equally plausible.

Antioxidants and neuroprotective agents

One of the key implications arising from the free radical theory of ageing is that of the possible neuroprotective and anti-ageing effects of antioxidants. A variety of compounds possess antioxidant activity, with demonstrable neuroprotective effects *in vitro* and in animal models. The most common of these can be grouped into vitamins (such as Vitamin E (alpha-tochopherol), Vitamin C (Ascorbic Acid), Beta Carotene, enzymes (e.g. superoxide dismutase, glutathione peroxidase), hormones (e.g. melatonin) and other neuroprotective agents (e.g. monoamine oxidase inhibitors). Some of these agents have been evaluated in clinical trials of various neuropsychiatric disorders. Trials of vitamin E have produced disappointing results, with no effect demonstrable in Parkinson's disease [211,212], equivocal results in treatment of tardive dyskinesia [213], and modest efficacy in AD [214]. Higher consumption of dietary and supplemental antioxidants in community samples has not shown to be of benefit in enhancing cognitive function in middle age [215] or elderly samples [216]. The monoamine oxidase inhibitor selegiline has shown modest benefit in slowing progression of AD [214], and may delay onset of disability in early Parkinson's disease [211]. The broader question of whether antioxidants or other neuroprotective agents modify the ageing process or age related diseases risk requires future long-term study.

Hormones

There are a number of basic science results which suggest that hormonal change with age may be associated with altered neuronal function [217]. Thyroid hormones, in particular T3, have been documented to stimulate proliferation and differentiation of neurones in development [218]. Direct mechanisms for this interaction have been proposed [219], in addition to indirect hypotheses mediated by nerve growth factor synthesis [220]. Clinical thyroid disease is well known to include cognitive and behaviour abnormalities [221] and this observation has been extended to the euthyroid, with researchers finding in normal younger subjects an inverse relationship between thyroid hormones and verbal performance [222]. More recently, in a larger sample of nondemented elderly, TSH levels (but not T4 levels) were positively related to episodic memory performance [223]. The magnitude of the relationship was approximately 9% after controlling for age and education. It is unclear whether medical supplementation of thyroid hormone in the euthyroid elderly is either safe or warranted and further studies are required.

Perhaps the best studied hormone in ageing is oestrogen because of the natural decrease in concentration in women after menopause. Oestrogens, like other hormones, can regulate the level of neuronal plasticity in the brain via direct and indirect routes [218]. Hypoestrogenism has been linked to age-related cognitive change and a higher risk of AD in postmenopausal women [224], making the efficacy of hormone replacement therapy an important issue. Recent systematic reviews [225,226] have provided cautious support for HRT in attenuating age-related cognitive decline and lowering AD risk. This is supported by a recent imaging study which has shown that hormone replacement therapy (HRT) users manifest better temporal lobe perfusion characteristics and cognitive performance over time than nonusers [227]. Large-scale longitudinal studies assessing side-effects and risks are required and a major Australian study is in preparation [228]. The attrition of testosterone levels in ageing men may in fact be a blessing in disguise! Wolf *et al*. [229] have found that a single injection of testosterone to supraphysiological levels in elderly men particularly inhibits verbal memory. Much further study is required on hormonal changes in ageing men and their putative behavioural or cognitive effects.

Conclusions

Beyond the age of 50 years there is a robust relationship between the passage of time and brain volume – the average person will lose about 5% per decade. Anatomical imaging studies have verified the regional nature of these changes *in vivo*. Importantly, neural loss can not adequately explain this phenomenon. Table 3 summarizes our understanding of brain ageing, which we suggest offers a more rational explanation. Brain ageing

Table 3. Summary of major neuropathological processes related to normal ageing in different atrophic-susceptible brain regions

Atrophic susceptible region	**Age-related neuro-pathological process**	**Mechanism for cellular dysfunction**	**Post-mortem structural change**	***In vivo* imaging evidence**	**Cognitive correlate**
Prefrontal cortex	Phylogenetic diaschisis: last in, first out? Cerebrovascular disease of MCA	Genetic? Ischaemic excito-toxicity	Robust and moderate volume decline	Robust and moderate volume decline rCBF decline*	'Executive' syndrome. Working memory decline.
Medial temporal lobe	Gluco-corticoid excess Neurofibrillary tangles	Apoptosis in certain hippocampal subregions Cytotoxicity	Neural loss* Volume decline*	NAA depletion Volume decline	Memory, spatial ability and new learning suffer. Loss of cholinergic hippocampal input may contribute.
Association cortex	Diffuse senile plaques	Immunoreactive oxidative cell membrane damage	Mild volume decline	Mild volume decline	Little change in ability to recall past experience or culture- and education-based information.
Neostriatum	Cerebrovascular disease Specific neurotransmitter receptor attrition	Ischaemic excito-toxicity? Synaptic loss?	Moderate volume decline	Moderate volume decline	Motor function decline.
White matter	Demyelination Cerebrovascular disease	Ischaemic excito-toxicity Free radical protein damage?	Robust volume decline	Mild volume decline and loss of tissue differentiation	Generalised slowing of information processing. Reaction time slows, motor speed slows. May contribute to executive dysfunction.
Mid-brain	Specific neurotransmitter nucleus attrition (eg., locus coerelus, substantia nigra)	Synaptic loss? Ischaemic excito-toxicity?	Robust and moderate volume decline Neural loss in specific nuclei	Robust and moderate volume decline	Decline in dopaminergic basal ganglia input will further affect motor speed and function.

Possible cellular mechanisms for cognitive decline are suggested and structural/functional imaging findings noted. *Indicates that reports demonstrate high variability.
? Signifies that process is conjectural. Middle Cerebral Artery (MCA); Regional Cerebral Blood Flow (rCBF); NAA, *N*-acetylaspartate.

follows a typical and topographically distinct course, with different pathological injuries tending to accumulate in regions of high vulnerability. Prefrontal, neostriatal, midbrain and the medial temporal regions contain cell bodies at most risk for age-related disease. In these regions, ischaemia, microglial reactivity and corticosteroid excess are important pathological processes. The ageing cell is therefore subject to a number of potentially damaging pathways, including oxidative stress, calcium excess and mitochondrial dysfunction. Axonal damage probably occurs from the combined effects of demylineation and cerebrovascular disease in the penetrating arteries. This can be detected by sensitive T2-weighted MRI techniques. Overall, cholinergic and dopaminergic neural systems seem to be affected to a greater extent than other neurotransmitters. Together this pattern of change may assist in explaining the preponderance of executive, working memory and information processing deficits which also begin to occur in the second half century of life. Obviously many questions remain unanswered.

In healthy ageing we pass on our wealth of knowledge and experience because long-term memory representation in the association cortex is not significantly disrupted. In AD, the appearance of neurofibrillary tangles and the more pernicious types of senile plaques in these areas, and their synergistic association with cerebrovascular disease, signifies that more devastating problems arise. Compensatory systems are overwhelmed as manifest by reduced cortical blood flow and accelerated atrophy of the temporal lobe accompanied by profound memory loss. Early detection of prodromal AD and those 'at risk' is required to allow intervention.

Interest in protective factors arose from the observation of no one-to-one relationship between pathological change and clinical manifestation. For example, we possess an elegant adaptive ability to overcome some aspects of normal brain ageing: we can recruit a larger neural substrate to solve a given problem. We may also be able to retard the onset and progression of some age-related diseases by careful attention to optimal lifestyle, nutrition and systemic health, although evidence for this is still preliminary. Mental activity over the lifespan potentially further reduces the impact of brain deterioration. Compensatory cellular, neuroplastic and strategic changes available to the individual means that age-related brain disease does not inevitably lead to significant mental impairment.

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