BRAIN AGEING — OPINION

Insights into CNS ageing from animal models of senescence

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Abstract | In recent years, novel model systems have made significant contributions to our understanding of the processes that control the ageing of whole organisms. However, there are limited data to show that the mechanisms that gerontologists have identified as having a role in organismal ageing contribute significantly to the ageing of the central nervous system. Two recent discoveries illustrate this particularly well. The first is the consistent failure of researchers to demonstrate a simple relationship between organismal ageing and oxidative stress — a mechanism often assumed to have a primary role in brain ageing. The second is the demonstration that senescent cells play a causal part in organismal ageing but remain essentially unstudied in a CNS context. We argue that the animal models now available (including rodents, flies, molluscs and worms), if properly applied, will allow a paradigm shift in our current understanding of the normal processes of brain ageing.

The ageing process is associated with increasing chances of both mortality and morbidity. Of these, morbidity, rather than mortality, is the primary challenge to health and social care systems. Within this arena, cognitive impairment, and the resulting reduction in activities of daily living, is a major cost component. It has been estimated that spending in this area will rise from 0.6% of UK gross domestic product (GDP) in 2002 to nearly 1% of GDP by 2031 (REF. 1). Thus, understanding the fundamental biology underlying brain ageing has the potential to produce a hugely beneficial impact on human welfare and the quality of later life.

Perhaps the two most obvious age-related changes to the functionality of the human CNS involve alterations to learning and memory, and motor performance. In the absence of distinct pathology, a small decline in cognitive performance (1 standard deviation below young adults on a standard memory function task) occurs in around 38% of 60–78-year-olds[2]. Specifically, episodic memory processing (explicit memory), working memory, spatial memory, processing speed and implicit memory function decline throughout normal brain ageing[3,4]. Motor function is also sensitive to the normal ageing process. Older adults show a slowing of motor movements and loss of fine motor control[5]. Motor tasks driven by central pattern generating circuits, such as those involving swallowing, also show age-related impairments[6].

These changes could be explained by two possible neural mechanisms: age-related neuronal loss or an age-related reduction in synaptic efficacy. At present, it is not possible to directly measure synaptic communication in the human brain. At an anatomical level, stereological imaging has shown a minor reduction in neuron number over the adult human lifetime, but whether this finding should be interpreted as strong evidence for a biologically significant age-related neuron decline remains open to debate[7]. Reductions in the supply of neurotransmitter, their reuptake and receptor number have also been seen in areas of the human brain that control motor function with advancing age[5], along with indicators of oxidative damage[8].

The obvious experimental impediments to working on the living human brain pose a fundamental question for researchers studying the biology of CNS ageing: to what degree are changes seen in the nervous systems of other ageing organisms reflective of the changes we know, or suspect, occur in the old human brain? Comparative data on organismal senescence show that species exhibiting highly divergent rates of ageing also show a high degree of conservation in their relative temporal patterns of mortality[9]. This conservation of age-related changes suggests that model systems should be reflective of many, but perhaps not all, of the changes seen in human brain ageing. TABLE 1 summarizes key changes seen with ageing in the brains of a variety of species. There is clearly a substantive degree of concordance between the changes that underlie the ageing of the CNS in these diverse organisms and those seen in *Homo sapiens*.

Classically, the ageing process was considered in an organ-specific manner[10], which finds an echo today in many articles that discuss brain ageing in isolation. Although there are obvious advantages to focusing on the key changes occurring within a single organ as it ages, this is not without cost. Failing to contextualize the changes taking place in the organ of interest with those occurring within the organism as a whole can be problematic. Not simply because the onset and progression of deficits within a specific organ can result from systemic problems (for example, the increase of Alzheimer's disease risk from hypertension and diabetes) but also because it fails to recognize the fundamental drivers, both evolutionary and mechanistic, that operate on the whole organism to produce the ageing phenotype. Accordingly, we attempt to discuss the ageing of the CNS in a somewhat broader context than it is often considered. In this Perspective article, we have simplified some subtleties of the biology of ageing in favour of a simple and (hopefully) coherent narrative that is intended to highlight key research questions.

Approaches to the use of model systems

For the purposes of this article, we propose to divide the available model systems into three major types: those used for cross-species comparisons intended to test specific

Table 1 | **Behavioural, anatomical and physiological changes during ageing in a range of vertebrate and invertebrate species**

Variable examined	Humans	Monkeys	Rabbits	Rats	Mice	*D. melanogaster*	Molluscs (*Lymnaea* spp. and *Aplysia* spp.)	*C. elegans*
MLSP	122.5 years[75]	30–40 years[75]	9 years[75]	3 years[76]	3 years[73]	3–4 months[75]	1–2 years[77] (*Lymnaea* spp.), 1 year[78] (*Aplysia* spp.)	27.3–30.4 days[79]
Behavioural changes								
Implicit learning	Declines[80–83]		Declines[84]	No impairment[85]; declines[42,86,87]	Declines[88]	Declines[89]	Declines[64,65]	Declines[90]
Spatial learning	Declines[91]	Declines[92,93]	NE	Declines[94–96]	Declines[97,98]	NE	NE	NE
Explicit learning	Declines[99,100]	Declines[92,93]	Declines[101]	Declines[86]	Declines[102]	NE	NE	NE
Motor function	Declines[103–105]	Declines[106–108]	NE	Declines[87]	Declines[88]	Declines[89]	NE	Declines[109]
Executive function	Declines[3]	Declines[110]	NA	NA	NA	NA	NA	NA
Fine motor control	Declines[103,104]	Declines[106,107]	NE	NE	NE	NE	NE	NE
Swallowing	Declines[6]	NE	NE	NE	NE	NE	Declines[66]	NE
Cerebellar function	Declines[4,105,111]	NE	Declines[84]	Declines[42]	Declines[112]	NA	NA	NA
Anatomical changes								
Neuronal number	Minimal change in NC[7]; reduction in Cb[113]	No loss in Hip or NC[114–116]; reduction in Cb[117]	Reduction in Cb[118]	No loss in Hip[119]; reduction in Cb[120]	No loss in Hip or dentate gyrus[121]	NE	Minimal loss of neurons controlling reproduction[122]	NE
Neuronal volume	Reduction[105,123,124]	NE	NE	NE	NE	NE	NE	NE
Neuronal arborization	Reduction[123]	NE	NE	NE	NE	Reduction[125]	Reduction[126]	NE
Spine density and/or connections	Reduction[127,128]	Reduction[129,130]	NE	Reduction[120,131]	Reduced[121]	NE	Reduction[67,132,133]	NE
Tangles	Positive[134]	Positive[136,137]	NA	NA	NA	NA	NA	NA
Senile plaques (Aβs)	Positive[134,135]	Positive[136,137]	NA	NA	NA	NA	NA	NA
Physiological changes								
LTP	NE	NE	NE	Impaired[91]	Impaired[91,97,138]	NE	NE	NE
LTD	NE	NE	NE	Increased[91]	NE	NE	NE	NE
Neuronal excitability	NE	Altered[139,140]	Decreased[99]	Decreased[100,141]	No change[142]	NE	Decreased[66]	Decreased[143]
Selective vulnerability	Yes[103,104]	SNc conserved[144]; SNc loss with age[145]; reduction in TH staining in SNc[146]	NE	NE	Yes[147]	NE	Yes[67]	NE
Baseline $[Ca^{2+}]_i$	NE	NE	NE	Increased[148]; decreased[149]	Decreased (whole brain)[150]; increased (CG)[151]	NE	NE	NE

Table 1 (cont.) | **Behavioural, anatomical and physiological changes during ageing in a range of vertebrate and invertebrate species**

Variable examined	Humans	Monkeys	Rabbits	Rats	Mice	*D. melanogaster*	Molluscs (*Lymnaea* spp. and *Aplysia* spp.)	*C. elegans*
NMDAR	NE	Decreased[152]	NE	Decreased[153]	Decreased[154,155]	NE	NE	NE
L-type Ca^{2+} channels	NE	NE	Increased[156]	Increased[157–159]	Unchanged in MSDB[160]	NE	Increased[68]	NE
Ryanodine receptors	NE	NE	NE	Increased conductance[23]; reduction in ageing biomarkers with ryanodine[161]	Reduced[151]; decreased caffeine stimulated release[162,163]	NE	NE	NE
Increased AHP	NE	Yes[164]	Yes[165]	Yes[22,23]	Yes[138]	NE	Yes[64,67]	NE

Although age-related changes for a particular brain region are consistent between species, there is a marked diversity in the changes that are observed between different CNS regions of the same species. Aβ, amyloid β-peptides; AHP, after-hyperpolarization; Cb, cerebellum; CG, cerebellar granule; Hip, hippocampus; LTD, long-term depression; LTP, long-term potentiation; MLSP, maximum lifespan potential; MSDB, medial septal diagonal band; NA, not applicable; NC, neocortex; NE, not examined; NMDAR, NMDA receptor; SNc, substantia nigra pars compacta; TH,tyrosine hydroxylase.

theories of ageing; mutants that have been typically used to determine the general mechanisms of ageing (most of these have been generated in flies, worms and mice); and novel animal models with specific useful features that have the potential to shed light on particular aspects of the ageing process, such as *Arctica islandica,* the longest living non-colonial animal. In order to do this, it is important to consider both why ageing exists and how it may work. The major theories dealing with these areas are briefly summarized in BOX 1, BOX 2 and FIG. 1.

Type I models: life on Earth

The use of cross-species comparisons is a long-standing gerontological approach dating back to the 1950s[11]. Cross-species comparisons attempt to make use of the fact that although ageing is a shared characteristic of all birds and mammals, a huge variation exists in both maximum and mean lifespans. Indeed, TABLE 1 is itself little more than a crude type 1 modelling approach, and we argue that a structured use of these approaches, with the caveats given below, would be of considerable benefit in understanding which processes are conserved within CNS ageing and which are species-specific.

Properly designed comparative biology studies also allow investigators to test hypotheses for the causal mechanisms of ageing. For instance, they have falsified simple explanations for ageing (for example, the 'rate of living' hypothesis that the rate of ageing correlates significantly with metabolic rate[12]). However, although they are potentially powerful, many older type I studies neglected two key problems that confound the use of simple correlations between the variable under study and the maximum lifespan potential (MLSP) of the species. The first of these is the co-variation of many traits with body mass (large animals tend to live longer). The second problem is more complex and results from a lack of true independence in cross-species data owing to the shared phylogenetic history of the animals under study[13]. However, when these are corrected for, exceptionally useful work can be undertaken.

Such corrections were made in a study across 42 different species to test the hypothesis that polyunsaturated fatty acids (PUFAs) have a causal role in regulating lifespan. This 'membrane pacemaker' hypothesis was based upon the potential for lipid peroxidation to cause tissue damage (a finesse of the 'oxidative damage' hypothesis relevant to CNS ageing) and the report that an inverse relationship existed between MLSP and the peroxidizability index of their lipids[14]. The expectation was that low levels of PUFAs would correlate with longer MLSP. However, when multiple linear regressions with body weight as a co-variate were combined with a phylogenetic generalized least square (GLS) model to minimize the chance of spurious co-variance, no relationship was apparent between MLSP and the PUFA content of membranes. These findings were inconsistent with the membrane pacemaker hypothesis and suggest that a fresh perspective may be required on the relationship between PUFAs and brain ageing.

Similarly, although many studies have reported an inverse relationship between key parameters relevant to oxidative damage and MLSP (for example, antioxidant enzyme levels in the brain), a large number of these did not correct for the potentially confounding co-variance of body mass and phylogeny. By contrast, when the rates of hydrogen peroxide production by heart mitochondria were compared across 11 species (including bats, mice, naked mole rats and pigeons) with similar body masses but widely varying maximum lifespans[15], the correlation between peroxide production rate and lifespan was barely significant when corrected for phylogeny. Thus, although the study gave some support to the idea that low rates of mitochondria radical production and MLSP were causally linked, a much stronger correlation might have been expected if reactive oxygen species (ROS) do indeed play the critical part proposed for them in both brain and organismal ageing[16]. These questions can be explored much more thoroughly in the context of the next class of models we consider.

Type II models: is oxidative stress is dead?

The classical laboratory model species (such as flies, worms and mice) have much to offer those seeking to understand the fundamental processes controlling ageing. New mutants and transgenics within these species have produced important insights into nervous system ageing.

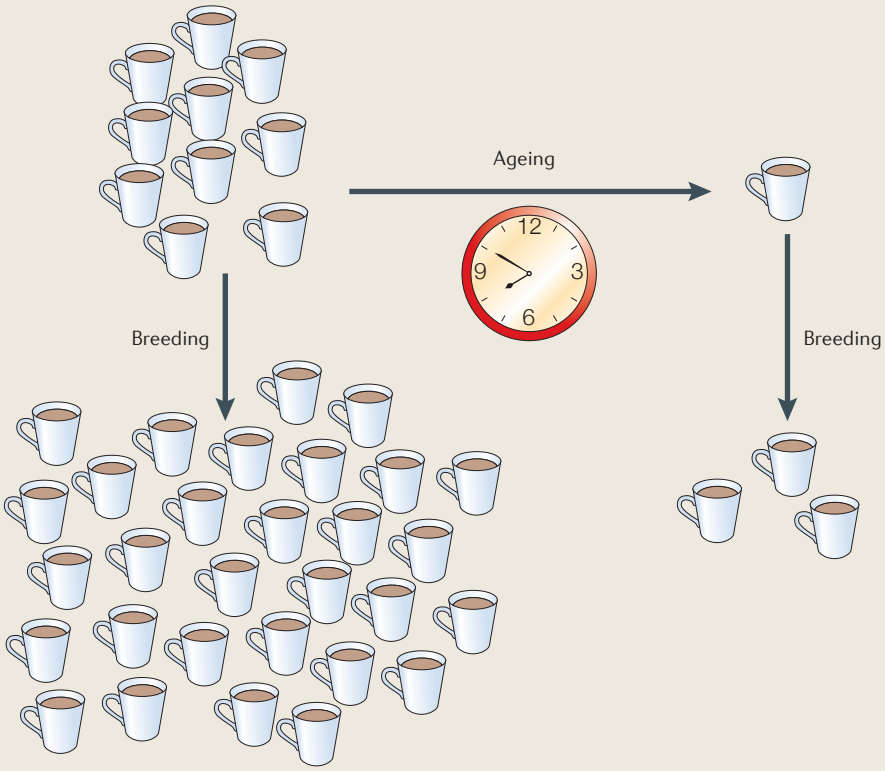
The first transgenic animals that were deemed relevant to CNS ageing are a series of mouse models (comprehensively reviewed in REF. 17), which are either knockouts or knockins for genes involved in antioxidant protection. If oxidative damage is indeed the major factor limiting lifespan, then animals with compromised antioxidant defences should show significant reductions in lifespan, and

those with increased protective or repair capacities should show extended lifespans. The general picture from these transgenics is that heterozygous C57BL/6 mouse knockouts of superoxide dismutase 2 (*Sod2*), glutathione peroxidase 4 (*Gpx4*) and thioredoxin 2 (*Trx2*) show no decrease in either mean or maximum lifespan even though markers of oxidative damage are increased. Although *Sod1* homozygous knockouts do show a decrease in lifespan, as the oxidative damage theory would predict, *Sod1* overexpression fails to lengthen it, and this pattern is replicated with *Sod2*, *Gpx4* or catalase (*Cat*), whereas expression of *Trx1* produces a small increase in lifespan. These data are paralleled by studies in *Caenorhabditis elegans* and, taken together, are inconsistent with any simple assertion that oxidative damage is the major constraint on lifespan. So, where does this the leave the idea that it is a primary cause of ageing in the brain?

In theory, the CNS should be particularly susceptible to damage by ROS because of its high demand for oxygen, the abundance of redox-active metals (iron and copper), the high levels of brain PUFAs and the fact that mature neurons are postmitotic[18]. In practice, increased oxidative damage is readily detectable in the ageing brain[19], which is paralleled by an increase in oxidative stress-response gene expression with ageing in several species[20]. Thus, markers of increased oxidative stress are present in the ageing brain, but whether these processes can drive alterations in neural function has been the subject of intense research.

There is some evidence to suggest that oxidative stress directly affects the mechanisms that determine neuronal excitability in the hippocampus. Some of these effects involve alterations in the way cells handle Ca^{2+} (the Ca^{2+} hypothesis of ageing[21]). Specifically, ageing is associated with a reduction in the excitability of hippocampal CA1 neurons owing to increases in both the amplitude and duration of the slow after-hyperpolarization (sAHP)[22]. Ageing in these neurons sees a decrease in Ca^{2+} entry via NMDA receptors, an increase in Ca^{2+} entry via L-type Ca^{2+} channels[23] and an increase in Ca^{2+} release from ryanodine-sensitive stores that activates the sAHP current (sI_{AHP}; a Ca^{2+}-activated K^+ current)[24], causing hyperpolarization of the postsynaptic neuron. The shift in Ca^{2+} entry from NMDA receptors to voltage-gated Ca^{2+} channels and the increase in ryanodine receptor-mediated Ca^{2+} release is driven by an alteration in the redox state of these proteins[23,25–27]. This ultimately alters the long-term potentiation (LTP)–long-term depression (LTD) transition threshold in mammalian models[26] (FIG. 2). Therefore, it does seem that the age-related changes observed in neuronal excitability can be produced by the action of ROS and reactive nitrogen species. However, a number of other interventions can also mimic age-related increases in

Box 1 | **Why does ageing happen?**



Ageing is not universal across all species but it is extremely common and thus provides a selective advantage to organisms, allowing them generally to out-compete organisms that do not age. One plausible driver for this advantage is as follows. Consider any given organism, even one which is immortal (in the same sense that a coffee cup is immortal, it 'lasts forever'). The longer this 'ageless' organism exists, the more likely it is to be eaten or die by accident. By the same token, the longer you have coffee cups in the canteen the more likely they are to break.

Thus, even in a population of innately immortal organisms, there are always far fewer chronologically old ones than young ones. This produces a situation in which chronologically 'old' organisms contribute fewer offspring to the next generation than chronologically 'young' organisms even when the reproductive ability of 'old' and 'new' immortal organisms is the same, simply because the young outnumber the old (illustrated in the accompanying cartoon which ratios the 'offspring' of the coffee cup population 3:1 with the parental mugs). This is the long-standing observation from evolutionary biology that the force of natural selection declines with age[69,70].

Under these conditions ageing processes can evolve in one of two ways. A mutation that favours early life fecundity will be selected for even if it results in deleterious effects later on in the lifetime (a type of gene action termed antagonistic pleiotropy)[71]. Alternatively, this situation allows the selection pressure against mutations that reduce either fecundity or viability to weaken at later life stages, allowing the entry into a population of mutations that are deleterious, expressed only in later life and do not confer a fitness advantage. Removal of such late-acting genes is not easy, as most of the individuals reproduce and die of other causes before the expression of these genes. These deleterious mutations accumulate during evolution and are responsible for ageing. Applying these situations to the CNS, the apolipoprotein-ε4 mutation would be an example of antagonistic pleiotropy if it conferred an advantage early in the life course but would be an example of mutation accumulation if its sole effect was to increase the risk of Alzheimer's disease in late life.

This view of ageing is inconsistent with the operation of a 'clock' controlling the ageing of individuals but suggests that ageing will result from an accumulation of faults at different rates in different tissues. Thus, ageing is not a programmed process, in the sense that no genes are known to have evolved specifically to cause it. It resembles a car breaking down rather than a bomb going off.

Box 2 | **How does ageing happen?**

Ageing is unprogrammed, in the sense that no genetic pathways appear to have evolved solely to trigger it. Thus, ageing mechanisms must either be the result of accidental damage or the side effects of processes that operate to a different, but beneficial, end. Perhaps more subtly, because ageing is the unprogrammed result of selection for early reproductive success, there is no *a priori* reason that a universal selection pressure should produce a set of ageing mechanisms that are uniform across species (by the same token, there is also nothing to exclude similar changes if they affect a common pathway)[46]. In fact, evidence exists across the biosphere for both mechanisms of ageing that are species-specific (for example, the observation that the toxic effect of compounds present in the seminal fluid of the male fruitfly cause ageing and death in female *Drosophila melanogaster*) and for those that appear to operate much more generally across the biosphere.

Perhaps the oldest theory of how ageing works that still has the capacity to do useful conceptual work is the oxidative damage theory[72]. This postulates that uncontrolled reactions involving reactive oxygen species are a major cause of ageing and age-related pathology. As radicals can cause damage to membrane lipids, proteins and DNA (giving rise to mutations), there is a significant amount of scope for radical damage either to kill cells or to alter their phenotype should they endure. However, simple interpretations of this idea do not seem entirely correct (see main text)[73].

The cell hypothesis of ageing proposes that the progressive accumulation of 'senescent cells' has a causal role in ageing and age-related pathology. Cell division to replace lost or damaged cells is an unavoidable process in any animal surviving longer than a few weeks, and entry into the senescent state occurs as a result of individual cells actively monitoring the number of times they have been called on to divide (probably as an anticancer mechanism). Senescent cells show a different, generally pro-inflammatory phenotype that disrupts the function of tissues[32]. Ongoing tissue turnover through life drives the accumulation of such cells and hence their degenerative effects. This idea has received a solid body of support in recent years and seems a plausible mechanism of ageing in many species (although obviously it would not apply to any species showing a genuinely postmitotic soma)[33].

The broad spectrum detoxification hypothesis (also known as the 'green theory' of ageing) is a much newer concept based on analysis of the processes that are conserved among the various extended healthy lifespan mutants[74]. It suggests that phase I and phase II detoxification, autophagy and recycling of damaged components prevents their build-up to levels that compromise cellular function, thus extending lifespan. It has some direct experimental support and allows for a contribution to the ageing process from oxidative damage without requiring it to be the major cause in every instance.

The Ca^{2+} hypothesis is an interesting example of a tissue-specific ageing mechanism. The basic concept is that age-dependent changes in calcium homeostasis drive both cognitive and motor decline associated with brain ageing. Calcium entry into neurons may alter synaptic strength and direction and, in extreme conditions, produce excitotoxicity (see main text)[21].

the AHP, including changes in the levels of corticosteroids and oestrogens[26], as well as the expression or activity of FK506-binding protein (FK506bp)[28]. Thus, simply ascribing the changes observed in the ageing brain to oxidative stress may be over simplistic.

The relationship between oxidative damage and neural function seems to be a key area to explore. The effects of overexpressing *SOD1* on LTP and learning and memory in old mice have been assessed, and the results suggest that both LTP and spatial learning improve[29]. In order to determine the relationship between altered redox handling, lifespan and brain ageing, we would urge a systematic and detailed behavioural and electrophysiological analysis of other antioxidant knockout and gain-of-function mutants. Ideally this would include a cross-species analysis, including both vertebrate and invertebrate species, to examine whether the mechanisms of brain ageing are conserved between organisms.

Recently, the effects of overexpressing a range of antioxidant genes using an innovative viral vector-mediated delivery system in the hippocampus of rats were examined[30]. Increasing the expression of *SOD1* and catalase in the hippocampus of aged (19-month-old) rats protected them against age-related cognitive decline. More interestingly, the authors observed that although overexpression of the antioxidant genes reduced hippocampal markers of oxidative damage, these did not correlate well with cognitive performance, raising the possibility that oxidative damage per se is not a major determinant of CNS ageing and that altered redox-sensitive signalling pathways may be more important.

Ultimately, if oxidative stress turns out to be a minor contributor to whole-organism ageing but is a major player in the ageing of the brain, the simplest explanation might be that in a protected environment, such as the one experienced by laboratory-reared animals, the CNS is not the major determinant of survival. However, if oxidative stress makes only a minor contribution to brain ageing, this will represent a significant paradigm shift in our understanding of CNS function. What then, would the paradigm shift to?

Although new models have failed to generate data supporting oxidative stress as a key mechanism in organismal ageing, a new transgenic mouse model has provided results that are consistent with the cell senescence hypothesis. Cellular senescence is the permanent entry of individual cells into a viable but non-dividing state (usually as the result of repeated cell division and probably as an anticancer mechanism[31]). Considered in these terms, senescence is essentially a process-level example of antagonistic pleiotropy. In the early part of the life course, senescence prevents tumour growth, thereby aiding survival. However, the onset of senescence is typically, but not always, associated with a shift to a pro-inflammatory phenotype marked by the secretion of a range of cytokines (for example, interleukin-6, interleukin-7, interleukin-8 and monocyte chemo-attractant protein 2) together with other changes in phenotype that have the potential to produce degenerative effects (for example, a pro-calcificatory phenotype in human vascular smooth muscle cells[32]). Until now, the majority of data consistent with this idea have come from *in vitro* studies.

A recent study[33] in which senescent cells were deleted *in vivo* using a promoter that drives expression of an FK506bp–caspase 8 fusion protein, which triggers apoptosis when exposed to the drug AP20187, indicated that senescent cells have a causal role in the ageing process. When cells expressing the transgene were eliminated in the BubR1 progeroid mouse background, the onset of a range of age-related pathologies, including cataracts and sarcopenia, was delayed. Lifespan was not extended in this model, which the authors plausibly attribute to mortality as a result of age-related phenotypes that were not attenuated by senescent cell deletion. Unfortunately, CNS phenotypes in this model remain unstudied at the time of writing. Given the widespread evidence for inflammation in the ageing brain and the pro-inflammatory phenotype of senescent cells, it seems possible that some aspects of brain ageing are due to astrocyte senescence. However, this should not be assumed because senescence in at least one human cell-type (corneal keratocytes) is not associated with inflammation but rather with dysdifferentiation[34]. A survey of the changes

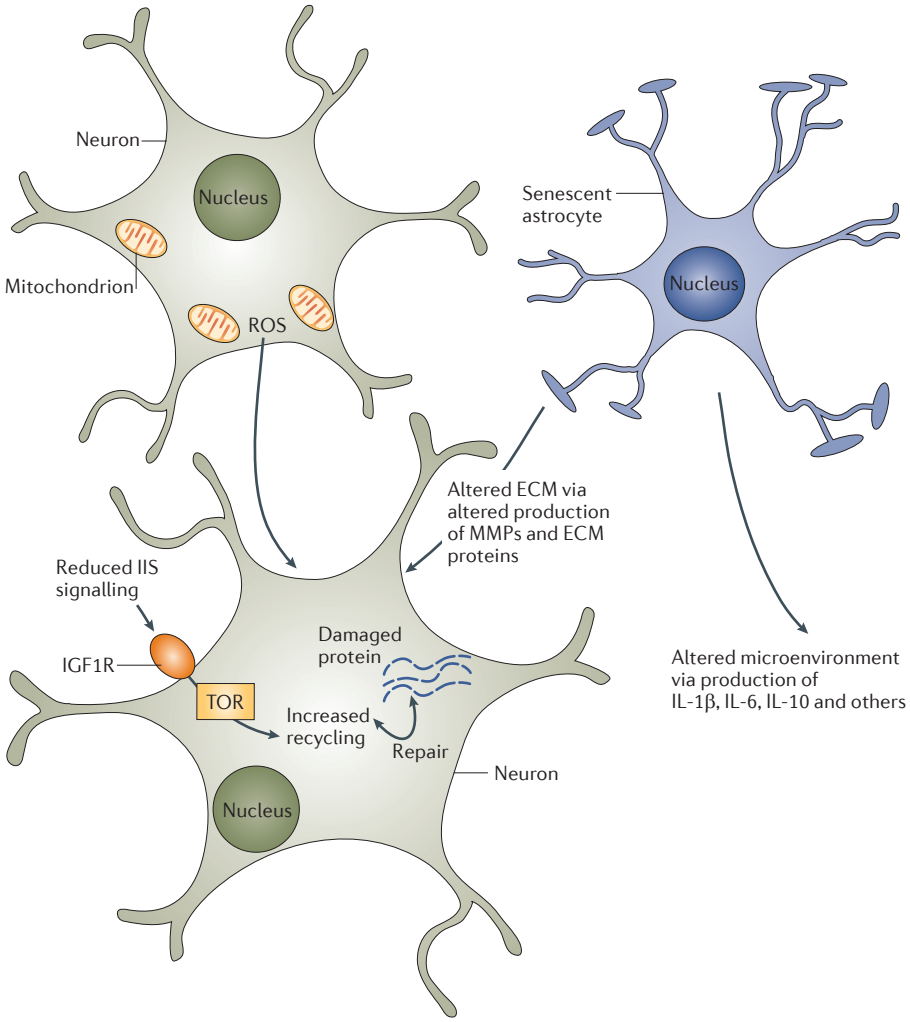


Figure 1 | **Schematic representation of the putative effects of the major ageing mechanisms on the CNS.** Reactive oxygen species (ROS) are produced as a by-product of oxidative metabolism and potentially add to the load of damaged macromolecules accumulated by a cell over time. Reduced signalling through the insulin/insulin-like growth factor signalling (IIS) pathway via mammlian target of rapamycin (mTOR) leads to the increased activity of recycling pathways dealing with oxidized or glycated proteins. Senescent cells in the vicinity produce a mixture of matrix-degrading enzymes and alter their production of matrix proteins. They also typically overproduce some pro-inflammatory cytokines and may cease to produce survival factors. However, many aspects of this signature are strongly cell-type-specific. ECM, extracellular matrix; IGF1R, insulin-like growth factor 1 receptor; IL, interleukin; MMPs, matrix metalloproteinases.

in phenotype seen at senescence in neuronal cell types is likely to shed light on this issue.

The last class of type II model includes mutants that were initially isolated because they displayed significantly extended lifespans compared to wild-type controls. Such mutations are typically in the insulin/insulin-like growth factor signalling (IIS) pathway and are found in all of the common model species. They include the *daf-2* mutant in *C. elegans* (a hypomorph in the insulin/insulin-like growth factor 1 (IGF1) receptor), *chico* (a *Drosophila melanogaster* insulin receptor substrate protein mutant) and a variety of mutations in mice (for example, insulin receptor susbstrate 1 (*Irs1*)) that affect the insulin receptor in selected tissues or global circulating insulin levels and insulin receptor substrates. In addition to being long lived, mammalian IIS mutants show retardation of multiple markers of the ageing process[35]. Thus, it appears that interference in IIS pathways can delay both age-associated pathology and morbidity[36]. An interesting feature of these mutants is that they show an extension of lifespan effect, which is more marked in female animals than in males[36]. By contrast, GHRKO mice (knockouts of both the growth hormone receptor and growth hormone binding protein) show a lifespan extension effect in both sexes but appear to share common mechanisms of action with the IIS mutants[37,38]. The pathways altered in these mutants strongly overlap with those associated with dietary restriction, an environmental intervention shown to extend lifespan by restricting the food intake of organisms normally fed *ad libitum*[39].

All of these mutants demonstrate that it may be possible to target multiple age-associated conditions via a single pathway, potentially improving health in later life. However, for this research to show eventual benefits, a better understanding of the effect of IIS mutations and dietary restriction on cognition (and by implication the morbidity concomitant with cognitive impairment) is required. We would argue that the potential rewards justify a sustained research effort.

Data from *C. elegans* suggest that cognition is indeed protected in some IIS mutants. *age-1* or *daf-2* worms showed improved thermotaxis[40] or salt chemotaxis[41] associative conditioning behaviour. More recently, a different model of associative conditioning (food–odorant association) was used to demonstrate that *daf-2* mutants show improved memory performance in early adulthood and maintain the ability to learn better with increasing age[42]. No studies as yet appear to have directly examined the effects of IIS mutants on learning and memory in *Drosophila* spp.

Conversely, increased insulin levels decrease locomotion and spatial memory in mice[43]. Selective knockout of *Irs2* in the basal forebrain of mice can improve hippocampal spatial memory and can increase the number of dendritic spines and synaptic connections in the CA1 region of the hippocampus, providing potential mechanisms for the improved spatial memory[44]. It would be interesting to determine whether the effects of this mutation improve the health span of aged animals.

Recent data suggest that the mammalian target of rapamycin (mTOR) pathway (which is downstream of the IIS pathway) may represent the best target for pharmacological intervention to increase lifespan. In the presence of nutrients, TOR promotes protein synthesis and inhibits autophagy. Knockout of the TOR target p70 S6 kinase extends lifespan significantly in female mice (possibly by upregulating detoxification and recycling pathways[45,46]). In addition, treatment of mice with the TOR inhibitor rapamycin leads to a significant increase in lifespan, even if it is administered late in life[47]. Potentially this provides

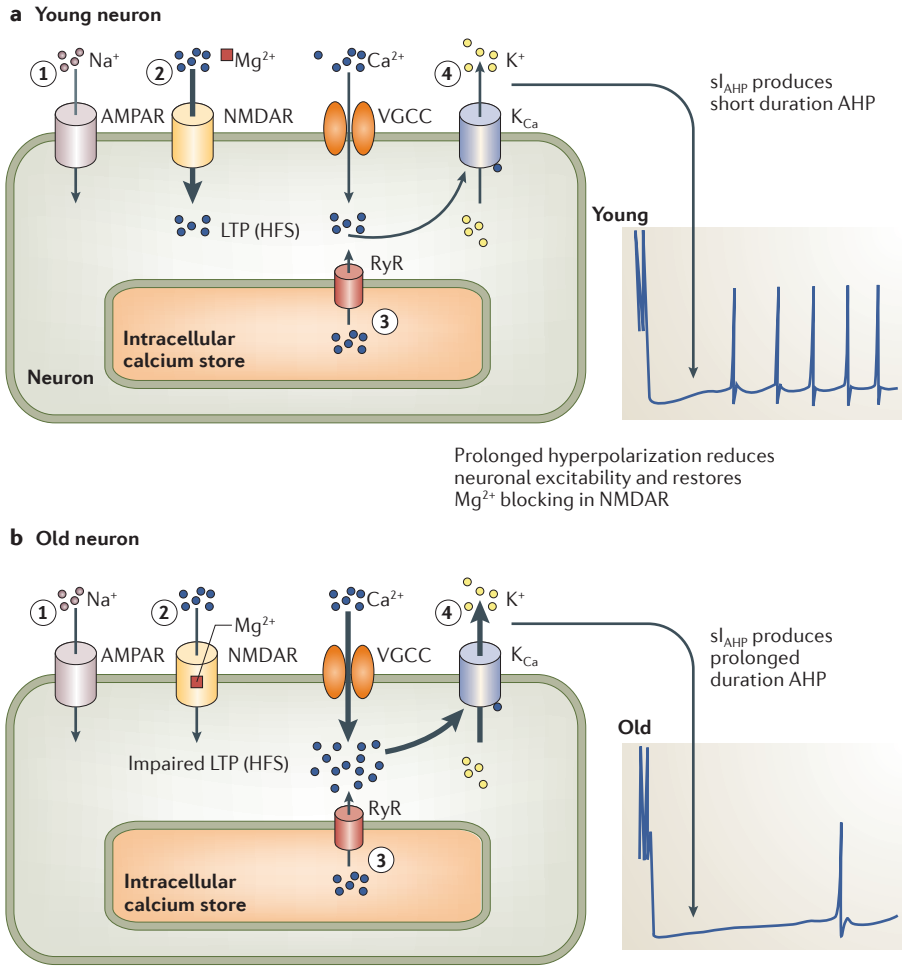


Figure 2 | **Schematic representation of the age-related changes to hippocampal neuronal biophysics. a** | In response to high-frequency stimulation (HFS), glutamate activates AMPA receptors (AMPARs), causing Na^+ influx and depolarization (step 1). Depolarization opens voltage-gated Ca^{2+} channels (VGCCs) and removes Mg^{2+} blocking from NMDA receptors (NMDARs), allowing Ca^{2+} influx (step 2). Ca^{2+} influx from NMDARs initiates long-term potentiation (LTP). Ca^{2+} influx through VGCCs induces Ca^{2+}-induced Ca^{2+} release (CICR) from ryanodine receptors (RyRs, step 3). CICR switches on the channels responsible for the slow after-hyperpolarization (sAHP, step 4), which hyperpolarizes the cell and restores Mg^{2+} blocking in the NMDAR. The sAHP current (sI_{AHP}) reduces excitability and delays recovery. In young hippocampal neurons, a relatively small amount of Ca^{2+} enters through VGCCs with little CICR, but a large influx is seen through NMDARs. These conditions favour LTP. **b** | As a consequence of ageing, depolarization favours Ca^{2+} entry through VGCCs over NMDARs. This leads to enhanced CICR and a consequential increase in the activity of sAHP channels. This dramatically decreases the excitability of hippocampal neurons, restores Mg^{2+} blocking of NMDARs and reduces Ca^{2+} entry through NMDARs, thus shifting the stimulation threshold for LTP induction to higher frequencies.

proof-of-principle that at least one drug licensed for clinical use could slow the ageing process. The central question then becomes whether this compound, or other 'rapalogues', have beneficial effects on cognition; this question remains open.

It is clear that rapamycin treatment has beneficial effects in transgenic mouse models of both Alzheimer's[48] and Huntington's disease[49], probably via the induction of autophagy[48,50,51]. It has also been shown that rapamycin can induce LTP during a weak stimulation protocol, possibly by increasing Ca^{2+} availability and lowering the threshold for LTP induction[52]. In themselves, these results are sufficient to justify sustained research into the therapeutic potential of compounds of this type. However, these results must be set against a series of studies showing that rapamycin may interfere with the molecular mechanisms of memory formation. For example, rapamycin has been shown to prevent 5-hydroxytryptamine-induced protein synthesis (a process required for long-term facilitation) in isolated synapses from *Aplysia* spp.[53], and in mice and rat hippocampal neurons, rapamycin has been shown to inhibit late-LTP[54,55]. Furthermore, there is interesting evidence to suggest that rapamycin may induce the aged phenotype in hippocampal neurons by uncoupling FK506bp from ryanodine receptors, with subsequent activation of the sI_{AHP}[28]. There is also evidence that the rapamycin–FK506bp complex may directly stimulate a Ca^{2+}-dependent K^+ channel that contributes to the AHP[56].

From a behavioural perspective, when rapamycin is administered systemically[55,57] or injected directly[58] into the hippocampus or amygdala of rodents, consolidation and reconsolidation of a contextual memory task is impaired. Thus, these data suggest that in adults with no neuronal pathology, treatment with rapamycin may impair memory trace formation and may be at odds with the potentially life- and health-extending benefits of oral rapamycin treatment observed in mice. The precise reasons for this inconsistency are unknown. One plausible explanation may be differences in the method of administration of rapamycin and the concentrations used across these different studies. The tissue concentrations of rapamycin were only known in the electrophysiological *ex vivo* experiments, and these were orders of magnitude above the dissociation constant (K_d) for rapamycin–FK506bp binding[59]. This raises questions about non-target-specific effects of the drug. Despite this, an interesting question remains unanswered: does rapamycin interfere positively with the process of normal brain ageing? Reconciling these areas of work will require a detailed investigation into the effects of mTOR inhibition (pharmacologically or otherwise) on deficits seen in normal brain ageing (for a schematic, see FIG. 3).

Type III models: do clams learn?

The final type of models we have chosen to discuss are those that are of particular interest to gerontologists because of their exceptionally long lives. Across the animal kingdom there are a few truly exceptional species that are either long lived for their body size compared to mammals (many birds), or long lived compared to other rodents (the naked mole rat). However, the most startling example of a long-lived organism is the clam *Arctica islandica*. This bivalve mollusc can reach ages close to 400 years and may be an example of a species that is non-ageing[60–62]. Regardless of whether *Arctica* spp. are ageless or simply exceptionally long

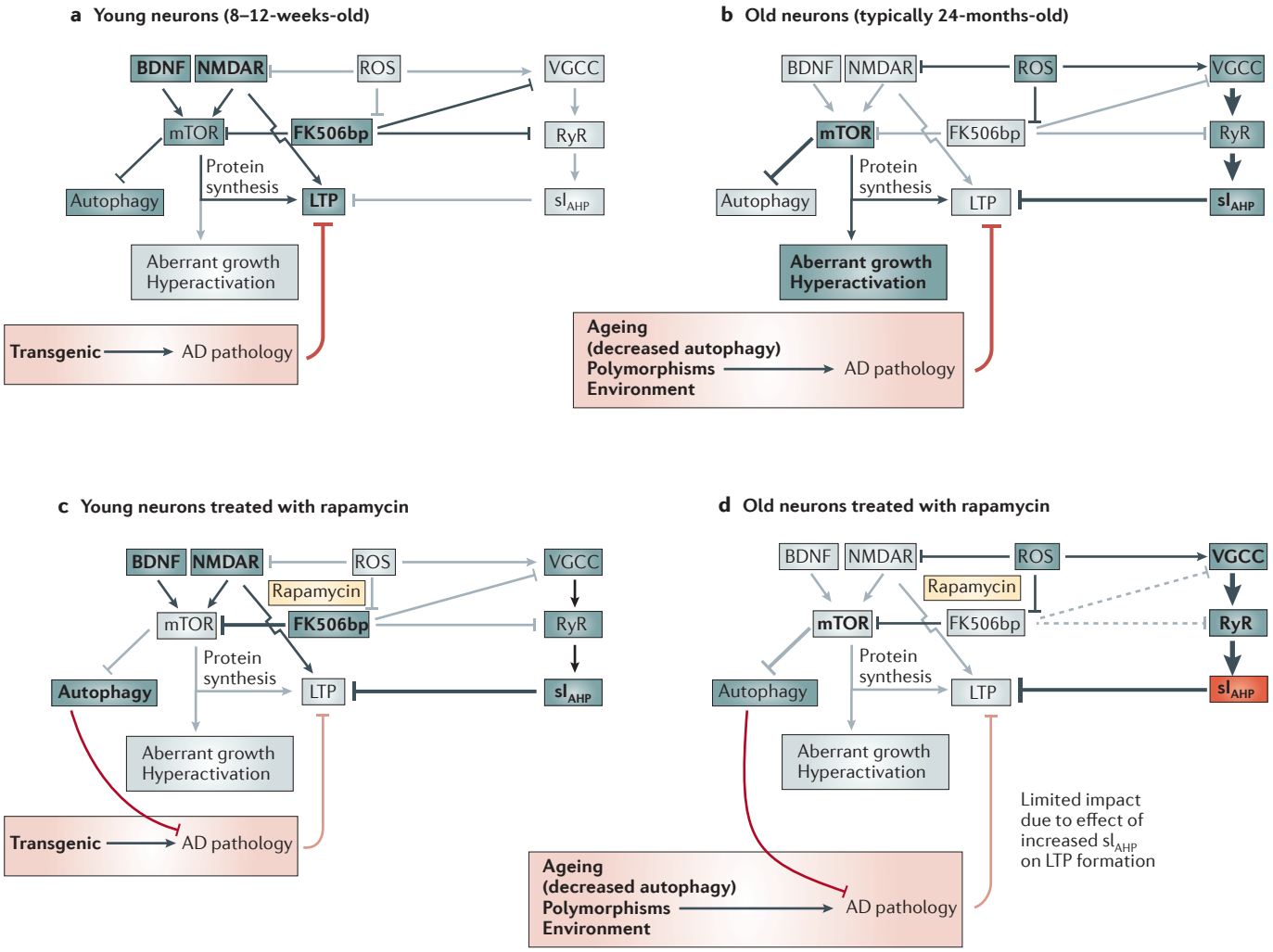


Figure 3 | **Schematic representations of the proposed interaction between ROS, mTOR and FK506bp and neuronal ageing in rat hippocampal neurons.** **a** | Mammalian target of rapamycin (mTOR) activity is 'normal', allowing protein synthesis at the neuronal synapse and long-term potentiation (LTP) to be induced. FK506-binding protein (FK506bp) level is 'normal', inhibiting both the voltage-gated calcium channels (VGCCs) and ryanodine receptors (RyRs) and therefore reducing the Ca^{2+}-activated slow after-hyperpolarization current (sI_{AHP}). **b** | FK506bp level is 'low', allowing activation of both the VGCC and RyR, with a marked increase in the Ca^{2+} activated sI_{AHP}, and a subsequent inhibition of LTP. Despite mTOR activity being 'high' in this scenario, which would allow protein synthesis at the neuronal synapse, LTP activation is impaired by the high-activity sI_{AHP}. **c** | Rapamycin causes a marked inhibition of mTOR activity and protein synthesis. Both VGCC and RyR activity is increased (owing to inhibition of FK506bp) compared to young controls, and there is a consequential increase in the sI_{AHP}, inhibiting LTP. **d** | Rapamycin causes a marked inhibition of mTOR activity and protein synthesis. Both VGCC and RyR activity and sI_{AHP} amplitude and duration are further increased from old controls (on top of the age-related increase; red box). Therefore, rapamycin does not improve the ageing phenotype, but may worsen it. The impact of Alzheimer's disease (AD) pathology on LTP (**a**–**d**). Superimposed on each diagram (pink), is the effect AD has on LTP and its interaction with mTOR. In non-aged (young) transgenic animals, AD pathology has a negative impact on LTP. When these animals are treated with rapamycin, AD pathology is reduced through increased autophagy, and LTP is improved when compared to controls. In old neurons, when AD pathology is present, it similarly contributes to reduced LTP. However, there is uncertainty over whether reduced AD pathology as a consequence of rapamycin treatment will be sufficient to overcome the negative effects of both rapamycin and ageing on an increased sI_{AHP}. BDNF, brain-derived neurotrophic factor; NMDAR, NMDA receptor; ROS, reactive oxygen species.

lived, they are unique both for their length of lifespan and the relative ease with which they can be kept under laboratory conditions (given that the next longest lived animal, with an MLSP of 211, is the bowhead whale[63]). Like all molluscs, *Arctica* spp. are complex creatures with a gut, muscles and, above all, a nervous system that appears to remain functional for periods of several centuries.

Ironically, other species of mollusc (*Aplysia* and *Lymnaea* spp.) have been used for over 40 years to unravel the mechanisms underlying synaptic communication in the CNS. Their large neurons and the restricted complexity of molluscan nervous systems (~25,000 neurons) have allowed neuronal circuits that regulate feeding, respiration, reproduction and withdrawal to be well described. In many instances single key neurons are responsible for coordinating the function of these circuits, and their properties have been well characterized. Crucially, the age-related changes seen in these molluscan species mimic those seen in higher organisms at both the behavioural level (associative conditioning[64,65]) and at the level of the individual neuron[64,66–68] (TABLE 1). Perhaps even more interesting is the observation that in both

molluscan species a dichotomy exists between identified synapses that appear sensitive to the effects of age and those that are age-resistant. Studies of these synapses should provide insight into the molecular mechanisms that determine vulnerability to age-related synaptic changes. The recent commitment to fund projects to sequence the genomes of both organisms will allow an analysis of whether the observed changes reflect on the mechanisms of ageing in higher organisms.

In evolutionary terms, *Arctica* spp. is far closer to both *Lymnaea* and *Aplysia* species than to humans. As these *Lymnaea* and *Aplysia* species have provided important insights into human neurobiology, it is very possible that some neuronal features are also conserved between humans and *Arctica* spp. Thus, a study to determine whether ageing changes are seen in the CNS of *Arctica* spp. would be of exceptional interest, regardless of the result.

Conclusions

In summary, several of the mechanisms that biogerontologists have identified as being probable players in the ageing of whole organisms remain relatively little studied within the context of the CNS. Considered within this context, several interesting paradoxes emerge. First, oxidative stress may have a significant role in neuronal ageing but seems less important in limiting lifespan. Second, treatment with rapamycin seems to improve cognition in animal models of disease states but seems to inhibit learning and memory in the normal situation. We argue that these gaps in our understanding could, and should, be closed. An intensive study of the effects of rapalogues in the normal ageing brain or the use of inducible transgenics for disease states, such as Alzheimer's disease, seem sensible places to start.

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doi:10.1038/nrn3230

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Acknowledgements
The authors gratefully acknowledge the financial support of the Glenn Foundation, Research Into Ageing and the Biotechnology and Biological Sciences Research Council (G015147/1).

Competing interests statement
The authors declare no competing financial interests.

DATABASES
Pathway Interaction Database: http://pid.nci.nih.gov

FURTHER INFORMATION
Mark Yeoman's homepage: http://www.brighton.ac.uk/pharmacy/contact/details.php?uid=msy3
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