Neural plasticity in the ageing brain

Sara N. Burke and Carol A. Barnes*‡§*

Abstract | The mechanisms involved in plasticity in the nervous system are thought to support cognition, and some of these processes are affected during normal ageing. Notably, cognitive functions that rely on the medial temporal lobe and prefrontal cortex, such as learning, memory and executive function, show considerable age-related decline. It is therefore not surprising that several neural mechanisms in these brain areas also seem to be particularly vulnerable during the ageing process. In this review, we discuss major advances in our understanding of age-related changes in the medial temporal lobe and prefrontal cortex and how these changes in functional plasticity contribute to behavioural impairments in the absence of significant pathology.

Stereological principles
A set of rules that allows objective counting of the number of objects in a three-dimensional structure independent of the size of the objects. Among these is the dissector principle, which ensures that objects are sampled with a probability that is proportional to their number and not their size.

**Neural Systems, Memory, and Aging Division, Life Sciences North Building, Room 384, University of Arizona, Tucson, Arizona 85724, USA. ‡Department of Psychology, 1503 East University Boulevard, University of Arizona, Tucson, Arizona 85721, USA. §Department of Neurology, 1501 North Campbell Avenue, University of Arizona, Tucson, Arizona 85724, USA. Correspondence to C.A.B. e-mail: carol@nsma.arizona.edu*
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Ageing is associated with a decline in cognitive function that can, in part, be explained by changes in neural plasticity or cellular alterations that directly affect mechanisms of plasticity. Although several age-related neurological changes have been identified during normal ageing, these tend to be subtle compared with the alterations that are observed in age-associated disorders, such as Alzheimer's disease and Parkinson's disease. Moreover, understanding age-related changes in cognition sets a background against which it is possible to assess the effects of pathological disease states.

In this review, we discuss functional alterations that occur during normal ageing in the medial temporal lobe and the prefrontal cortex (PFC) and how these age-associated changes might contribute to the selective cognitive impairments that occur in advanced age. We first discuss data that suggest that profound loss of neurons does not significantly contribute to age-related cognitive impairments. We then review the subtle changes in neuronal morphology, cell–cell interactions and gene expression that might contribute to alterations in plasticity in aged animals and how these changes disrupt the network dynamics of aged neuronal ensembles that ultimately contribute to selective behavioural impairments.

Morphology of the ageing brain

Age-related changes in the morphology of neurons are selective and it seems that there is no universal pattern across the entire brain. However, one finding that does seem to be consistent is that in most brain areas neuronal loss does not have a significant role in age-related cognitive decline. Rather, small, region-specific changes in dendritic branching and spine density are more characteristic of the effects of ageing on neuronal morphology (FIG. 1). This is contrary to early investigations of aged nervous tissue in which profound neuron loss was reported to occur in advanced age.

In 1955, Brody was the first to suggest that age-related reductions in brain weight were due, in part, to a decline in neuron number in all cortical layers[1]. Subsequent investigations corroborated this work, reporting a 10–60% decline in cortical neuron density between late childhood and old age[2]. In addition, profound cell loss was found in the hippocampus of ageing humans[3] and the hippocampus and PFC of non-human primates[4]. The data obtained from these early reports, however, were confounded by various technical and methodological issues, such as tissue processing and sampling design, that later called into question their accuracy[5].

In the 1980s, when new stereological principles were developed, it became possible to identify and eliminate many of the confounding factors of the previous studies that had indicated a profound decline in neuron number occurring in advanced age[6]. The resulting conclusion was that in humans[7,8], non-human primates[9–12] and rodents[13–15], significant cell death in the hippocampus and neocortex is not characteristic of normal ageing. A notable exception to this idea, however, has recently been reported. In aged non-human primates, there is a ~30% reduction in neuron number in all layers in area 8A of the dorsolateral PFC, which significantly correlates with impaired performance on a working memory task. By contrast, area 46 of the PFC shows conservation of neuron number[16].

Similar to early reports of a decline in neuronal density with ageing, early investigations of dendritic branching suggested massive deterioration in the human entorhinal cortex and hippocampus[17,18] (FIG. 1a). These experiments,

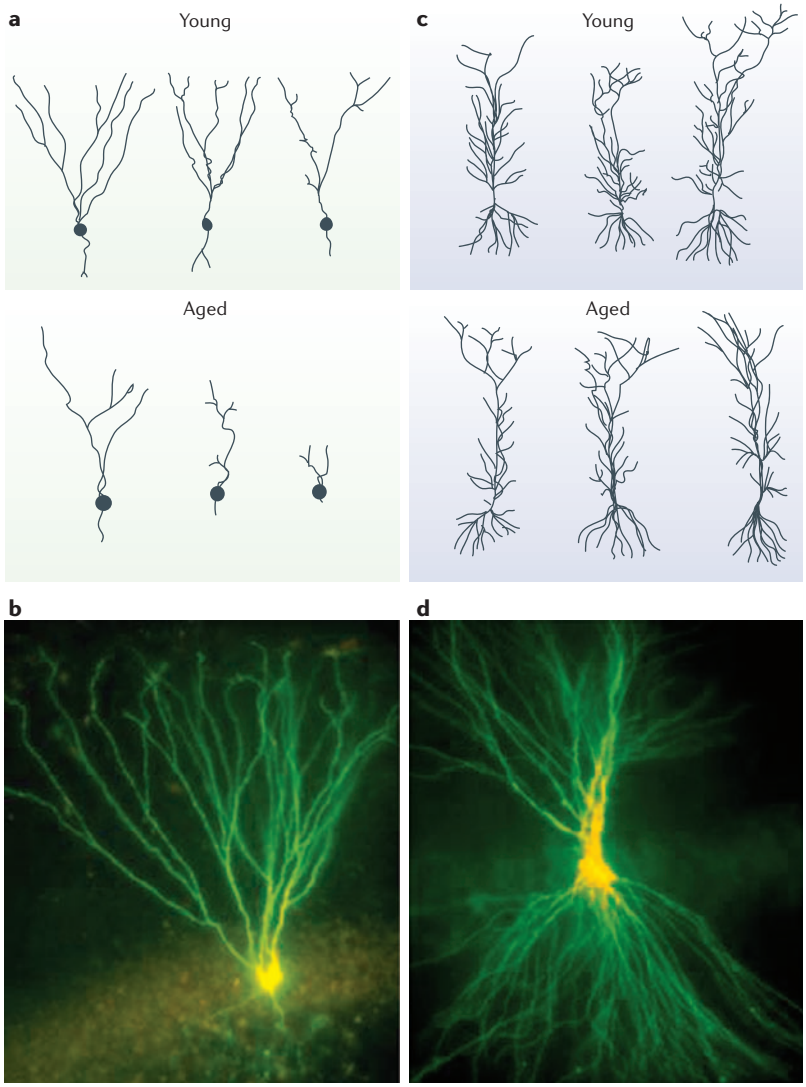


Figure 1 | **The myth of brain ageing.** A common misconception about normal ageing is that significant cell loss and dramatic changes in neuronal morphology occur. **a** | This example shows progressive loss of the dendritic surface in aged human dentate gyrus granule cells. These data do not accurately reflect the subtle and selective morphological alterations that actually occur in aged neurons, however. Age-associated loss of dendritic extent in the dentate gyrus and CA1 was exaggerated by including healthy aged individuals and those with dementia in the same experimental group, and not using stereological controls. **b** | Two representative granule cells filled with 5,6-carboxyfluorescein from the dentate gyrus of a 24-month-old rat. In the rat dentate gyrus, there is no significant change in dendritic extent between young and old animals, but there is a significant increase in electrotonic coupling (REF. 71; C.A.B., unpublished observations). **c** | Reconstructions of representative hippocampal CA1 neurons from young rats (2 months) and old rats (24 months). There is no reduction in dendritic branching or length with age in area CA1. **d** | A CA3 neuron filled with 5,6-carboxyfluorescein from a 24-month-old rat. There is no regression of dendrites but the aged cells show a significant increase in the number of gap junctions compared with young cells[72]. Panel **a** modified, with permission, from REF. 17 © (1976) Elsevier Science. Panel **c** reproduced, with permission, from REF. 28 © (1996) Elsevier Science. Panel **d** reproduced, with permission, from REF. 168 © (1986) Elsevier Science.

however, included both healthy individuals and people with dementia. Subsequent investigations, which were more precisely controlled for the participants' mental status and applied stereological controls, found that normal aged individuals had extensive dendritic branching in layer II of the parahippocampal gyrus, the origin of the perforant pathway to the dentate gyrus[19,20]. Moreover, dendritic branching and length appeared to be greater in aged individuals than in younger adults or patients with senile dementia. Other investigations have reported increased dendritic extent in the dentate gyrus of old compared with middle-aged humans[21,22]. In other subregions of the human hippocampus, however, including areas CA1 (REF. 23) and CA3 (REF. 24), and the subiculum[25], there is no change in dendritic branching with age.

Studies of dendritic extent in other animals have, in general, confirmed that there is no regression of dendrites with age. In rats, there is no significant change in dendritic length of hippocampal granule cells between young (3 months), middle-aged (12–20 months) and aged (27–30 months) rats, with a trend towards an increase between middle-age (20 months) and old age (27 months)[26]. There is also no decrease in dendritic extent between young (3 months) and old rats (26 months) in area CA1 (REFS 27,28), although there is some evidence that a small subset of CA1 neurons from 24-month-old rats have increased basilar dendritic length and branching compared with 2-month-old rats[28] (but see also REF. 29).

The morphology of PFC neurons seems to be more vulnerable to the effects of ageing than that of hippocampal neurons. In rats, dendritic branching of pyramidal neurons decreases with age for both apical and basal dendrites in superficial cortical layers[30]. A reduction in dendritic branching with age has also been observed in anterior cingulate layer V of the rat[31] and the human medial PFC[32,33].

Similar to the investigations on dendritic branching during ageing, the data on spine density suggest that age-associated alterations are also region-specific. Even in the hippocampus, changes in spine density are not consistent across subregions. In the dentate gyrus, there is no significant reduction in spine density in aged humans[34] or rats[35]. There is also no reduction in spine density in area CA1 in aged compared with young rats[29]. In the subiculum of non-human primates, however, significant reductions in spine density with age have been observed in monkeys between the ages of 7 and 28 years[36].

Biophysical properties of aged neurons

In all subregions of the hippocampus, most electrical properties remain constant over the lifespan[37]. These include resting membrane potential[27,38–45]; membrane time constant[27,46,47]; input resistance[39–48] (but see also REF. 49); threshold to reach an action potential[42,47]; and the width and amplitude of Na^+ action potentials[27,40,42–45,47,50]. Numerous studies, however, have shown an increase in Ca^{2+} conductance in aged neurons. CA1 pyramidal cells in the aged hippocampus have an increased density of L-type Ca^{2+} channels[51] that might lead to disruptions in Ca^{2+} homeostasis[52], contributing to the plasticity deficits that occur during ageing[53,54]. Moreover, Ca^{2+} activates outward K^+ currents that are responsible for the after-hyperpolarizing potential (AHP) that follows a burst of action potentials[41,43]. Aged neurons in areas CA1 and CA3

have an increase in the amplitude of the AHP that results, at least in part, from age-related increases in Ca^{2+} conductance[41,50]. Other factors that might contribute to the larger AHP in aged animals include reduced basal cyclic AMP (cAMP) levels[55].

The larger AHP observed in aged hippocampal neurons suggests that aged CA1 pyramidal cells are less excitable, as they are further from action potential threshold than are young neurons during the AHP. The only evidence that supports this idea is the finding that, in an *in vitro* hippocampal slice preparation, aged CA1 neurons fire fewer action potentials than do young neurons in response to a prolonged depolarization[50]. This is not the case, however, when pyramidal neurons are recorded *in vivo* under normal physiological conditions. In awake, behaving rats, there is no difference in the firing rates of CA1 pyramidal neurons according to age[56–64], and, in fact, the firing rates of CA3 pyramidal neurons are actually slightly higher in aged than young rats[64].

Similar to neurons in the hippocampus, many electrophysiological properties of neurons in the PFC remain the same during normal ageing, including resting membrane potential; membrane time constant; threshold to elicit an action potential; and rise time and duration of an action potential[65]. There is some evidence of a small increase in the input resistance in PFC neurons of aged monkeys as well as a decrease in the amplitude and fall time of action potentials[65]. However, cognitive performance is not related to action potential amplitude, action potential fall time or input resistance[65]. Neurons in the PFC of aged monkeys also have a significantly larger AHP compared with young neurons[65], which suggests that Ca^{2+} homeostasis might also be disrupted in PFC neurons in advanced age.

Changes in cell–cell interactions

Aged animals have alterations in the mechanisms of plasticity that contribute to cognitive functions. One functional alteration that could directly affect plasticity is reduced synapse number, which could make it more difficult to attain the sufficient amount of cooperatively active synapses that is necessary to lead to network modification. An early electron microscopic investigation at the perforant path–granule cell synapse showed that aged rats have a 27% decrease in axodendritic synapse number in the middle molecular layer of the dentate gyrus compared with young rats[66,67]. Moreover, spatial memory deficits have been shown to correlate with a reduction in perforated synapses at the medial perforant path–granule cell synapse[68]. When these results were replicated with stereologically controlled measures of synapse number, the total number of synaptic contacts per neuron was found to be diminished significantly in the dentate gyrus middle molecular layer and inner molecular layer of aged rats relative to young adults. Both perforated and non-perforated axospinous synapses showed age-dependent decreases in numbers[69]. The primary difference between the new stereological synapse count data and the old synapse count data is the observation that age-related synaptic loss involves axospinous, but not axodendritic, junctions.

Electrophysiological data support the anatomical observation that there is a reduction in synapse number in the dentate gyrus of older animals. In the aged rat, the field excitatory postsynaptic potential (EPSP) recorded in the dentate gyrus is reduced[38,47]. This reduction is accompanied by a decrease in the presynaptic fibre potential amplitude at the perforant path–dentate gyrus granule cell synapse[47,70]. Because there is no loss of entorhinal cells[13], this decrease is probably due to a reduction in axon collaterals from layer II of the entorhinal cortex to the granule cells. Interestingly, whereas the field EPSP in the aged dentate gyrus decreases, for a given magnitude of afferent fibre response, old animals show a larger synaptic field potential[47], which indicates that fewer fibres are able to elicit larger postsynaptic currents in aged animals. Consistent with this, the unitary EPSP is increased in old granule cells in response to stimulation of single afferent perforant path fibres[71]. The increase in unitary EPSP size in the dentate gyrus is probably mediated by an increase in AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor currents and is suggestive of a compensatory mechanism that increases postsynaptic sensitivity in response to the reduced medial perforant path input[39,47,71]. Another possible mediator of the larger currents is the increase in electrotonic coupling that is observed between aged hippocampal neurons. In fact, there is a 15% increase in the number of gap junctions between granule cells in aged compared with young rats (FIG. 1b). Old rats also show an increase in electrotonic coupling between neurons in areas CA1 (15% more gap junctions; FIG. 1d) and CA3 (18% more gap junctions) compared with young rats[72].

A reduction in axospinous synapses in the dentate gyrus is correlated with spatial memory deficits in aged rats[68]. This is not the case for Schaffer collateral–CA1 synapses, as the total synapse number remains the same across different age groups[73]. When the postsynaptic density area of axospinous synapses in area CA1 is compared between aged learning-impaired and learning-unimpaired rats, however, the impaired animals show a profound reduction in the postsynaptic density area of perforated synapses[74]. These findings support the idea that many hippocampal perforated synapses become non-functional or silent in aged learning-impaired rats, and this loss of functional synapses might contribute to cognitive decline during normal ageing. Electrophysiological data also support the hypothesis that there is a loss of functional synapses in area CA1. The amplitude of the field EPSP recorded in area CA1 of aged rats is reduced compared with young rats[39,75,76]. The unitary EPSP size in the CA1 region of the hippocampus in aged rats is preserved[39], however, and there is no change in the amplitude of the Schaffer collateral presynaptic fibre potential[77–79], which indicates that there is no axonal pruning of aged Schaffer collateral fibres. Interestingly, the postsynaptic density area of perforated synapses declines most significantly in aged learning-impaired Long Evans rats[74]. In an electrophysiological study, however, the field EPSP amplitude was found to be reduced in both impaired and unimpaired aged Fischer 344 rats[80], whereas plasticity mechanisms were

defective only in the impaired group[80]. Combined, these data implicate the perforated synapse postsynaptic density area in plasticity mechanisms independent of fast synaptic transmission processes.

The effects of altered morphology, biophysical properties and synaptic connections of aged neurons on plasticity can be assessed by measuring age-associated alterations in long-term potentiation (LTP) and long-term depression (LTD).

LTP can be divided into an induction phase (early-phase LTP) and a maintenance phase (late-phase LTP). The induction phase involves the temporal association of presynaptic glutamate release with postsynaptic depolarization (necessary to eject Mg^{2+} from the pores of NMDA (*N*-methyl-D-aspartate) receptors), which results in an increase in intracellular Ca^{2+} (REF. 81). LTP maintenance is the continued expression of increased synaptic efficacy that persists after induction. It probably involves changes in gene expression and insertion of AMPA receptors into the postsynaptic membrane[82]. Aged rats have deficits in both LTP induction and maintenance. These deficits, however, are complex, protocol-dependent and region-specific.

Although there is a reduction in the field EPSPs recorded both in the dentate gyrus[47,71] and in area CA1 (REFS 39,75,76), aged animals can show intact LTP induction at the perforant path–granule cell synapse[38,83,84], the CA3–CA1 Schaffer collateral synapse[85,86] and the perforant path–CA3 pyramidal cell synapse[87] when robust high-frequency, high current amplitude stimulation protocols are used (FIG. 2a). Even when supra-threshold stimulation parameters are used, however, aged rats have a deficit in the maintenance of LTP in both the dentate gyrus[38,47] and CA3 compared with young rats[87].

When peri-threshold stimulation parameters are used, LTP induction deficits can be observed in both the dentate gyrus and CA1. In the dentate gyrus, when weak presynaptic stimulation is combined with direct depolarization of the granule cell, larger amplitude current injection is required to elicit LTP at the perforant path–granule cell synapse of aged rats compared with young rats[88]. This indicates that aged granule cells in the dentate gyrus have an increased threshold for LTP induction.

The pattern of age-related LTP deficits in CA1 pyramidal cells is different from that observed in the dentate gyrus. Aged neurons in area CA1 do not have an increased threshold for LTP[89], but when peri-threshold stimulation parameters are used, the level of LTP induction in aged rats is less than in young rats[78,80,90,91] (FIG. 2b). For example, when LTP induction is measured in young and old rat hippocampal slices using four-pulse stimulation at 100 Hz[90] or the primed-burst protocol, in which a single priming pulse is followed 170 ms later by four stimulus pulses at 200 Hz[91], the increase in field EPSP slope is less in the aged rats than in the young rats. These induction deficits occur even if the stimulus intensity of the Schaffer collaterals is increased to match field EPSP amplitudes between young and aged rats[78]. Although the aged rats' Schaffer collateral axons can follow high-frequency stimulation as well as those of young rats, aged CA1 neurons show weaker temporal summation of the multiple EPSPs induced by high-frequency stimulation. Therefore, during high-frequency bursts, CA1 pyramidal cells are less depolarized, which explains the age-related LTP induction impairment in CA1 (REF. 78).

It is possible that age-related changes in Ca^{2+} regulation cause some portion of the observed age-related plasticity deficits. In particular, it has been proposed that postsynaptic intracellular Ca^{2+} concentrations are involved in setting the synaptic modification threshold. This threshold might then affect the probability that a synapse will be depressed or potentiated at a given time[53,92,93]. Ca^{2+} dyshomeostasis in aged animals[51,53,54] could, therefore, alter the probability that synaptic activity will induce either LTP or LTD. This idea is supported by Ca^{2+} imaging studies, which have shown that the resting Ca^{2+} concentration does not differ substantially with age in area CA1. Greater elevation of somatic Ca^{2+} and greater depression of EPSP frequency facilitation, however, develop in aged CA1 neurons in response to stimulation[94].

In line with the Ca^{2+} hypothesis of age-related plasticity impairments is the finding that aged rats are more susceptible than are young rats to LTD[95] (FIG. 2c) and to the reversal of LTP[53] (FIG. 2d). Moreover, it was recently shown that inhibition of Ca^{2+} release from intracellular Ca^{2+} stores attenuated LTD induction in aged CA1 neurons[96] (FIG. 2c).

Age-related changes in gene expression

It is known that the maintenance of LTP requires gene expression and *de novo* protein synthesis; therefore, it is not surprising that aged animals also show alterations in these processes. The investigation of the role of immediate-early genes (IEGs) in neural plasticity began in 1987 with the observation that the IEG *c-fos* is rapidly induced in neurons following seizures[97]. Subsequent investigations showed that IEGs are expressed following LTP induction[98–100], which led to the hypothesis that IEGs are dynamically regulated by specific forms of patterned synaptic activity believed to underlie information storage[98]. It was later shown that IEGs are expressed by cells that are activated during behaviour such as spatial exploration[101].

A crucial event for the induction of expression of IEGs is the phosphorylation of CREB (cAMP-responsive element-binding protein). Once phosphorylated, CREB promotes the transcription of IEG mRNA that may then be translated into protein. On the basis of the functional role of the protein, IEGs can be grouped into two classes: inducible transcription factors and effector proteins. Among the inducible transcription factors are c-*jun*, c-*fos* and *zif*268. After c-*jun* and c-*fos* mRNA are translated into protein, their protein products can form a heterodimer called the activator protein 1 (AP1) complex. AP1 is a transcription factor that promotes the expression of late-response genes, some of which are important for the growth of new synapses or the modification of synaptic structure[102,103]. The expression of *zif*268 is necessary, although probably not sufficient, for the maintenance of LTP and long-term memory[104].

Long-term potentiation
The physiological mechanism for selectively increasing synaptic weight distributions to develop the associations between neurons that are necessary for learning and memory.

Long-term depression
A mechanism for selectively decreasing synaptic weights so that new associations can be stored in the network without reaching saturation.

Immediate-early gene
(IEG). Any gene whose expression does not require the activation of any other responsive genes or *de novo* protein synthesis.

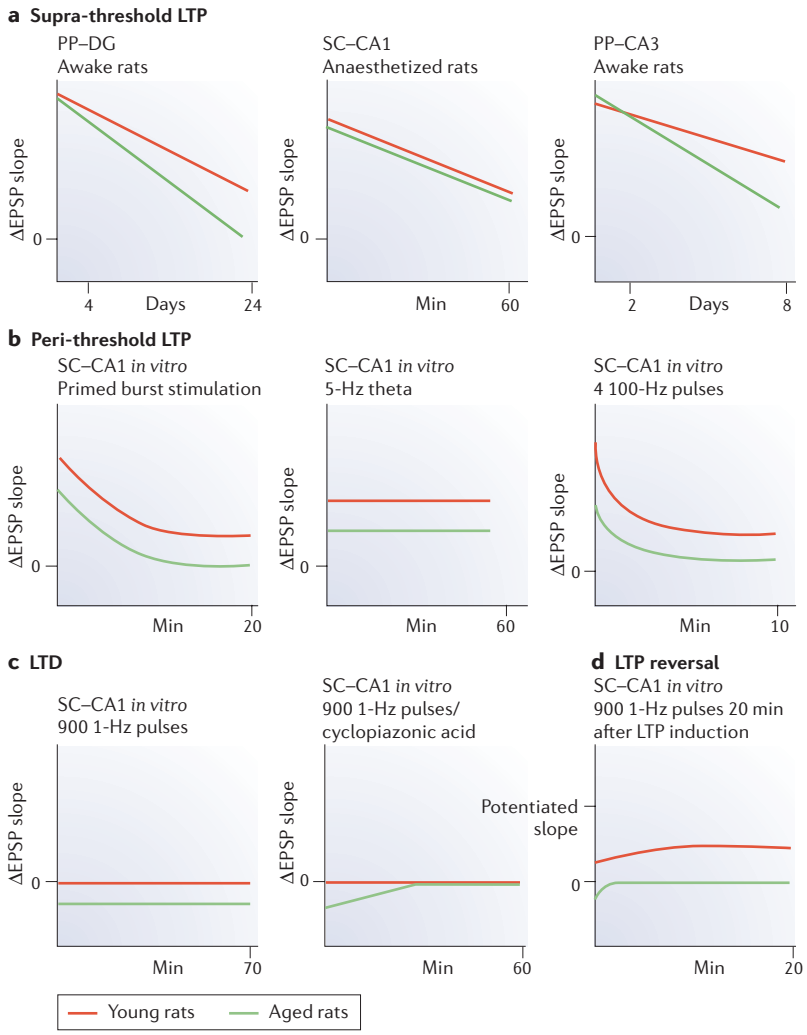


Figure 2 | **Summary of age-related alterations in long-term potentiation and long-term depression between young and aged animals.** The *y* axes show the change in excitatory postsynaptic potential (EPSP) slope following the induction of long-term potentiation (LTP) or long-term depression (LTD), and the *x* axes show the retention intervals for maintenance of LTP or LTD. Red lines, young rats; green lines, aged rats. **a** | When supra-threshold stimulation parameters are used, LTP induction is intact at old hippocampal synapses[38,86,87] but decay over days in the dentate gyrus (DG)[38] and area CA3 (REF. 87) is faster in aged rats. **b** | When peri-threshold stimulation parameters are used, aged rats can show LTP induction deficits[89–91]. **c** | In area CA1, aged rats are more susceptible to LTD induction[95]. In old rats, however, LTD induction with low-frequency stimulation (LFS) can be attenuated by agents such as cyclopiazonic acid that prevent the release of Ca^{2+} from internal Ca^{2+} stores[96]. **d** | Aged rats are also more susceptible than are young rats to the reversal of LTP. The increase in EPSP slope that results from LTP-inducing stimuli can be attenuated by the application of LFS to the potentiated pathway. In young rats, LTP is not completely reversed by LFS and there is still some residual potentiation. In old rats, however, LFS returns the EPSP slope to the baseline pre-LTP levels[95]. PP, perforant path; SC, Schaffer collateral. Data in panel **a** from REF. 38 (left), REF. 86 (centre) and REF. 87 (right). Data in panel **b** from REF. 91 (left), REF. 89 (centre) and REF. 90 (right).

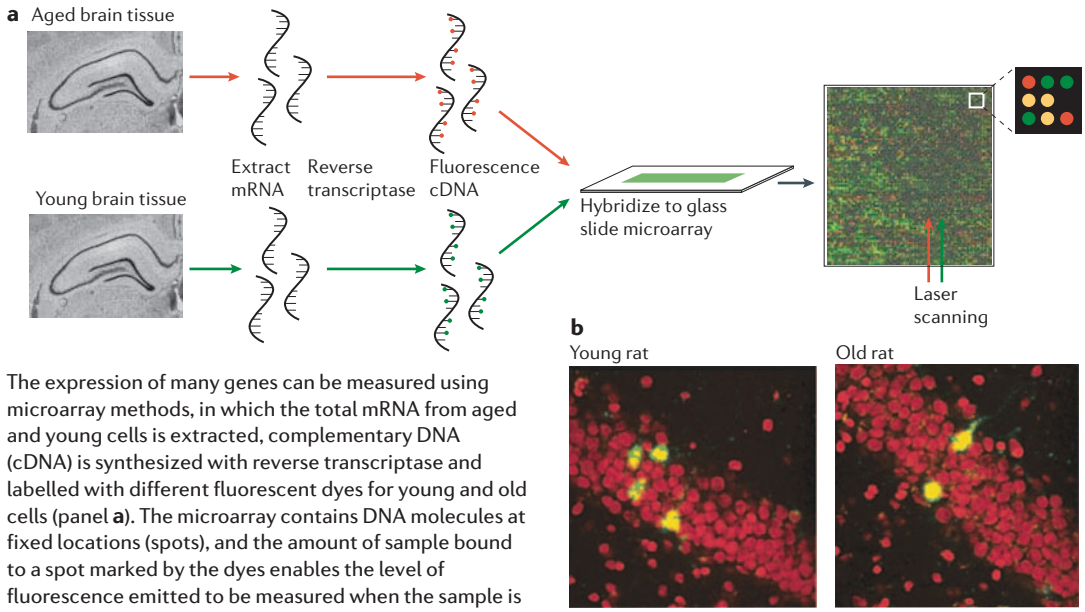
Among the effector IEGs are *Narp* (neuronal activity-regulated pentraxin) and *Arc* (activity-regulated cytoskeletal gene). After transcription, *Narp* mRNA translocates to the synapse[105], where it is released and may act to cluster AMPA receptors on the postsynaptic membrane[106]. After transcription, *Arc* mRNA localizes selectively to the region of the dendrite that receives the synaptic input that initiated transcription[107], and is proposed to be involved in the structural rearrangement of activated dendrites[108]. This probably involves AMPA receptor trafficking, as Arc protein has also been shown to reduce AMPA receptor currents (P. F. Worley, personal communication). Finally, *Arc* expression is necessary for the maintenance, but not the induction, of LTP and long-term memory[109].

Age-associated changes in gene expression have been investigated using several techniques (BOX 1), each of which has specific advantages and disadvantages. Gene microarray technology allows researchers to monitor the expression level of thousands of genes in a given brain region and make comparisons between young and old animals. The initial use of this method, in mice, showed age-related alterations in the expression of hundreds of genes[110,111], but did not involve the use of formal statistical tools to evaluate age effects and their behavioural relevance. In a later study that used behaviourally characterized rats, gene expression alterations in area CA1 were found to correlate with age-related cognitive decline. The behaviourally relevant upregulated genes included several that are associated with inflammation and intracellular Ca^{2+} release pathways, whereas genes associated with energy metabolism, biosynthesis and activity-regulated synaptogenesis were downregulated. *Arc* and *Narp* were two of the genes that were shown to be downregulated[112]. These results should be interpreted with caution, however, as the data reflect resting levels of gene expression. As many of the genes that are necessary for learning and memory are only robustly expressed after synaptic activity, resting levels of expression might not capture an age difference that may occur in gene expression during behaviour.

Northern blots can be used to measure the amount of RNA transcribed from a particular gene. When this technique was used to compare resting levels of c-*fos*, c-*jun* and *AP1* activity between young and aged rats there was no age-associated difference[113]. The expression levels of *Arc*, c-*fos*, c-*jun*, *zif*268 and *Narp* mRNA have been measured following LTP-inducing stimuli using a reverse northern strategy. In adult and aged memory-impaired rats, the induced levels of ARC, c-jun, junB, Zif268 and NARP mRNA are similar but the amount of c-fos mRNA is significantly higher in aged animals[114]. Both the microarray and northern blot techniques are limited, however, by their lack of cell specificity.

Changes in the proportion of cells that express a gene can be assessed using fluorescence *in situ* hybridization. This allows exact determination of which individual cells are expressing which genes. For example, in aged rats, granule cells of the dentate gyrus, but not the pyramidal cells of areas CA1 and CA3, have a significantly smaller proportion of neurons that express *Arc* following spatial exploration[115]. Interestingly, in studies using MRI methods in humans and monkeys, the granule cells also seem to be particularly vulnerable to the effects of normal ageing[115,116]. Fluorescence *in situ* hybridization alone, however, does not allow determination of the magnitude of expression of a particular gene in a cell.

Box 1 | **Measuring age-associated changes in gene expression**



The expression of many genes can be measured using microarray methods, in which the total mRNA from aged and young cells is extracted, complementary DNA (cDNA) is synthesized with reverse transcriptase and labelled with different fluorescent dyes for young and old cells (panel **a**). The microarray contains DNA molecules at fixed locations (spots), and the amount of sample bound to a spot marked by the dyes enables the level of fluorescence emitted to be measured when the sample is excited by a laser. In traditional paired-subject comparisons, the old and young tissue is bound to a single array and if the mRNA from the young cells is in abundance the spot will be green, whereas if the aged cells have more mRNA it will be red, and if both are equal the spot will be yellow. Note that other approaches have been used in which samples from single animals are placed on a single chip, and comparisons are made across chips[112]. Microarrays have been used to reveal that rats show age-related differences in the expression of several genes.

Following behavioural induction, the proportion of cells expressing a gene in a specific brain structure can be measured with fluorescence *in situ* hybridization. Panel **b** shows confocal images of fluorescence *in situ* hybridization for *Arc* mRNA in the dentate gyrus of a young rat and an old rat. Granule cells are shown in red and *Arc* mRNA in yellow. After spatial exploration, more granule cells are positively labelled for *Arc* in young than old rats[115]. The quantity of mRNA transcribed from a gene following behavioural induction can be measured using real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) methods. Again, mRNA is extracted from brain tissue of young and old rats, synthesized to cDNA and fluorescently labelled using reverse transcriptase. The cDNA is then logarithmically amplified with the RT-PCR reaction. Interestingly, when RT-PCR is combined with *in situ* hybridization, old CA1 pyramidal cells have less c-*fos* per cell than the cells of young animals. Hippocampal images in panel **a** reproduced, with permission, from REF. 164 © (1998) Academic Press; panel **b** reproduced, with permission, from REF. 115 © (2004) National Academy of Sciences.

By using real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) concurrently with fluorescence *in situ* hybridization, changes in gene expression levels within a single cell can be determined. For example, in area CA1, the proportion of cells that express c-*fos* mRNA is similar between young and aged rats but when RT-PCR is carried out, young rats are found to have higher levels of c-*fos* mRNA compared with the old animals (M. K. Chawla, unpublished observations). This indicates that although a similar number of pyramidal neurons express c-*fos* across different age groups, the individual cells from old animals transcribe less c-*fos* mRNA, which may lead to dysregulation of other genes that depend on the AP1 transcription factor.

Reverse northern strategy
A technique in which levels of tissue mRNA are assessed by monitoring the intensity of the hybridization signal of radiolabelled cDNA prepared from tissue RNA to Southern blots containing cloned cDNAs of multiple candidate genes. The hybridization signal for each gene is indicative of the tissue mRNA level.

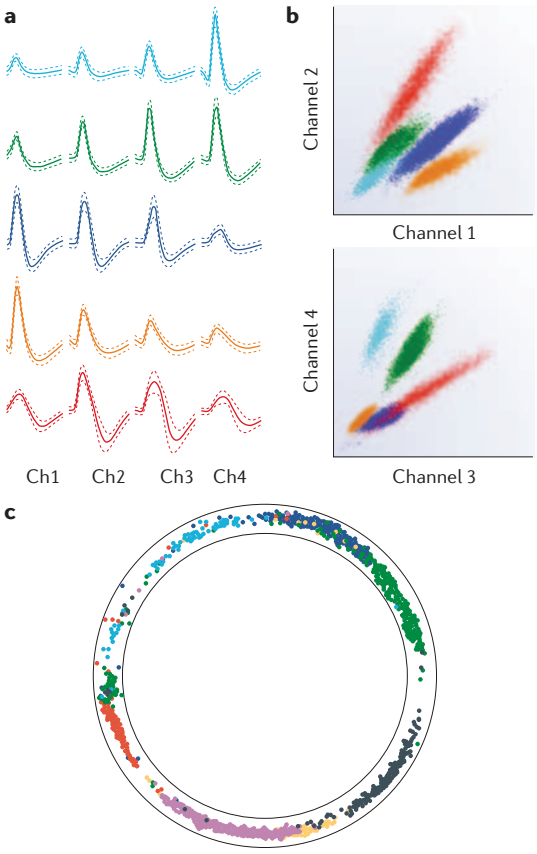
Dynamics of aged neural ensembles

It is widely agreed that modifiable neuronal ensembles support cognition. Therefore, alterations in these networks could be responsible for the behavioural impairments observed with ageing. Advances in multiple single unit recording methods (BOX 2) have allowed the dynamics of hippocampal cell populations to be investigated in behaving rats, and studies using these methods have shown that certain properties of these networks are compromised during ageing. Interestingly, many of the age-related changes that have been discovered can be linked to plasticity deficits, as blockade of NMDA receptors in young rats results in ensemble dynamics that resemble those of aged rats[117,118].

Neuronal recordings from the hippocampus of adult rats reveal that when a rat explores an environment, pyramidal[119] and granule[120] cells show patterned neural activity that is highly correlated with a rat's position in space (that is, the 'place field' of the cell; BOX 2). Between 30% and 50% of CA1 pyramidal cells show place-specific firing in a given environment[101,121], which has earned these neurons the name 'place cells'. When the firing patterns of many hippocampal neurons are recorded simultaneously, it is possible to reconstruct the position of a rat in an environment from the place cell firing patterns alone[121]. The composite cell activity is 'map-like' and, in different environments, hippocampal

Box 2 | **Investigating neuronal ensembles with multiple single unit recording methods**

Recordings of more than 100 cells can be obtained from a 'hyperdrive' device that is permanently mounted on a rat's head[120,165], enabling the recording of extracellular action potentials in freely moving animals. The tetrode recording probe used consists of four twisted 13-μm wires, each providing a different recording channel[120,166,167]. Cells can be distinguished from each other offline on the basis of the relative amplitude differences of their spikes. Panel **a** shows analogue waveforms from five hippocampal cells recorded from the four tetrode channels (different cells are shown in different colours). Panel **b** shows the amplitude distributions of the neurons from panel **a**. The top panel shows the peak amplitude on channel 1 compared with channel 2, and the bottom panel shows the peak amplitude on channel 3 compared with channel 4. Note that individual cell amplitudes cluster distinctively for the different cells. Statistical clustering methods can be applied to data to identify individual cells, enabling the rat's behaviour to be correlated with the activity of single neurons. For example, principal cells of the hippocampus will fire selectively when a rat is in a specific region of the environment[118,119]. The area of the environment where a hippocampal principal cell is active is referred to as the cell's 'place field'. Panel **c** shows the place fields of seven CA1 pyramidal neurons when the rat traversed a circular track. Small dots correspond to individual spikes and the spikes from different neurons are shown in different colours (S.B. *et al.*, unpublished observations). Multiple single unit recordings have been used to reveal differences in place cell ensemble dynamics between young and aged



place maps change markedly. Although these maps can be driven by external environmental features, internal events are also important and a new map might be generated in the same environment if the demands of the task change[122–124].

In young rats, CA1 place fields expand asymmetrically during repeated route following (for example, traversing a circular track), which results in a shift in the centre of mass of place fields in the direction opposite to the rat's trajectory[125]. This observation is consistent with neural network models dating back to Hebb's 1949 concept of the 'phase sequence' of cell assemblies, which suggested that an associative, temporally asymmetric synaptic plasticity mechanism could serve to encode sequences or episodes of experience[126]. The magnitude of this place field expansion, however, significantly decreases in aged rats[60]. It is likely that this age-associated reduction in experience-dependent plasticity is due to LTP deficits, as it does not occur when the NMDA receptor antagonist CPP (3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) is administered to young rats[118].

In addition to age-related alterations in experience-dependent place field expansion, the maintenance of place maps also differs between young and old animals. In normal young rats, a place map for a given environment can remain stable for months[127]. Therefore, when a rat is returned to the same environment, the same place map is retrieved. A similar stability of CA1 place maps in aged rats is observed within and between episodes of behaviour in the same environment. Occasionally, however, if the old rat is removed from the environment and returned later, the original place map is not retrieved and an independent population of place cells may be activated even in a familiar room[59]. This 'remapping' predicts that rats should show bimodal performance on tasks that require the functional integrity of the hippocampus. For spatial tasks, good performance should correspond to retrieval of the original map, and poor performance should correspond to retrieval of an incorrect map. This prediction seems to be correct. When trained on the spatial version of the Morris swim task, the performance of both young and aged rats is bimodal in early trials. This means that for some trials rats find the hidden escape platform with a short path but for other trials the rats do not recall the location of the platform and take a longer path. By the final training trials, however, the young rats' performance is unimodal, with most rats taking a direct path to the platform. By contrast, the aged rats' performance remains bimodal. The trials on which the old rats fail to correctly remember the location of the hidden escape platform could correspond to map retrieval failures[59].

A probable mechanism for map retrieval failures is defective LTP in aged rats. Although place-map stability within an episode does not require plasticity, the maintenance of place maps between episodes depends on an

Morris swim task
The most widely used test of spatial learning and memory in rats. In this task, rats are placed into a tank of cloudy water. To escape from the water the rats need to find the location of a platform hidden just below the surface. The platform is always in the same location relative to the room and the distal cues.

LTP-like mechanism. In young rats, blockade of NMDA receptors[117] or protein synthesis inhibition[128] has been shown to result in map retrieval errors when the rat is returned to the same environment.

When CA1 and CA3 place cell recordings are pooled it seems that spatial representations in old rats do not change when they should (for example, in response to major changes in the environment)[61,129]. Combining results across areas is problematic, however, as it has recently been shown that ensemble activity in these different subregions is dissociable[130–132]. This dissociation could reflect two competing functions of the hippocampal network: pattern completion versus pattern separation[133,134]. Moreover, there is evidence of a dissociation of the effects of ageing on CA1 and CA3 ensembles. In area CA1, spatial representations are less stable in aged compared with young rats[59]. By contrast, spatial representations in CA3 seem to be more rigid in aged rats. When an aged rat explores a familiar environment for 7 min and is then placed into a novel environment, spatial representations in area CA3 remain the same even though the environment has changed[64]. In young rats, however, CA3 place maps are independent between familiar and new environments[64,131]. A disruption in the ensemble characteristics of dentate gyrus granule cells, a structure known to be particularly vulnerable to the ageing process[115,116], could contribute to the failure of aged CA3 networks to form new spatial representations. It is believed that the dentate gyrus makes information stored in hippocampal networks more dissimilar (that is, it is involved in pattern separation), thereby increasing storage capacity[133]. Because the transfer of information between granule cells and CA3 pyramidal cells declines, this might contribute to the inability of the aged CA3 network to form new spatial representations when required.

Age-related changes in behaviour

Because the hippocampus and the PFC are particularly vulnerable to the ageing process, it is not surprising that performance on tasks that require information processing in these brain regions declines with age. Below is a brief discussion of selective examples of these behavioural changes in humans, non-human primates and rats. One such example is an age-related decline in spatial memory, which is a key element of most episodic experience. Compared with younger adults, episodic memory declines in aged humans, who show deficits in retrieving the contextual details of these memories[135,136]. In addition, aged humans[137,138], monkeys[139,140], dogs[141], rats[38,142,143] and mice[144] all show deficits on tasks designed to test spatial navigation. This is consistent with the neurobiological data that suggest that hippocampal function is compromised with age[145].

An additional hippocampal-dependent impairment that is consistently observed during ageing across species is a deficit in trace eyeblink conditioning. The trace eyeblink conditioning task tests associative learning using a classical conditioning paradigm. In this task, a neutral or conditioned stimulus, which is usually a tone, is predictive of an aversive unconditioned stimulus (air puff or stimulation of eye). The time taken to acquire the eyeblink reflex to the neutral stimulus alone and the retention of this association is measured. Acquisition and retention of the learned eyeblink response both require the involvement of the hippocampus, as well as other brain structures such as the cerebellum[146]. Aged mice[147], rats[148], rabbits[149,150] and humans[151] are impaired in the acquisition of trace eyeblink conditioning.

Whereas the hippocampus is crucially involved in spatial and episodic memory, the PFC is necessary for working memory[152,153] and executive function[154]. In animals and humans, working memory function can be measured using the delayed non-matching-to-sample (DNMS) task. Aged rats[155] and non-human primates[156–158] show time-dependent deficits on the DNMS task, with the magnitude of the deficit increasing as the delay increases. Humans also show working memory impairments in advanced age[159]. The DNMS task also tests recognition memory, which depends on the perirhinal cortex[153,160,161]. It is therefore possible that age-related changes in this task could involve additional plasticity deficits in the perirhinal cortex, although little is known about the impact of ageing on the functional integrity of perirhinal circuits.

In addition to its involvement in working memory, the PFC is considered to be the neural substrate of executive function, which also declines during normal ageing. One way to measure executive function is with the Wisconsin card sorting task (WCST). Aged humans are impaired on the WCST and make more perseverative errors[162]. Animal analogues of the WCST have been designed, which also show that normal ageing leads to a decline in executive function. For example, relative to young adult monkeys, aged monkeys show a significant difficulty in the acquisition of a conceptual set shifting task and demonstrate a high degree of perseverative responding[163].

Conclusions

In summary, during the normal ageing process, animals experience age-related cognitive decline. Historically, it was thought that primary contributions to the aetiology of this decline were massive cell loss[1] and deterioration of dendritic branching[17,18]. However, we now know that the changes occurring during normal ageing are more subtle and selective than was once believed. In fact, the general pattern seems to be that most age-associated behavioural impairments result from region-specific changes in dendritic morphology, cellular connectivity, Ca^{2+} dysregulation, gene expression or other factors that affect plasticity and ultimately alter the network dynamics of neural ensembles that support cognition.

Of the brain regions affected by ageing, the hippocampus and the PFC seem to be particularly vulnerable, but even within and between these regions the impact of ageing on neuronal function can differ. The morphology of neurons in the PFC is more susceptible to age-related change, as these cells show a decrease in dendritic branching in rats[30,31] and humans[32,33]. There is also evidence of a small but significant decline in cell number in area 8A of monkeys that is correlated with working

Pattern completion
The ability of a network to retrieve an entire stored pattern when only a fragment of the pattern is presented.

Pattern separation
The ability of a network to make the stored representations of similar input patterns more dissimilar.

Delayed non-matching-to-sample task
(DNMS task). A sample stimulus is presented to the subject. After a delay, the sample is presented again, along with a new stimulus. The subject is rewarded for selecting the new stimulus.

Perirhinal cortex
High level association cortex in the medial temporal lobe that receives highly processed polymodal information from the entire cortical mantel and sends direct projections to the entorhinal cortex and hippocampus as well as back-projections to the cortex.

Wisconsin card sorting task
(WCST). Participants are required to sort response cards of different dimensions (shape, colour and number) by a particular category, which is determined by an experimenter-defined rule. Card sorting principles must be inferred. Once the sorting rule is discovered and a determined number of correct responses are made, the experimenter changes the rule and the subject must then infer the new rule.

memory impairments[16]. Although there is evidence of Ca^{2+} dysregulation in aged PFC neurons[65], the functional consequences of this are not yet known. Moreover, so far, there are no reports of multiple single unit recordings in the PFC of awake behaving animals. More is known about the impact of ageing on hippocampal function. Ca^{2+} dysregulation[51,53,54] and changes in synaptic connectivity[69,74] might affect plasticity and gene expression, resulting in altered dynamics of hippocampal neuronal ensembles. Because more is known about the neurobiology of ageing in this brain region, there are therapeutic approaches on the horizon that might modify hippocampal neurobiology and slow age-related cognitive decline or partially restore mechanisms of plasticity. For example, agents that reduce intracellular Ca^{2+} concentration following neural activity could modulate the ratio of LTD and LTP induction, thereby partially restoring normal network dynamics. Considering that the average lifespan is increasing worldwide, understanding the brain mechanisms that are responsible for age-related cognitive impairment, and finding therapeutic agents that might curb this decline, becomes increasingly important.

1. Brody, H. Organization of the cerebral cortex. III. A study of aging in the human cerebral cortex. *J. Comp. Neurol.* **102**, 511–516 (1955).
2. Coleman, P. D. & Flood, D. G. Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. *Neurobiol. Aging* **8**, 521–545 (1987).
3. Ball, M. J. Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia. A quantitative study. *Acta Neuropathol. (Berl.)* **37**, 111–118 (1977).
4. Brizzee, K. R., Ordy, J. M. & Bartus, R. T. Localization of cellular changes within multimodal sensory regions in aged monkey brain: possible implications for age-related cognitive loss. *Neurobiol. Aging* **1**, 45–52 (1980).
5. Morrison, J. H. & Hof, P. R. Life and death of neurons in the aging brain. *Science* **278**, 412–419 (1997).
6. West, M. J. New stereological methods for counting neurons. *Neurobiol. Aging* **14**, 275–285 (1993).
7. Pakkenberg, B. & Gundersen, H. J. Neocortical neuron number in humans: effect of sex and age. *J. Comp. Neurol.* **384**, 312–320 (1997).
8. West, M. J., Coleman, P. D., Flood, D. G. & Troncoso, J. C. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* **344**, 769–772 (1994).
A stereologically controlled investigation reporting preserved neuron number in most subregions of the hippocampus of healthy aged humans, which is distinct from individuals with Alzheimer's disease who show a significant decline in cell number.
9. Merrill, D. A., Roberts, J. A. & Tuszynski, M. H. Conservation of neuron number and size in entorhinal cortex layers II, III, and V/VI of aged primates. *J. Comp. Neurol.* **422**, 396–401 (2000).
10. Peters, A., Leahu, D., Moss, M. B. & McNally, K. J. The effects of aging on area 46 of the frontal cortex of the rhesus monkey. *Cereb. Cortex* **4**, 621–635 (1994).
11. Gazzaley, A. H., Thakker, M. M., Hof, P. R. & Morrison, J. H. Preserved number of entorhinal cortex layer II neurons in aged macaque monkeys. *Neurobiol. Aging* **18**, 549–553 (1997).
12. Keuker, J. I., Luiten, P. G. & Fuchs, E. Preservation of hippocampal neuron numbers in aged rhesus monkeys. *Neurobiol. Aging* **24**, 157–165 (2003).
13. Merrill, D. A., Chiba, A. A. & Tuszynski, M. H. Conservation of neuronal number and size in the entorhinal cortex of behaviorally characterized aged rats. *J. Comp. Neurol.* **438**, 445–456 (2001).
14. Rapp, P. R. & Gallagher, M. Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc. Natl Acad. Sci. USA* **93**, 9926–9930 (1996).
Similar to findings in humans, this stereologically controlled experiment reveals that aged rats have no neuronal loss and so cell death cannot account for age-related behavioural impairments.
15. Rasmussen, T., Schliemann, T., Sorensen, J. C., Zimmer, J. & West, M. J. Memory impaired aged rats: no loss of principal hippocampal and subicular neurons. *Neurobiol. Aging* **17**, 143–147 (1996).
16. Smith, D. E., Rapp, P. R., McKay, H. M., Roberts, J. A. & Tuszynski, M. H. Memory impairment in aged primates is associated with focal death of cortical neurons and atrophy of subcortical neurons. *J. Neurosci.* **24**, 4373–4381 (2004).
17. Scheibel, M. E., Lindsay, R. D., Tomiyasu, U. & Scheibel, A. B. Progressive dendritic changes in the aging human limbic system. *Exp. Neurol.* **53**, 420–430 (1976).
18. Scheibel, A. B. The hippocampus: organizational patterns in health and senescence. *Mech. Ageing Dev.* **9**, 89–102 (1979).
19. Buell, S. J. & Coleman, P. D. Quantitative evidence for selective dendritic growth in normal human aging but not in senile dementia. *Brain Res.* **214**, 23–41 (1981).
20. Buell, S. J. & Coleman, P. D. Dendritic growth in the aged human brain and failure of growth in senile dementia. *Science* **206**, 854–856 (1979).
21. Flood, D. G., Buell, S. J., Defiore, C. H., Horwitz, G. J. & Coleman, P. D. Age-related dendritic growth in dentate gyrus of human brain is followed by regression in the 'oldest old'. *Brain Res.* **345**, 366–368 (1985).
22. Flood, D. G., Buell, S. J., Horwitz, G. J. & Coleman, P. D. Dendritic extent in human dentate gyrus granule cells in normal aging and senile dementia. *Brain Res.* **402**, 205–216 (1987).
23. Hanks, S. D. & Flood, D. G. Region-specific stability of dendritic extent in normal human aging and regression in Alzheimer's disease. I. CA1 of hippocampus. *Brain Res.* **540**, 63–82 (1991).
24. Flood, D. G., Guarnaccia, M. & Coleman, P. D. Dendritic extent in human CA2–3 hippocampal pyramidal neurons in normal aging and senile dementia. *Brain Res.* **409**, 88–96 (1987).
25. Flood, D. G. Region-specific stability of dendritic extent in normal human aging and regression in Alzheimer's disease. II. Subiculum. *Brain Res.* **540**, 83–95 (1991).
26. Flood, D. G. Critical issues in the analysis of dendritic extent in aging humans, primates, and rodents. *Neurobiol. Aging* **14**, 649–654 (1993).
27. Turner, D. A. & Deupree, D. L. Functional elongation of CA1 hippocampal neurons with aging in Fischer 344 rats. *Neurobiol. Aging* **12**, 201–210 (1991).
28. Pyapali, G. K. & Turner, D. A. Increased dendritic extent in hippocampal CA1 neurons from aged F344 rats. *Neurobiol. Aging* **17**, 601–611 (1996).
29. Markham, J. A., McKian, K. P., Stroup, T. S. & Juraska, J. M. Sexually dimorphic aging of dendritic morphology in CA1 of hippocampus. *Hippocampus* **15**, 97–103 (2005).
30. Grill, J. D. & Riddle, D. R. Age-related and laminar-specific dendritic changes in the medial frontal cortex of the rat. *Brain Res.* **937**, 8–21 (2002).
31. Markham, J. A. & Juraska, J. M. Aging and sex influence the anatomy of the rat anterior cingulate cortex. *Neurobiol. Aging* **23**, 579–588 (2002).
32. de Brabander, J. M., Kramers, R. J. & Uylings, H. B. Layer-specific dendritic regression of pyramidal cells with ageing in the human prefrontal cortex. *Eur. J. Neurosci.* **10**, 1261–1269 (1998).
33. Uylings, H. B. & de Brabander, J. M. Neuronal changes in normal human aging and Alzheimer's disease. *Brain Cogn.* **49**, 268–276 (2002).
34. Williams, R. S. & Matthysse, S. Age-related changes in Down syndrome brain and the cellular pathology of Alzheimer disease. *Prog. Brain Res.* **70**, 49–67 (1986).
35. Curcio, C. A. & Hinds, J. W. Stability of synaptic density and spine volume in dentate gyrus of aged rats. *Neurobiol. Aging* **4**, 77–87 (1983).
36. Uemura, E. Age-related changes in the subiculum of *Macaca mulatta*: synaptic density. *Exp. Neurol.* **87**, 403–411 (1985).
37. Barnes, C. A. Normal aging: regionally specific changes in hippocampal synaptic transmission. *Trends Neurosci.* **17**, 13–18 (1994).
A comprehensive review of hippocampal region-specific changes in synaptic transmission, along with functional sparing, which challenged the traditional concept of ageing as a process of general deterioration.
38. Barnes, C. A. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J. Comp. Physiol. Psychol.* **93**, 74–104 (1979).
The first report in aged rats of an increased LTP decay rate at the perforant path–granule cell synapse that correlates with the rate of forgetting a spatial problem on the Barnes circular platform task.
39. Barnes, C. A., Rao, G., Foster, T. C. & McNaughton, B. L. Region-specific age effects on AMPA sensitivity: electrophysiological evidence for loss of synaptic contacts in hippocampal field CA1. *Hippocampus* **2**, 457–468 (1992).
40. Segal, M. Changes in neurotransmitter actions in the aged rat hippocampus. *Neurobiol. Aging* **3**, 121–124 (1982).
41. Landfield, P. W. & Pitler, T. A. Prolonged Ca^{2+}-dependent afterhyperpolarizations in hippocampal neurons of aged rats. *Science* **226**, 1089–1092 (1984).
The first report of a significant increase in the K^+-dependent afterhyperpolarization of aged hippocampal CA1 pyramidal cells that is blocked by low concentrations of Ca^{2+}.
42. Niesen, C. E., Baskys, A. & Carlen, P. L. Reversed ethanol effects on potassium conductances in aged hippocampal dentate granule neurons. *Brain Res.* **445**, 137–141 (1988).
43. Kerr, D. S., Campbell, L. W., Hao, S. Y. & Landfield, P. W. Corticosteroid modulation of hippocampal potentials: increased effect with aging. *Science* **245**, 1505–1509 (1989).
44. Potier, B., Lamour, Y. & Dutar, P. Age-related alterations in the properties of hippocampal pyramidal neurons among rat strains. *Neurobiol. Aging* **14**, 17–25 (1993).
45. Potier, B., Rascol, O., Jazat, F., Lamour, Y. & Dutar, P. Alterations in the properties of hippocampal pyramidal neurons in the aged rat. *Neuroscience* **48**, 793–806 (1992).
46. Pitler, T. A. & Landfield, P. W. Aging-related prolongation of calcium spike duration in rat hippocampal slice neurons. *Brain Res.* **508**, 1–6 (1990).
47. Barnes, C. A. & McNaughton, B. L. Physiological compensation for loss of afferent synapses in rat hippocampal granule cells during senescence. *J. Physiol. (Lond.)* **309**, 473–485 (1980).
48. Reynolds, J. N. & Carlen, P. L. Diminished calcium currents in aged hippocampal dentate gyrus granule neurones. *Brain Res.* **479**, 384–390 (1989).
49. Luebke, J. I. & Rosene, D. L. Aging alters dendritic morphology, input resistance, and inhibitory signaling in dentate granule cells of the rhesus monkey. *J. Comp. Neurol.* **460**, 573–584 (2003).
50. Moyer, J. R. Jr, Thompson, L. T., Black, J. P. & Disterhoft, J. F. Nimodipine increases excitability of rabbit CA1 pyramidal neurons in an age- and concentration-dependent manner. *J. Neurophysiol.* **68**, 2100–2109 (1992).
51. Thibault, O. & Landfield, P. W. Increase in single L-type calcium channels in hippocampal neurons during aging. *Science* **272**, 1017–1020 (1996).
The first evidence that the increase in voltage-activated Ca^{2+} influx in aged CA1 hippocampal neurons is due to an age-related increase in L-type Ca^{2+} channels.
52. Toescu, E. C., Verkhratsky, A. & Landfield, P. W. Ca^{2+} regulation and gene expression in normal brain aging. *Trends Neurosci.* **27**, 614–620 (2004).

53. Foster, T. C. & Norris, C. M. Age-associated changes in Ca^{2+}-dependent processes: relation to hippocampal synaptic plasticity. *Hippocampus* **7**, 602–612 (1997).
54. Landfield, P. W. Hippocampal neurobiological mechanisms of age-related memory dysfunction. *Neurobiol. Aging* **9**, 571–579 (1988).
55. Tombaugh, G. C., Rowe, W. B. & Rose, G. M. The slow afterhyperpolarization in hippocampal CA1 neurons covaries with spatial learning ability in aged Fisher 344 rats. *J. Neurosci.* **25**, 2609–2616 (2005).
56. Barnes, C. A., McNaughton, B. L. & O'Keefe, J. Loss of place specificity in hippocampal complex spike cells of senescent rat. *Neurobiol. Aging* **4**, 113–119 (1983).
57. Markus, E. J., Barnes, C. A., McNaughton, B. L., Gladden, V. L. & Skaggs, W. E. Spatial information content and reliability of hippocampal CA1 neurons: effects of visual input. *Hippocampus* **4**, 410–421 (1994).
58. Mizumori, S. J., Lavoie, A. M. & Kalyani, A. Redistribution of spatial representation in the hippocampus of aged rats performing a spatial memory task. *Behav. Neurosci.* **110**, 1006–1016 (1996).
59. Barnes, C. A., Suster, M. S., Shen, J. & McNaughton, B. L. Multistability of cognitive maps in the hippocampus of old rats. *Nature* **388**, 272–275 (1997).
The first report of altered stability of hippocampal place maps in aged rats, which correlates with the bimodal performance of aged rats on the spatial version of the Morris swim task.
60. Shen, J., Barnes, C. A., McNaughton, B. L., Skaggs, W. E. & Weaver, K. L. The effect of aging on experience-dependent plasticity of hippocampal place cells. *J. Neurosci.* **17**, 6769–6782 (1997).
Many hippocampal place cells were recorded simultaneously in young and aged rats to reveal that behaviourally induced plasticity mechanisms are defective in aged rats.
61. Tanila, H., Shapiro, M., Gallagher, M. & Eichenbaum, H. Brain aging: changes in the nature of information coding by the hippocampus. *J. Neurosci.* **17**, 5155–5166 (1997).
62. Smith, A. C., Gerrard, J. L., Barnes, C. A. & McNaughton, B. L. Effect of age on burst firing characteristics of rat hippocampal pyramidal cells. *Neuroreport* **11**, 3865–3871 (2000).
63. Oler, J. A. & Markus, E. J. Age-related deficits in the ability to encode contextual change: a place cell analysis. *Hippocampus* **10**, 338–350 (2000).
64. Wilson, I. A., Ikonen, S., Gallagher, M., Eichenbaum, H. & Tanila, H. Age-associated alterations in place cells are subregion specific. *J. Neurosci.* **25**, 6877–6886 (2005).
The first paper to report a distinction between the impact of ageing on CA1 and CA3 networks. This makes it clear that these areas cannot be combined when age comparisons are made.
65. Chang, Y. M., Rosene, D. L., Killiany, R. J., Mangiamele, L. A. & Luebke, J. I. Increased action potential firing rates of layer 2/3 pyramidal cells in the prefrontal cortex are significantly related to cognitive performance in aged monkeys. *Cereb. Cortex* **15**, 409–418 (2005).
66. Bondareff, W. & Geinisman, Y. Loss of synapses in the dentate gyrus of the senescent rat. *Am. J. Anat.* **145**, 129–136 (1976).
67. Geinisman, Y., Bondareff, W. & Dodge, J. T. Partial deafferentation of neurons in the dentate gyrus of the senescent rat. *Brain Res.* **134**, 541–545 (1977).
68. Geinisman, Y., de Toledo-Morrell, L. & Morrell, F. Loss of perforated synapses in the dentate gyrus: morphological substrate of memory deficit in aged rats. *Proc. Natl Acad. Sci. USA* **83**, 3027–3031 (1986).
69. Geinisman, Y., de Toledo-Morrell, L., Morrell, F., Persina, I. S. & Rossi, M. Age-related loss of axospinous synapses formed by two afferent systems in the rat dentate gyrus as revealed by the unbiased stereological dissector technique. *Hippocampus* **2**, 437–444 (1992).
70. Barnes, C. A., Rao, G. & Houston, F. P. LTP induction threshold change in old rats at the perforant path–granule cell synapse. *Neurobiol. Aging* **21**, 613–620 (2000).
71. Foster, T. C., Barnes, C. A., Rao, G. & McNaughton, B. L. Increase in perforant path quantal size in aged F-344 rats. *Neurobiol. Aging* **12**, 441–448 (1991).
72. Barnes, C. A., Rao, G. & McNaughton, B. L. Increased electrotonic coupling in aged rat hippocampus: a possible mechanism for cellular excitability changes. *J. Comp. Neurol.* **259**, 549–558 (1987).
73. Geinisman, Y. *et al.* Aging, spatial learning, and total synapse number in the rat CA1 stratum radiatum. *Neurobiol. Aging* **25**, 407–416 (2004).
74. Nicholson, D. A., Yoshida, R., Berry, R. W., Gallagher, M. & Geinisman, Y. Reduction in size of perforated postsynaptic densities in hippocampal axospinous synapses and age-related spatial learning impairments. *J. Neurosci.* **24**, 7648–7653 (2004).
75. Landfield, P. W., Pitler, T. A. & Applegate, M. D. The effects of high Mg^{2+}-to-Ca^{2+} ratios on frequency potentiation in hippocampal slices of young and aged rats. *J. Neurophysiol.* **56**, 797–811 (1986).
76. Deupree, D. L., Bradley, J. & Turner, D. A. Age-related alterations in potentiation in the CA1 region in F344 rats. *Neurobiol. Aging* **14**, 249–258 (1993).
77. Barnes, C. A., Rao, G. & Shen, J. Age-related decrease in the *N*-methyl-D-aspartate$_R$-mediated excitatory postsynaptic potential in hippocampal region CA1. *Neurobiol. Aging* **18**, 445–452 (1997).
78. Rosenzweig, E. S., Rao, G., McNaughton, B. L. & Barnes, C. A. Role of temporal summation in age-related long-term potentiation-induction deficits. *Hippocampus* **7**, 549–558 (1997).
79. Barnes, C. A., Rao, G. & Orr, G. Age-related decrease in the Schaffer collateral-evoked EPSP in awake, freely behaving rats. *Neural Plast.* **7**, 167–178 (2000).
80. Tombaugh, G. C., Rowe, W. B., Chow, A. R., Michael, T. H. & Rose, G. M. Theta-frequency synaptic potentiation in CA1 *in vitro* distinguishes cognitively impaired from unimpaired aged Fischer 344 rats. *J. Neurosci.* **22**, 9932–9940 (2002).
81. Bliss, T. V. & Collingridge, G. L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31–39 (1993).
82. Malinow, R. & Malenka, R. C. AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* **25**, 103–126 (2002).
83. Diana, G., Domenici, M. R., Loizzo, A., Scotti de Carolis, A. & Sagratella, S. Age and strain differences in rat place learning and hippocampal dentate gyrus frequency-potentiation. *Neurosci. Lett.* **171**, 113–116 (1994).
84. Diana, G., Scotti de Carolis, A., Frank, C., Domenici, M. R. & Sagratella, S. Selective reduction of hippocampal dentate frequency potentiation in aged rats with impaired place learning. *Brain Res. Bull.* **35**, 107–111 (1994).
85. Landfield, P. W. & Lynch, G. Impaired monosynaptic potentiation in *in vitro* hippocampal slices from aged, memory-deficient rats. *J. Gerontol.* **32**, 523–533 (1977).
86. Landfield, P. W., McGaugh, J. L. & Lynch, G. Impaired synaptic potentiation processes in the hippocampus of aged, memory-deficient rats. *Brain Res.* **150**, 85–101 (1978).
87. Dieguez, D. Jr & Barea-Rodriguez, E. J. Aging impairs the late phase of long-term potentiation at the medial perforant path–CA3 synapse in awake rats. *Synapse* **52**, 53–61 (2004).
88. Barnes, C. A., Rao, G. & Houston, F. P. LTP induction threshold change in old rats at the perforant path–granule cell synapse. *Neurobiol. Aging* **21**, 613–620 (2000).
89. Barnes, C. A., Rao, G. & McNaughton, B. L. Functional integrity of NMDA-dependent LTP induction mechanisms across the lifespan of F-344 rats. *Learn. Mem.* **3**, 124–137 (1996).
90. Deupree, D. L., Turner, D. A. & Watters, C. L. Spatial performance correlates with *in vitro* potentiation in young and aged Fischer 344 rats. *Brain Res.* **554**, 1–9 (1991).
91. Moore, C. I., Browning, M. D. & Rose, G. M. Hippocampal plasticity induced by primed burst, but not long-term potentiation, stimulation is impaired in area CA1 of aged Fischer 344 rats. *Hippocampus* **3**, 57–66 (1993).
In contrast to earlier reports of intact hippocampal LTP induction (see references 38 and 83), the results of this study suggest that engaging plasticity-inducing mechanisms around threshold becomes more difficult with age.
92. Bear, M. F., Cooper, L. N. & Ebner, F. F. A physiological basis for a theory of synapse modification. *Science* **237**, 42–48 (1987).
93. Bear, M. F. & Malenka, R. C. Synaptic plasticity: LTP and LTD. *Curr. Opin. Neurobiol.* **4**, 389–399 (1994).
94. Thibault, O., Hadley, R. & Landfield, P. W. Elevated postsynaptic $[Ca^{2+}]_i$ and L-type calcium channel activity in aged hippocampal neurons: relationship to impaired synaptic plasticity. *J. Neurosci.* **21**, 9744–9756 (2001).
95. Norris, C. M., Korol, D. L. & Foster, T. C. Increased susceptibility to induction of long-term depression and long-term potentiation reversal during aging. *J. Neurosci.* **16**, 5382–5392 (1996).
Provides the first characterization of homosynaptic LTD/LTP reversal in aged rats and shows that plasticity induced by low-frequency stimulation is increased during ageing, probably as a result of Ca^{2+} dysregulation.
96. Kumar, A. & Foster, T. C. Intracellular calcium stores contribute to increased susceptibility to LTD induction during aging. *Brain Res.* **1031**, 125–128 (2005).
97. Morgan, J. I., Cohen, D. R., Hempstead, J. L. & Curran, T. Mapping patterns of c-fos expression in the central nervous system after seizure. *Science* **237**, 192–197 (1987).
98. Cole, A. J., Saffen, D. W., Baraban, J. M. & Worley, P. F. Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature* **340**, 474–476 (1989).
99. Dragunow, M. *et al.* Long-term potentiation and the induction of c-fos mRNA and proteins in the dentate gyrus of unanesthetized rats. *Neurosci. Lett.* **101**, 274–280 (1989).
100. Wisden, W. *et al.* Differential expression of immediate early genes in the hippocampus and spinal cord. *Neuron* **4**, 603–614 (1990).
101. Guzowski, J. F., McNaughton, B. L., Barnes, C. A. & Worley, P. F. Environment-specific expression of the immediate-early gene *Arc* in hippocampal neuronal ensembles. *Nature Neurosci.* **2**, 1120–1124 (1999).
102. Platenik, J., Kuramoto, N. & Yoneda, Y. Molecular mechanisms associated with long-term consolidation of the NMDA signals. *Life Sci.* **67**, 335–364 (2000).
103. Clayton, D. F. The genomic action potential. *Neurobiol. Learn. Mem.* **74**, 185–216 (2000).
104. Jones, M. W. *et al.* A requirement for the immediate early gene *Zif268* in the expression of late LTP and long-term memories. *Nature Neurosci.* **4**, 289–296 (2001).
105. Reti, I. M., Reddy, R., Worley, P. F. & Baraban, J. M. Prominent Narp expression in projection pathways and terminal fields. *J. Neurochem.* **82**, 935–944 (2002).
106. O'Brien, R. J. *et al.* Synaptic clustering of AMPA receptors by the extracellular immediate-early gene product Narp. *Neuron* **23**, 309–323 (1999).
107. Steward, O., Wallace, C. S., Lyford, G. L. & Worley, P. F. Synaptic activation causes the mRNA for the IEG *Arc* to localize selectively near activated postsynaptic sites on dendrites. *Neuron* **21**, 741–751 (1998).
108. Lyford, G. L. *et al. Arc*, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron* **14**, 433–445 (1995).
109. Guzowski, J. F. *et al.* Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *J. Neurosci.* **20**, 3993–4001 (2000).
110. Jiang, C. H., Tsien, J. Z., Schultz, P. G. & Hu, Y. The effects of aging on gene expression in the hypothalamus and cortex of mice. *Proc. Natl Acad. Sci. USA* **98**, 1930–1934 (2001).
111. Lee, C. K., Weindruch, R. & Prolla, T. A. Gene-expression profile of the ageing brain in mice. *Nature Genet.* **25**, 294–297 (2000).
112. Blalock, E. M. *et al.* Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. *J. Neurosci.* **23**, 3807–3819 (2003).
A report of the first gene-expression microarray experiment in behaviourally characterized rats, which shows an age-associated change in the resting levels of expression of several genes that correlates with cognitive decline.
113. Smith, D. R., Hoyt, E. C., Gallagher, M., Schwabe, R. F. & Lund, P. K. Effect of age and cognitive status on basal level AP-1 activity in rat hippocampus. *Neurobiol. Aging* **22**, 773–786 (2001).
114. Lanahan, A., Lyford, G., Stevenson, G. S., Worley, P. F. & Barnes, C. A. Selective alteration of long-term potentiation-induced transcriptional response in hippocampus of aged, memory-impaired rats. *J. Neurosci.* **17**, 2876–2885 (1997).
115. Small, S. A., Chawla, M. K., Buonocore, M., Rapp, P. R. & Barnes, C. A. Imaging correlates of brain function in monkeys and rats isolates a hippocampal subregion differentially vulnerable to aging. *Proc. Natl Acad. Sci. USA* **101**, 7181–7186 (2004).

The authors used different imaging methods in rats and monkeys and report a cross-species consensus that the dentate gyrus of the hippocampus is particularly vulnerable to the impact of ageing. These results are consistent with a previous report in humans.
116. Small, S. A., Tsai, W. Y., DeLaPaz, R., Mayeux, R. & Stern, Y. Imaging hippocampal function across the human life span: is memory decline normal or not? *Ann. Neurol.* **51**, 290–295 (2002).
117. Kentros, C. *et al.* Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. *Science* **280**, 2121–2126 (1998).
118. Ekstrom, A. D., Meltzer, J., McNaughton, B. L. & Barnes, C. A. NMDA receptor antagonism blocks experience-dependent expansion of hippocampal 'place fields'. *Neuron* **31**, 631–638 (2001).
119. O'Keefe, J. & Dostrovsky, J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* **34**, 171–175 (1971).
120. Jung, M. W. & McNaughton, B. L. Spatial selectivity of unit activity in the hippocampal granular layer. *Hippocampus* **3**, 165–182 (1993).
121. Wilson, M. A. & McNaughton, B. L. Dynamics of the hippocampal ensemble code for space. *Science* **261**, 1055–1058 (1993).
122. McNaughton, B. L., Barnes, C. A. & O'Keefe, J. The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. *Exp. Brain Res.* **52**, 41–49 (1983).
123. Muller, R. U., Bostock, E., Taube, J. S. & Kubie, J. L. On the directional firing properties of hippocampal place cells. *J. Neurosci.* **14**, 7235–7251 (1994).
124. Markus, E. J. *et al.* Interactions between location and task affect the spatial and directional firing of hippocampal neurons. *J. Neurosci.* **15**, 7079–7094 (1995).
125. Mehta, M. R., Barnes, C. A. & McNaughton, B. L. Experience-dependent, asymmetric expansion of hippocampal place fields. *Proc. Natl Acad. Sci. USA* **94**, 8918–8921 (1997).
126. Hebb, D. *The Organization of Behavior: A Neurophysiological Theory* (Wiley, New York, 1949).
127. Thompson, L. T. & Best, P. J. Long-term stability of the place-field activity of single units recorded from the dorsal hippocampus of freely behaving rats. *Brain Res.* **509**, 299–308 (1990).
128. Agnihotri, N. T., Hawkins, R. D., Kandel, E. R. & Kentros, C. The long-term stability of new hippocampal place fields requires new protein synthesis. *Proc. Natl Acad. Sci. USA* **101**, 3656–3661 (2004).
129. Tanila, H., Sipila, P., Shapiro, M. & Eichenbaum, H. Brain aging: impaired coding of novel environmental cues. *J. Neurosci.* **17**, 5167–5174 (1997).
130. Lee, I., Yoganarasimha, D., Rao, G. & Knierim, J. J. Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. *Nature* **430**, 456–459 (2004).
131. Leutgeb, S., Leutgeb, J. K., Treves, A., Moser, M. B. & Moser, E. I. Distinct ensemble codes in hippocampal areas CA3 and CA1. *Science* **305**, 1295–1298 (2004).
132. Vazdarjanova, A. & Guzowski, J. F. Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *J. Neurosci.* **24**, 6489–6496 (2004).
133. Marr, D. Simple memory: a theory for archicortex. *Phil. Trans. R. Soc. Lond. B* **262**, 23–81 (1971).
134. McNaughton, B. L. & Morris, R. G. Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends Neurosci.* **10**, 408–415 (1987).
135. Spencer, W. D. & Raz, N. Differential effects of aging on memory for content and context: a meta-analysis. *Psychol. Aging* **10**, 527–539 (1995).
136. McIntyre, J. S. & Craik, F. I. Age differences in memory for item and source information. *Can. J. Psychol.* **41**, 175–192 (1987).
137. Wilkniss, S. M., Jones, M. G., Korol, D. L., Gold, P. E. & Manning, C. A. Age-related differences in an ecologically based study of route learning. *Psychol. Aging* **12**, 372–375 (1997).
138. Newman, M. & Kasznaik, A. Spatial memory and aging: performance on a human analog of the Morris water maze. *Aging Neuropsychol. Cogn.* **7**, 86–93 (2000).
Similar to rats and monkeys, in a dry version of the Morris swim task, healthy aged humans are impaired in remembering the location of a landmark in relation to room cues.
139. Lai, Z. C., Moss, M. B., Killiany, R. J., Rosene, D. L. & Herndon, J. G. Executive system dysfunction in the aged monkey: spatial and object reversal learning. *Neurobiol. Aging* **16**, 947–954 (1995).
140. Rapp, P. R., Kansky, M. T. & Roberts, J. A. Impaired spatial information processing in aged monkeys with preserved recognition memory. *Neuroreport* **8**, 1923–1928 (1997).
These results reveal substantial correspondence between rat and monkey data, showing that aged animals are impaired in tasks that test spatial memory.
141. Head, E. *et al.* Spatial learning and memory as a function of age in the dog. *Behav. Neurosci.* **109**, 851–858 (1995).
142. Markowska, A. L. *et al.* Individual differences in aging: behavioral and neurobiological correlates. *Neurobiol. Aging* **10**, 31–43 (1989).
143. Gallagher, M. & Rapp, P. R. The use of animal models to study the effects of aging on cognition. *Annu. Rev. Psychol.* **48**, 339–370 (1997).
144. Bach, M. E. *et al.* Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation *in vitro* and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc. Natl Acad. Sci. USA* **96**, 5280–5285 (1999).
145. Rosenzweig, E. S. & Barnes, C. A. Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. *Prog. Neurobiol.* **69**, 143–179 (2003).
146. Christian, K. M. & Thompson, R. F. Neural substrates of eyeblink conditioning: acquisition and retention. *Learn. Mem.* **10**, 427–455 (2003).
147. Kishimoto, Y., Suzuki, M., Kawahara, S. & Kirino, Y. Age-dependent impairment of delay and trace eyeblink conditioning in mice. *Neuroreport* **12**, 3349–3352 (2001).
148. Knuttinen, M. G., Gamelli, A. E., Weiss, C., Power, J. M. & Disterhoft, J. F. Age-related effects on eyeblink conditioning in the F344 x BN F1 hybrid rat. *Neurobiol. Aging* **22**, 1–8 (2001).
149. Thompson, L. T., Moyer, J. R. Jr & Disterhoft, J. F. Trace eyeblink conditioning in rabbits demonstrates heterogeneity of learning ability both between and within age groups. *Neurobiol. Aging* **17**, 619–629 (1996).
150. Solomon, P. R. & Groccia-Ellison, M. E. Classic conditioning in aged rabbits: delay, trace, and long-delay conditioning. *Behav. Neurosci.* **110**, 427–435 (1996).
151. Finkbiner, R. G. & Woodruff-Pak, D. S. Classical eyeblink conditioning in adulthood: effects of age and interstimulus interval on acquisition in the trace paradigm. *Psychol. Aging* **6**, 109–117 (1991).
152. Funahashi, S., Bruce, C. J. & Goldman-Rakic, P. S. Dorsolateral prefrontal lesions and oculomotor delayed-response performance: evidence for mnemonic 'scotomas'. *J. Neurosci.* **13**, 1479–1497 (1993).
153. Mair, R. G., Burk, J. A. & Porter, M. C. Lesions of the frontal cortex, hippocampus, and intralaminar thalamic nuclei have distinct effects on remembering in rats. *Behav. Neurosci.* **112**, 772–792 (1998).
154. Godefroy, O., Cabaret, M., Petit-Chenal, V., Pruvo, J. P. & Rousseaux, M. Control functions of the frontal lobes. Modularity of the central-supervisory system? *Cortex* **35**, 1–20 (1999).
155. Dunnett, S. B., Evenden, J. L. & Iversen, S. D. Delay-dependent short-term memory deficits in aged rats. *Psychopharmacology (Berl.)* **96**, 174–180 (1988).
156. Moss, M. B., Rosene, D. L. & Peters, A. Effects of aging on visual recognition memory in the rhesus monkey. *Neurobiol. Aging* **9**, 495–502 (1988).
157. Rapp, P. R. & Amaral, D. G. Evidence for task-dependent memory dysfunction in the aged monkey. *J. Neurosci.* **9**, 3568–3576 (1989).
158. Moss, M. B., Killiany, R. J., Lai, Z. C., Rosene, D. L. & Herndon, J. G. Recognition memory span in rhesus monkeys of advanced age. *Neurobiol. Aging* **18**, 13–19 (1997).
159. Lyons-Warren, A., Lillie, R. & Hershey, T. Short- and long-term spatial delayed response performance across the lifespan. *Dev. Neuropsychol.* **26**, 661–678 (2004).
160. Wiig, K. A. & Burwell, R. D. Memory impairment on a delayed non-matching-to-position task after lesions of the perirhinal cortex in the rat. *Behav. Neurosci.* **112**, 827–838 (1998).
161. Buffalo, E. A. *et al.* Dissociation between the effects of damage to perirhinal cortex and area TE. *Learn. Mem.* **6**, 572–599 (1999).
162. Rhodes, M. G. Age-related differences in performance on the Wisconsin card sorting test: a meta-analytic review. *Psychol. Aging* **19**, 482–494 (2004).
When educational status and test modality are considered, compared with younger adults, aged humans show deficits on measures of executive function as assessed by the Wisconsin card sorting task, which correlates with an age-related decline in prefrontal cortex functioning.
163. Moore, T. L., Killiany, R. J., Herndon, J. G., Rosene, D. L. & Moss, M. B. Impairment in abstraction and set shifting in aged rhesus monkeys. *Neurobiol. Aging* **24**, 125–134 (2003).
Relative to young adult monkeys, aged monkeys are impaired on an animal analogue of the Wisconsin card sorting task, which suggests an age-related decline in prefrontal cortex functioning. This is consistent with human studies.
164. Paxinos, G. & Watson, C. *The Rat Brain in Stereotaxic Coordinates* 4th edn (Academic, San Diego, 1998).
165. Gothard, K. M., Skaggs, W. E., Moore, K. M. & McNaughton, B. L. Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task. *J. Neurosci.* **16**, 823–835 (1996).
166. McNaughton, B. L., O'Keefe, J. & Barnes, C. A. The stereotrode: a new technique for simultaneous isolation of several single units in the central nervous system from multiple unit records. *J. Neurosci. Methods* **8**, 391–397 (1983).
167. Gray, C. M., Maldonado, P. E., Wilson, M. & McNaughton, B. Tetrodes markedly improve the reliability and yield of multiple single-unit isolation from multi-unit recordings in cat striate cortex. *J. Neurosci. Methods* **63**, 43–54 (1995).
168. Rao, G., Barnes, C. A. & McNaughton, B. L. Intracellular fluorescent staining with carboxyfluorescein: a rapid and reliable method for quantifying dye-coupling in mammalian central nervous system. *J. Neurosci. Methods* **16**, 251–263 (1986).

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