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Normal brain ageing: models and mechanisms

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Normal ageing is associated with a degree of decline in a number of cognitive functions. Apart from the issues raised by the current attempts to expand the lifespan, understanding the mechanisms and the detailed metabolic interactions involved in the process of normal neuronal ageing continues to be a challenge. One model, supported by a significant amount of experimental evidence, views the cellular ageing as a metabolic state characterized by an altered function of the metabolic triad: mitochondria–reactive oxygen species (ROS)–intracellular Ca^{2+}. The perturbation in the relationship between the members of this metabolic triad generate a state of decreased homeostatic reserve, in which the aged neurons could maintain adequate function during normal activity, as demonstrated by the fact that normal ageing is not associated with widespread neuronal loss, but become increasingly vulnerable to the effects of excessive metabolic loads, usually associated with trauma, ischaemia or neurodegenerative processes. This review will concentrate on some of the evidence showing altered mitochondrial function with ageing and also discuss some of the functional consequences that would result from such events, such as alterations in mitochondrial Ca^{2+} homeostasis, ATP production and generation of ROS.

Keywords: ageing; neurons; mitochondria; Ca^{2+} homeostasis; neuronal vulnerability; homeostatic reserve

There is always a murmur of expectation and a frisson of curiosity when the discussion reaches the issue of supercentenarians. To date, the human being with the oldest certified lifespan is Madame Jeanne Calment who, at the time of her death in August 1997, was 122 years and 164 days old, according to the Guiness World Book or Records. According to the other important reference source, The Bible, apart from the described instances of immortality, almost the whole of the Old Testament is full of supercentenarians. Adam lived 930 years and the record pertains to Methuselah who lived to 969 years. After the biblical flood, the situation on Earth deteriorated significantly, such that Abraham lived only to the age of 275, and Joseph died at a more realistic 110.

Historical antiquity artefacts record a relatively short lifespan, estimated in pre-Roman Italy at between 28 and 42 years (Capasso *et al.* 2003), and the increase in lifespan had been rather slow. However, as stressed in a number of recent reports, an analysis of life expectancy from 1840 to present shows a linear, steady-pace increase of almost three months per year, standing currently, at least for women, at 85 years (the men's life expectancy also increased, but at a slower rate, determining a slowly increasing gender gap; Oeppen & Vaupel 2002). Clearly, life expectancy is different in concept from the maximal age at death, keenly recorded by the books of records, but the 'case' of Mme. Calment raises an important question to which there is not yet an agreed answer. Was she the exception, a living example of the maximal life span of the human beings, as would be sustained by the gerontologists such as S. J. Olshansky (Carnes *et al.* 2003), or was she just a pioneer on the road to an ever-expanding life-expectancy, as would be argued by gerontologists such as Vaupel and Carey (Vaupel *et al.* 1998), with further prospects of immortality in the shape of various strategies for engineering negligible senescence, as coined by deGrey (de Grey *et al.* 2002)?

Disentangling reality from wishfulness is sometimes difficult, especially in the case of processes as multi-factorial and multi-layered as ageing. As a result, sometime significant paradigm shifts takes place. In the process of understanding the mechanisms that regulate ageing in the brain, an organ in which the principal cells are post-mitotic and thus outside the rules regulating replicative senescence, such an important paradigm shift resulted from the development of better morphological methods for volumetric counting of neurons. As presented in a seminal review in 1997 (Morrison & Hof 1997), normal brain ageing is not, as previously dogmatically thought, associated or explained by a decrease in neuronal numbers. Many studies since then (reviewed in Hof & Morrison 2004) have confirmed this fact and few brain regions show significant neuronal losses. Since normal ageing is, nevertheless, associated with a small decline in cognitive and memory functions (Rosenzweig & Barnes 2003), the substrate for such dysfunctions must be functional, at the level of synaptic activity.

1. MITOCHONDRIAL STATUS

All these comments point to the fact that valid explanations require proper understanding of the

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One contribution of 18 to a Theme Issue 'Reactive oxygen species in health and disease'.

mechanisms involved, in a bottom-up fashion, from the cellular/sub-cellular level to the network level. At cellular level, particularly for post-mitotic cells, an important theory that is able to explain a variety of the experimental observations is the 'mitochondrial theory of ageing' (MTA). A role for the mitochondria was mentioned initially in 1972 by Harman, within the context of his 'free radicals theory of ageing' (Harman 1972), but the MTA had been formally introduced in 1980 having as its central plank the view that ageing is, essentially, a consequence of mitochondrial DNA damage by mutation, inactivation or loss (Miquel *et al.* 1980). Even now, after more than 20 years, normal neuronal ageing can be seen, from a cellular physiology stand-point, as a metabolic state, characterized by the status of mitochondria.

(a) *Polarization status*

Several lines of experimental evidence show that in the aged tissues the mitochondria are chronically depolarized. In some studies, FACS analysis of the distribution of rhodamine-123 (R123) labelled mitochondria obtained from aged liver showed decreased labelling (Hagen *et al.* 1997). Other studies have demonstrated quantitatively that the proton leakage of the respiratory chain (resulting in a mitochondrial depolarization) is increased and ATP synthesis is decreased with age in the liver mitochondria of the mouse (Harper *et al.* 1998). Using a protonophore to release the rhodamine-123 accumulated in the mitochondria of cerebellar neurons in brain slices, we have shown more recently (Xiong *et al.* 2002) that in the aged neurons there is a significant age-dependent decrease in the amount of mitochondrial dye accumulated in the aged neurons, consistent with the concept of age-induced mitochondrial depolarization. Using a ratiometric mitochondrial dye (JC-1) under confocal microscopy imaging, a similar result was reported for acutely dissociated basal forebrain neurons (Murchison *et al.* 2004). One possible confounding factor in interpreting the data from such experiments is that ageing can induce also a decrease in the number of mitochondria. Thus, studies in both humans (Miquel 1992) and rats (Bertoni-Freddari *et al.* 1994; Sastre *et al.* 1998) show that in young cells there are a relatively large number of small mitochondria. In aged rats and humans, however, there are a smaller number of larger mitochondria; however, the total volume of mitochondria (up to 20% of cell volume) remains roughly the same in young and old rats/humans. These larger, mega-mitochondria are not as bio-energetically efficient as the small mitochondria (Sastre *et al.* 1998; Wakabayashi 2002).

2. FUNCTIONAL CONSEQUENCES

From the observation that mitochondria in the aged tissues are chronically depolarized, a number of functional consequences must follow. Mitochondrial chronic depolarization should affect the Ca^{2+} gradient across the mitochondrial membrane system and thus either reduce the effectiveness of the mitochondrial Ca^{2+} stores during normal Ca^{2+} signalling or increase the threshold required for the activation of the mitochondrial Ca^{2+} uptake. Also, mitochondrial depolarization should affect their capacity to produce ATP, as well as reducing their production of free radicals (Nicholls 2004). All together, such changes in the metabolic status would decrease the homeostatic reserve of the neurons and increase their susceptibility to injury.

(a) *Calcium uptake and mitochondrial Ca^{2+} stores*

During neuronal activity that involves increases of cytosolic Ca^{2+}, mitochondria can take up significant loads of Ca^{2+} (Nicholls & Budd 2000), that can play either a beneficial role of coupling increased metabolic demands with increased oxidative phosphorylation activity (McCormack & Denton 1994) or a deleterious one, activating processes that lead ultimately to cell death (Duchen 1999; Nicholls & Budd 2000). Until recently, the nature of this mitochondrial Ca uniporter was not well established, apart from being defined by a relatively low Ca^{2+} affinity (in the low micromolar range) and a high capacity (Gunter *et al.* 2000). Although the initial estimations of the apparent affinity were in the range of 200–300 nM Ca^{2+} (Gunter & Gunter 1994), and thus precluding an intervention of this Ca^{2+} transport system at resting concentrations of cytosolic Ca^{2+} (between 50 and 100 nM Ca^{2+}), other studies, using mitochondrially targeted Ca^{2+}-sensitive dyes, showed rapid Ca^{2+} transients that do not require large cytoplasmic Ca^{2+} increases (Duchen 1999; Rizzuto *et al.* 1993). Very little is known about the effects of age on this Ca^{2+} uniporter. By use of simultaneous recordings of both cytosolic Ca^{2+} and mitochondrial depolarization, we were able to show that with ageing, either in brain slices or in a model of ageing in primary neuronal cell cultures, there is an increase in the threshold required to activate mitochondrial Ca^{2+} uptake, as measured by the level of cytosolic Ca^{2+} at which mitochondrial depolarization is initiated (396 ± 33 in young and 563 ± 43 in old neurons; Xiong *et al.* 2002, 2004). Recently, by use of direct patch clamp electrophysiological measurements on mitoplasts formed from the inner mitochondrial membranes, a highly specific Ca^{2+} channel on the inner mitochondrial membrane, with a high Ca^{2+} affinity (in the nanomolar range) and displaying the pharmacological properties earlier established for the Ca^{2+} uniporter has been described (Kirichok *et al.* 2004). A high affinity, coupled with the rather small Ca^{2+}-induced inactivation and together with the fact that the significant electrochemical gradient across the mitochondrial membranes is the driving force and modulator of this transport mode, would support our view that the age-dependent increase in the threshold level for mitochondrial Ca^{2+} uptake is a result of a decreased electrochemical gradient for Ca^{2+} secondary to the chronic mitochondrial depolarization, rather than due to a change in the Ca^{2+} affinity of the transporter.

However, the increase of the Ca^{2+} threshold for the activation of the mitochondrial Ca^{2+} uptake does not imply a decrease in the effectiveness of the mitochondrial Ca^{2+} store. The participation of the mitochondrial Ca^{2+} stores can be assessed at two levels: (i) effects on the rates of cytosolic $[Ca^{2+}]_i$ increase that follow stimulation and (ii) amount of Ca^{2+} taken into

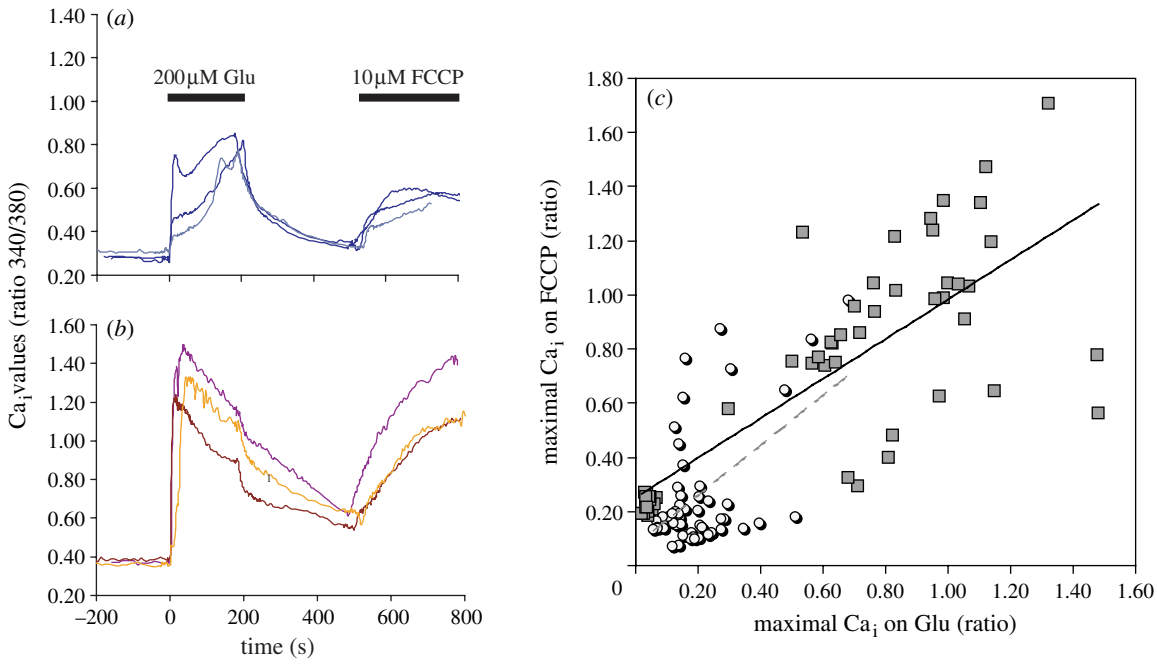

Figure 1. Assessment of mitochondrial Ca^{2+} stores. Cerebellar granule neurons were prepared and maintained as previously described (Xiong *et al.* 2004). The protocol for assessing the size of the mitochondrial Ca^{2+} pools on neuronal activation involved a 3 min stimulation of the neurons by bath perifusion with 200 μM glutamate (in the presence of 10 μM glycine and in the absence of added Mg^{2+}). After removal of the stimulus and a standard period of recovery (5 min) the neurons were perifused with 10 μM CCCP, a protonophore that dissipates the mitochondrial membrane potential and activates the release of the mitochondrial Ca^{2+}. Intracellular free Ca^{2+} was measured using fura-2AM as the Ca^{2+}-sensitive fluorescent dye and the methodologies and technologies described previously (Xiong *et al.* 2002, 2004). Panels (*a*) and (*b*) show average traces from individual experiments, performed either on 'young' neurons (Panel (*a*), neurons at 7–10 DIV) or on 'old' neurons (Panel (*b*), neurons at >28 DIV). In the older cultures, the amplitude of the Ca^{2+} signal evoked by 200 μM was significantly larger. Panel (*c*) describes the correlation between the value of the maximal $[Ca^{2+}]_i$ on Glu stimulation (on the abscissa, and expressed in 340/380 nm ratio units) against the 5 min values of $[Ca^{2+}]_i$ recorded after the addition of the protonophore. The light, shadowed circles represent the correlation values obtained for the young neurons, while the darker squares represent the values of the older neurons. For both populations ('young' and 'old') there is a highly significant correlation between the two $[Ca^{2+}]_i$ parameters, indicating that the level of cytosolic Ca^{2+} load is a very important factor in regulating the size of the mitochondrial Ca^{2+} stores. Furthermore, the slopes of the linear fit for the two populations are very similar ('young': 0.978 and 'old': 0.834), despite the fact that, as discussed in the text, there are significant difference in the level of mitochondrial polarization in the two populations.

the mitochondrial stores. When measured at $[Ca^{2+}]_i$ values above the mitochondrial threshold for Ca^{2+} uptake, mitochondria decreased the rate of cytosolic $[Ca^{2+}]_i$ increase, an effect that was not affected by ageing (Murchison *et al.* 2004; Toescu 2000). Some reports suggest that in the aged neurons the mitochondrial Ca^{2+} handling properties are less important in shaping the depolarization-induced Ca^{2+} signals (i.e. when the buffering role of the mitochondria is eliminated by use of a protonophore, the Ca^{2+} signal evoked by depolarization is almost doubled in the young neurons, and increased only by 30% in the aged neurons; (Murchison *et al.* 2004). Using again a protonophore to assess the size of the mitochondrial Ca^{2+} pool following neuronal stimulation and activation of the mitochondrial Ca^{2+} uptake, we showed that upon glutamatergic stimulation the major determinant controlling the size of the releasable mitochondrial Ca^{2+} pool is the size of the Ca^{2+} load to which a particular neuron has been exposed to, independent of age (figure 1). This is despite the fact that, as described above, mitochondria in the aged neurons are chronically depolarized and that there is an increase of the threshold value of activation of Ca^{2+} uptake, but is in agreement with the fact that an important determinant of the size of the mitochondrial Ca^{2+} store is the amount of phosphate available in the mitochondrial matrix (Nicholls & Budd 2000).

(b) *ATP production*

Mitochondrial dysfunction should also result in possible changes in ATP production or, more importantly, in the ATP/ADP ratio. Albeit extremely important, these issues are, particularly in the context of ageing research, rather under-studied and most of the conclusions are inferred from the study of mitochondrial dysfunction rather than from direct measurements of ATP production. The danger in these conditions is that the multi-factorial nature of the coupling between the oxidative cycles of the respiratory chain and the adenosine disphosphate phosphorylation may lead to significant miscalculations. Thus, Davey *et al.* showed that in rat brain synaptosomes, differential levels of inhibition of the complexes I, III and IV were required to induce significant decreases of ATP levels: while only 20% inhibition of the complex I was sufficient for the inhibition of ATP production, the complex III was much more resilient, and required a drastic inhibition of up to 80% to achieve the same block of ATP production (Davey *et al.* 1998). It is also

important to note that neurons should have a certain hierarchy of ATP-consuming processes, quite possibly similar to that described for thymocytes (Buttgereit & Brand 1995), where the synthetic processes (protein and nucleic acids synthesis) take priority and use about 50% of the resting ATP production. Another important feature is that neuronal stimulation activates a massive increase in energetic demand, based mainly on the requirements of the Na^{2+}/K^{2+} ATPase for restoring the ionic balance in the wake of action potentials (Attwell & Laughlin 2001). Despite these significant energetic requirements of neuronal activity, studies on cerebellar granule neurons in primary cultures suggest that ATP supply is not the triggering factor in initiating the delayed Ca^{2+} dysregulation (DCD) associated with glutamate excitotoxicity (Castilho *et al.* 1998; Nicholls & Budd 2000). Furthermore, for primary culture models of excitotoxicity a certain degree of mitochondrial depolarization is protective against neuronal death, through preventing Ca^{2+} overload of the mitochondria (Castilho *et al.* 1998; Stout *et al.* 1998). Whether these considerations are applicable to the metabolic state of the aged neurons is not yet clear, and only few direct data are available. Recent direct luminometric measurements of the ATP content and rate of ATP production in resting conditions using mitochondria acutely obtained from Fischer 344 rats showed no difference between adult (12 months) and old (24 months) animals (Drew & Leeuwenburgh 2003). In measuring ATP from whole brain slices (Xiong & Toescu unpublished results) we observed little difference in ATP content between young and old cerebellar slices, but following neuronal activity (45 min after a 5 min pulse of 75 mM KCl) the ATP content in the old slices (from 20 to 23 months old animals) was about 50% less than in the younger slices (12 months old), indicating a decreased functional reserve.

(c) *Free radical production*

Mitochondria are also a major site of free radical production, and the relationship between mitochondrial functional status and free radical production is a complex and subtle one. Although mitochondria have a high rate of oxygen consumption, careful studies of mitochondria isolated from brain or other organs showed that only a small proportion of this oxygen (less than 3%) generate free radicals (aka, reactive oxygen species, ROS; e.g. Floyd & Hensley 2002; Sastre *et al.* 2003). For a long period of time, stemming from the two important functional theories discussed above aiming at explaining the process of cellular ageing through a functional/metabolic perspective (the 'free radicals' and the 'mitochondrial' theories of ageing), a commonly held paradigmatic view was that of 'live fast, die young'. This was based on the observation of the inverse relationship between mitochondrial ROS production and longevity in mammal species (Ku *et al.* 1993), and implicit in it was the fact that an activation of the respiratory chain activity will inherently increase the rate of electrons slippage at complex III (predominantly, although a similar event can take place at complex I (Nicholls & Budd 2000)). However, as demonstrated experimentally (Korshunov *et al.* 1997) and modelled theoretically (Nicholls 2004), the relationship is inversely proportional and at fast mitochondrial respiratory rates (state 3, high substrates levels, high ADP concentration, low ATP/ADP ratio) fewer free radicals are produced than in state 4 ('resting' state, with low ADP and high ATP/ADP ratio; Nicholls 2004). However, there are a variety of other reasons for which the role of oxidative stress in the ageing brain is of relevance: a high content of unsaturated fatty acids, that are more liable to peroxidation, a high content of pro-oxidant iron ions and a low reserve of antioxidant defences (Floyd & Hensley 2002). If it is still unclear whether the resting or stimulated rate of free radicals production is affected by the ageing process, what is clear and demonstrated by numerous studies is that ageing is associated with a significant accumulation of markers of oxidative stress. Once generated, free radicals can oxidize proteins, and accumulation of oxidized proteins in many tissues is considered a hallmark of ageing (Stadtman 1992). Other targets of free radicals' action are the lipids, and peroxidation of biological lipids yields a large number of compounds. Amongst them, 4-hydroxynonenal (4-HNE), resulting from the peroxidation of omega-6-conjugated fatty acids (e.g. arachidonic and linoleic acids; Esterbauer *et al.* 1991), is one of the most studied aldehydes. HNE is very active in biological systems and can react with many types of biological molecules: amino acids in proteins, bases in DNA and other lipid amino groups (Esterbauer *et al.* 1991), explaining its widespread range of actions from effects on synaptic function (Keller *et al.* 1997) to mediating excitotoxicity and neuronal death (Mark *et al.* 1997). Increased levels of lipid peroxidation and particularly HNE adduct products have been demonstrated in the brain of human subjects with neurodegenerative diseases (Yoritaka *et al.* 1996; Markesbery & Lovell 1998). Another important and recognized target of free radicals attack is the DNA. Many oxidatively damaged DNA bases have been identified, but one of the most widely studied is the 8-OHdG (8-hydroxy-2′-deoxyguanosine; aka, 8-oxo-2-deoxiguanosine, oxo8dG; Floyd *et al.* 1990). A recent study (Hamilton *et al.* 2001) showed significant age-dependent increases in 8-OHdG in various tissues in the rat and mouse that resulted not from a decrease in the antioxidant defences, but from an increased sensitivity of the DNA oxidation process.

(d) *Increased vulnerability*

Thus, with the accumulation of functional defects at different levels, underlined by the activity of the triad Ca^{2+}–mitochondria–ROS, the issue of cellular and neuronal vulnerability and susceptibility to damage as a function of age becomes important. Taking a lead from the physical model of ageing, characterized by frailty, it is a common sense assertion that age brings vulnerability also in the domain of cellular physiology. Indeed, using the traumatic brain injury as an experimental model in rodents, it has been long demonstrated that age is associated with a significantly increased mortality and, for smaller levels of injury, with a greater level of acute neurological deficits (Hamm *et al.* 1991), with comparable equivalent results in human studies

(Susman *et al.* 2002). However, the issue is fraught with some difficulties of interpretation and assessment, since there is a certain developmentally controlled maturation of vulnerability to excitotoxic insults. Thus, in short-term cultures of hippocampal neurons, an *N*-methyl-D-aspartate (NMDA)-induced excitotoxic lesion resulted in a minimal degree of neuronal death in neurons derived from 3 day old animals, whereas more that 90% of the neurons obtained from 21 day old animals died (Marks *et al.* 2000), reproducing *in vitro* results obtained *in vivo* in rodents (Liu *et al.* 1996). This dramatic difference was not due to differences in $[Ca^{2+}]_i$ signalling, since both populations of neurons gave similar values, but to significant differences in the mitochondrial depolarization response—much larger in the older population (Marks *et al.* 2000). Another study, using a similar experimental model and looking at the NMDA-evoked electrophysiological and $[Ca^{2+}]_i$ responses, showed that with ageing (26 months old rodents) there was a significant increase in responsiveness when compared with the 'middle-aged' (nine months old) neurons (Cady *et al.* 2001). Interestingly, during attempts to develop an 'ageing in the dish' experimental model, it was reported that several of the functional features of the aged neurons as recorded in acute brain slice preparations from the aged animals (either hippocampal or cerebellar) developed during longer term primary neuronal cultures (Blalock *et al.* 1999; Vergun *et al.* 1999; Xiong *et al.* 2004).

It is important to understand that increased vulnerability, as that associated with ageing, does not mean increased neuronal death. Many times the word and the concept of 'ageing' are used in the same context as used for 'neurodegeneration', and attempts to explain the mechanisms involved in various neurodegenerative diseases are extrapolated to the explanation of the normal process of ageing. Furthermore, there are some who suggest that neurodegeneration and one of its resulting dysfunctions, dementia, are inevitable and will follow inexorably from the advanced age (Terry & Katzman 2001). To counteract these views, others are pointing out significant morphological and functional differences between the aged brain and that afflicted by neurodegeneration (Morrison & Hof 1997; Morrison 2001). Another argument, from the realm of basic neurophysiology, is the observation, confirmed by more and more labs, that the values of resting $[Ca^{2+}]_i$ are not affected by age (Thibault *et al.* 2001; Xiong *et al.* 2002; Murchison *et al.* 2004). Within the context of the 'Ca^{2+} hypothesis of ageing', the dysfunction of the aged brain was seen as a result of alterations in one or another of the Ca^{2+} homeostatic processes (Khachaturian 1994). The value of the resting $[Ca^{2+}]_i$ represents a steady-state balance between the Ca^{2+} entry and Ca^{2+} extrusion mechanisms (Toescu & Verkhratsky 2000). Alterations, even minimal, in one or another of these Ca^{2+} flux processes should affect, especially if expressed over a long period of time, the value of the resting $[Ca^{2+}]_i$ in the aged neurons. The observation of the stability of the resting $[Ca^{2+}]_i$ values across the lifespan is important not only in the context of the older 'Ca^{2+} hypothesis of ageing' (Verkhratsky & Toescu 1998; Toescu *et al.* 2004) but also in the wider context of the significant deleterious effects of increased cytosolic Ca^{2+} (Duchen 1999; Nicotera *et al.* 1999; Nicholls & Budd 2000).

3. AGEING AS PHYSIOLOGICAL STATE OF DECREASED HOMEOSTATIC RESERVE

What all this data indicate is that ageing means a decrease in the homeostatic reserve (or, according to other nomenclature, allostatic load (McEwen 2000)). This state can be defined as a decrease in the capacity to oppose the damaging effects of strong, excessive stressors and is entirely compatible with functioning at normal levels of functional load. An example of this process is the response of neurons to different levels of stimulation. One of the most consistent proofs of age-dependent dysregulation of $[Ca^{2+}]_i$ homeostasis is the delayed recovery of the resting $[Ca^{2+}]_i$ values following large stimulation-evoked Ca^{2+} signals (Toescu & Verkhratsky 2000; Xiong *et al.* 2002). The decrease in the $[Ca^{2+}]_i$ clearance rate could be explained either by metabolic limitations, as discussed above, or by functional or biological changes in the properties of the Ca^{2+} removal systems. Evidence for age-dependent alterations in the function of the plasma membrane Ca^{2+}ATPase (PMCA) has been presented, and might involve changes in the phosphorylation properties or calmodulin-binding properties (Zaidi *et al.* 1998). Another Ca^{2+} removal system of significance in neuronal physiology is the PM Na^+/Ca^{2+} exchanger (Hoyt *et al.* 1998; Kiedrowski 1999). Recent exciting data revealed a new mechanism through which the activity of the Na^+/Ca^{2+} exchanger can be modulated, involving a Ca^{2+}-dependent activation of proteolysis and resulting in a loss of function (Bano *et al.* 2005). This mechanism has been proposed to explain the DCD associated with late, post-ischaemic lesions; whether similar, but subtler, changes in either the types of Na^+/Ca^{2+} exchanger present or in the sensitivity of the proteolytic mechanism to Ca^{2+} are taking place during the ageing process is not at all clear. The issue of whether the delayed recovery of $[Ca^{2+}]_i$ in the aged neurons is due to a functional or an irreversible change is illuminated by the fact that when the cytosolic Ca^{2+} load is reduced by decreasing the level of stimulation, the rate of recovery in the aged neurons is significantly improved and approaches the values recorded in the young neurons (Toescu & Xiong 2004). Similarly, when maintained in *in vitro* conditions, for the first 2–3 h, there are no significant differences in the number of compromised neurons between slices obtained from young or old mice; however, by 5 h a significantly higher number of non-viable neurons was recorded in the aged slices (Xiong *et al.* 2002), reflecting an increased susceptibility of the aged neurons to the metabolically demanding conditions of *in vitro* maintenance.

In conclusion, it is our view that normal neuronal ageing is a metabolic state characterized by a decreased homeostatic reserve, defined as the capacity of the cells (neurons) to oppose the destabilizing effects of metabolic stressors. Implicit in this definition, and in keeping with the wealth of experimental evidence, is that in the aged neurons normal metabolic activity is maintained at rest or during moderate levels of activity.

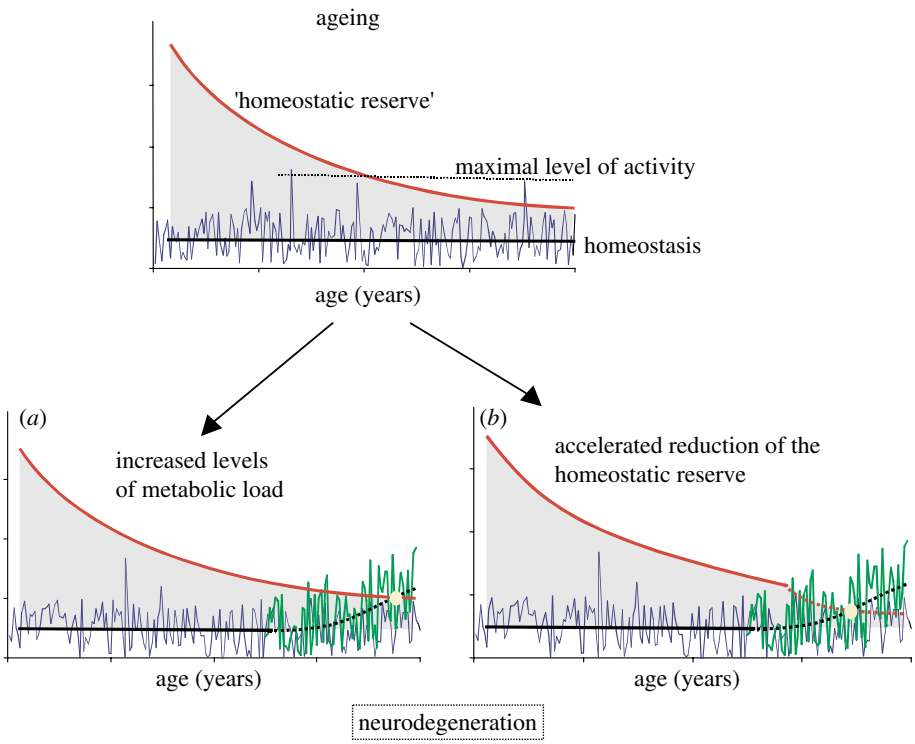

Figure 2. Relationship between ageing and neurodegeneration. The process of ageing (top panel) can be seen as a continuous decrease of the homeostatic reserve, defined as the capacity of cells to fight various metabolic stressors and maintain the cells/organ on the steady-state level of homeostasis. With age, acute surges of metabolic activity become more dangerous as they reach the limits of homeostatic reserve defences, a process that underlies the age-dependent increased in vulnerability. When the homeostasis line intersects the homeostatic reserve line, the biological system becomes unstable and severe dysfunction/death ensues. The process of neurodegeneration, characterized by extensive neuronal death, becomes mostly manifest at the older ages, on the background of decreased homeostatic reserve, and could result either from (*a*) an increased level of metabolic load or from (*b*) an accelerated reduction of the homeostatic reserve or from a combination of both.

Functional deficits of the aged neurons are expressed, in a use- and level-dependent manner, only at higher levels of stimulation, usually associated with clinical or subclinical instances of trauma, ischaemia or other excitotoxic events (figure 2). Results obtained in a number of laboratories including ours indicate that a major factor underlying the decreased homeostatic reserve is a change in the mitochondrial status in the aged neurons, resulting in a number of down-stream functional effects. Understanding the intimate relationship that links the members of the functional triad Ca^{2+}–mitochondria–ROS is an important challenge, but should provide new avenues not only for a better understanding of the ageing process, but also for allowing more successful therapeutic interventions in a variety of pathological instances associated with ageing.

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