Behavour, learning and perception

The subjectivity of odour perception was emphasized by several speakers, and this was highlighted by Robyn Hudson (Munich, Germany). She reported that Mexican, Japanese and German subjects differed in their ratings of the pleasantness of a range of culturally-specific, common odours, and that odours that were more familiar, or very pleasant or unpleasant, tended to be rated as more intense. This subjectivity of odour perception might in part reflect genetic differences in receptor complement, but it is also influenced by experience. For instance, pre-natal experience of the odour of food, eaten by a gestating rabbit, increases the electro-olfactogram response to that odour, recorded subsequently from the olfactory epithelium of her pups. Although some effects of experience could be mediated at the level of the receptor input itself, the more important central mechanisms underlying chemosensory learning were illustrated by two other speakers. Barry Keever (Cambridge, UK) presented evidence that pheromonal learning in mice requires neural changes in the accessory olfactory bulb, specifically at the reciprocal synapses between the M/T cells and their inhibitory interneurons. Yadin Dudai (Rehovot, Israel), showed that the tyrosine phosphorylation of the NR2B subunit of the NMDA receptor is increased transiently in the insular cortex, following taste-aversion training. Moreover, the level of phosphorylation depends on the novelty and saliency of the conditioned stimulus and this might be mediated via the cholinergic system.

Susan Schiffman (Durham, NC, USA) reminded us of the importance of olfaction for human health. Many factors might be responsible for the decline of olfactory function with age, including diseases, drug treatments and the deleterious effects of environmental pollutants. This loss of olfactory function can have profound negative effects on mood and lead to loss of appetite in pets, retarding their clinical progress. In these cases, the enhancement of the flavor of foods can lead to improved mood and is correlated with enhancement of immune system function. A more subtle example of the role of olfaction in humans was provided by Roman Furstl (Kiel, Germany) who presented results showing that major histocompatibility type can influence attractiveness ratings for body odours of members of the opposite sex.

Gordon Shepherd concluded events by stressing the importance of the integrated approach to olfaction exemplified by this meeting. To achieve an understanding of the olfactory system, it is vital to work across different levels of analysis, different stages of olfactory processing and different phyla. Considering the rapid progress being made on all these fronts, it is an exciting time to be working in olfaction.

Selected references

Viewpoint

Calcium and neuronal ageing
Alexej Verkhratsky and Emil C. Toescu

Brain ageing is associated with a marked decline in mental faculties. One hypothesis postulates that sustained changes in the regulation of intracellular Ca²⁺ concentration, [Ca²⁺], are the major cause of neuronal degeneration. This ‘calcium hypothesis’ is supported by demonstrations of the impairment in aged neurones of molecular cascades that regulate [Ca²⁺]. However, the number of direct measurements of [Ca²⁺] in senescent neurones is limited, and the hypothesis cannot be regarded as fully confirmed. Furthermore, physiological brain ageing, at least in certain regions, need not necessarily be a degenerative process accompanied by neuronal loss. Pharmacological manipulation of Ca²⁺ entry has been shown to be effective in increasing some aspects of cognitive function of the aged brain. Therefore, further exploration of Ca²⁺ homeostasis and signalling might reveal the mechanisms involved in the age-dependent decline in neuronal performance, and might aid the search for new therapeutic treatments.


We all have a powerful image of human ageing:

‘...old men have grey beards...their faces are wrinkled, their eyes purging thick amber and plumtree gum, and they have a plentiful lack of wit, together with most weak hams...’

(Shakespeare, Hamlet, 2.2).

However, the basic mechanisms responsible for the age-dependent decrease in human mental faculties remain almost as much of a mystery now as in the days of Shakespeare. All ageing brains show a decrease in the ability to handle and retain both new and existing knowledge. One of the current hypotheses postulates that age-dependent alterations in the cellular
mechanisms of Ca$^{2+}$ homeostasis result in sustained changes in the regulation of intracellular Ca$^{2+}$ concentration, [Ca$^{2+}$], and that this is the main cause of the neuronal degeneration. The conceptual pillars of this 'calcium hypothesis of ageing' are: dysfunction of [Ca$^{2+}$], homeostasis, and neuronal loss.

This canonical view of the ageing brain is that the decrease in cognitive function results mainly from neuronal death and that this leads to a decrease in the number of brain cells. Strong support for this hypothesis comes from studies of neurodegenerative diseases, such as Alzheimer’s disease (AD). In AD there is an increase in the loss of neurones that correlates well with the decrease in learning abilities and memory functions. In addition, a key element of AD pathology, the β-amyloid protein, has been shown to disrupt neuronal [Ca$^{2+}$], homeostasis. The role of neuronal [Ca$^{2+}$] overload in initiating [Ca$^{2+}$]-dependent neurotoxicity and death is well documented (see Refs 9 and 10 for review), and thus a link between [Ca$^{2+}$] and morphological or functional losses in AD has been proposed.

However, this link does not necessarily apply during the physiological process of ageing. It is established that even slight symptoms of AD can be accompanied by a marked loss of neurones in various brain regions. For many years it was also believed that neuronal loss in the hippocampus was directly responsible for age-related deterioration of cognitive function. However, recent morphological studies have demonstrated that the number of neurones is preserved in the CA1–CA3 hippocampal regions of humans undergoing normal ageing. Similarly, the number of neurones in the hippocampal area of aged rats remains unaltered, despite deficits in spatial learning. Thus, the decline in cognitive function that accompanies the normal ageing process is probably associated with more subtle changes that are the result of impaired neuronal performance rather than neuronal loss. Reduced synaptic efficacy or alterations in neuronal plasticity might play a significant role. The involvement of [Ca$^{2+}$], in the regulation of synaptic plasticity is well documented, and therefore age-dependent impairment in both intracellular Ca$^{2+}$ homeostasis and signalling reduces the efficiency of this process.

Age-dependent changes in systems that regulate intracellular Ca$^{2+}$ concentrations

The mechanisms involved in neuronal Ca$^{2+}$ signalling and [Ca$^{2+}$], homeostasis have been established (see Refs 14 and 17 for review). The Ca$^{2+}$ signal is determined by: Ca$^{2+}$ inflow into the cytoplasm from either extracellular space or intracellular Ca$^{2+}$ stores; the amount of cytoplasmic Ca$^{2+}$ buffering; and Ca$^{2+}$-clearance systems (either expulsion of Ca$^{2+}$ from the cell or accumulation into the internal Ca$^{2+}$-stores). The impairment of any of these components can affect [Ca$^{2+}$], regulation, although the existence of numerous feedback mechanisms might compensate for defects in any single pathway.

Plasmalemmal Ca$^{2+}$ channels

The most important mechanism for the generation of a Ca$^{2+}$ signal during neuronal activity is the inflow of Ca$^{2+}$ ions from the extracellular space that takes place through plasmalemmal Ca$^{2+}$-permeable channels, of which there are several superfamilies. Depending on the mechanism of activation, the plasmalemmal Ca$^{2+}$ channels are subclassified into, for example, voltage-operated, ligand-operated and store-operated. The majority of studies on age-dependent alterations of the plasmalemmal Ca$^{2+}$ influx have been confined to the investigation of voltage-operated Ca$^{2+}$ channels.

Biochemical studies on brain tissue from aged rats, using the Ca$^{2+}$-antagonist nitrendipine, revealed an increase in the number of binding sites, but a decrease in affinity of approximately 50% (Ref. 21). In the brains of senescence-prone mice, which have an accelerated age-dependent decline in learning and memory, a significant decrease in the number of binding sites for both the L-type Ca$^{2+}$-channel antagonist PN 200-110 and N-type Ca$^{2+}$-channel antagonist, α-conotoxin, has been reported. Early experiments on brain synaptosomes showed a significant age-dependent decrease in depolarization-induced Ca$^{2+}$ uptake, but more recent studies have reported the opposite. However, neither Ca$^{2+}$-antagonist-binding assays (due to nonspecific binding) nor radiotracer experiments on synaptosomes directly indicate the number and properties of neuronal Ca$^{2+}$ channels; however, direct measurements can be obtained electrophysiologically.

The first attempt to address the issue of possible changes in Ca$^{2+}$ entry or [Ca$^{2+}$], homeostasis in aged neurones was done by Landfield and Pitler, who found prolonged Ca$^{2+}$-dependent afterhyperpolarization (AHP) in the hippocampal neurones of aged rats. Electrophysiological measurements performed by other research groups yielded disparate findings (Table 1): both current-clamp and voltage-clamp experiments performed on aged neurones from different regions of the nervous system, demonstrated that Ca$^{2+}$ currents can be either increased, unchanged, or decreased. These changes were not caused by alterations in single-channel conductance or voltage-dependence, but were related to differences in channel density.

The limited information available raises an important question: are the changes in Ca$^{2+}$ current similar throughout the brain or region-specific? The direct measurement of Ca$^{2+}$ currents in various parts of the CNS and PNS seems an obvious direction for future experiments. An important consideration in the design of these experiments is the type of preparation used for the electrophysiological measurements and, in this case, the use of the brain-slice preparations is appropriate.

Data concerning age-dependent changes in ligand-gated Ca$^{2+}$ channels is even more limited. Currently, the major ligand-operated, Ca$^{2+}$-influx pathway is considered to be associated with the ionotropic glutamate receptors (iGluRs). Depending on their subunit composition, these receptors possess different permeabilities for Ca$^{2+}$ (Ref. 37). The number of NMDA-iGluRs, which are believed to have the highest Ca$^{2+}$ permeability, was unchanged in the senescent human hippocampus and entorhinal cortex, but significantly decreased in the cortex and hippocampus of aged mice. The Ca$^{2+}$ impermeability of another set of iGluRs, the AMPA receptors, is determined by the degree of expression of the GluR-B subunit. In the hippocampus of aged rats, the expression of all AMPA-GluR subunits, but particularly the GluR-B subunit, was decreased. Thus, AMPA receptors in the aged brain might be more Ca$^{2+}$-permeable.
TABLE 1. Ca\(^{2+}\) currents in neurones from aged rats

<table>
<thead>
<tr>
<th>Source</th>
<th>Preparation</th>
<th>Method of measurement</th>
<th>Ca(^{2+})-current change</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>Slice</td>
<td>Current clamp/microelectrodes/AHP</td>
<td>AHP increase</td>
<td>27, 28</td>
</tr>
<tr>
<td></td>
<td>Slice</td>
<td>Current clamp/microelectrodes/Ca(^{2+})-AP</td>
<td>Increased duration of the Ca(^{2+}) spike</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Slice</td>
<td>Voltage clamp/microelectrodes/Ca(^{2+})-AP/Ca(^{2+})-current amplitude</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>‘Zipped’ slice</td>
<td>Voltage clamp/cell-attached patch clamp/uniary Ca(^{2+})-current</td>
<td>[I_{\text{NMDA}}] (caused by an increase in channel density)</td>
<td>31</td>
</tr>
<tr>
<td>DG</td>
<td>Freshly isolated</td>
<td>Voltage clamp/microelectrodes/ Ca(^{2+})-current amplitude</td>
<td>[I_{\text{NMDA}}] decrease (caused by Ca(^{2+})-dependent inactivation)</td>
<td>32</td>
</tr>
<tr>
<td>MSDB</td>
<td>Freshly isolated</td>
<td>Voltage clamp/whole-cell patch clamp/Ca(^{2+})-current amplitude</td>
<td>[I_{\text{NMDA}}] increase</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Freshly isolated</td>
<td>Voltage clamp/whole-cell patch clamp, perforated patch clamp/Ca(^{2+})-current amplitude</td>
<td>[I_{\text{NMDA}}] unchanged</td>
<td>34</td>
</tr>
<tr>
<td>Striatum</td>
<td>Slice</td>
<td>Voltage clamp/microelectrodes/ AP duration</td>
<td>[I_{\text{NMDA}}] decrease</td>
<td>35</td>
</tr>
<tr>
<td>DRG</td>
<td>Culture</td>
<td>Voltage clamp/whole-cell patch clamp/Ca(^{2+})-current amplitude</td>
<td>[I_{\text{NMDA}}] decrease</td>
<td>36</td>
</tr>
</tbody>
</table>

Abbreviations: AHP, afterhyperpolarization; AP, action potential; Ca\(^{2+}\)-AP, calcium action potential; DG, dentate gyrus; DRG, dorsal root ganglia; \[I_{\text{NMDA}}\], high-threshold calcium current; \[I_{\text{LVA}}\], low-threshold calcium current; L, L-type Ca\(^{2+}\) channel; MSDB, medial septum, nucleus of the diagonal band; T, T-type Ca\(^{2+}\) channel; ?, Ca\(^{2+}\) channel type unknown.

Intracellular Ca\(^{2+}\) channels

Another important Ca\(^{2+}\) signalling event is the release of Ca\(^{2+}\) from stores in the intracellular endoplasmic reticulum. These possess Ca\(^{2+}\) pumps of the SERCA [sarc(o)endoplasmic reticulum calcium ATPase] type that allow Ca\(^{2+}\) accumulation in the stores and Ca\(^{2+}\)-release channels that mediate the release of Ca\(^{2+}\) from the stores. Intracellular Ca\(^{2+}\) channels are activated in two ways: Ca\(^{2+}\)-gated Ca\(^{2+}\)-release channels (ryanodine receptors, RyR) are controlled by Ca\(^{2+}\) ions; and the intracellular messenger inositol (1,4,5)-trisphosphate \([\text{Ins(1,4,5)P}_3]\) controls Ins(1,4,5)P\(_4\)-gated channels and Ins(1,4,5)P\(_2\) receptors. RyRs amplify the entry of plasmalemmal Ca\(^{2+}\) (Ref. 41), whereas the activation of Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\) release results from an intracellular signal-transduction chain that is controlled by specific plasmalemmal, metabolotropic receptors that are coupled to phospholipase C and Ins(1,4,5)P\(_3\) production\(^{42}\). Neither the density nor the dissociation constant (K\(_d\)) of ryanodine binding sites was affected in the cerebral and cerebellar cortex of aged rats\(^{43}\). Conversely, the density of Ins(1,4,5)P\(_3\) receptors was decreased by almost 50% in the cerebral cortex of old rats\(^{44,45}\), and in a separate study, a decrease in Ins(1,4,5)P\(_3\)R density and an increase in its binding affinity were observed in the spinal cord\(^{46}\). Additionally, the amount of Ins(1,4,5)P\(_3\) produced by receptor activation might be altered in the aged brain\(^{47}\). However, the variation in the number of Ins(1,4,5)P\(_3\) receptors does not necessarily indicate changes in the efficacy of the Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\)-release mechanism. It is widely accepted that Ins(1,4,5)P\(_3\) receptors are controlled by the amount of cytoplasmic Ca\(^{2+}\) (Ref. 47), and because this might be increased in aged neurones, it has to be considered when designing experiments to characterize functionally Ins(1,4,5)P\(_3\)-induced Ca\(^{2+}\) release.

Intracellular Ca\(^{2+}\) buffers

The expression of various Ca\(^{2+}\)-binding proteins that are present within the cell is down-regulated in certain brain regions of old animals. The degree of expression of two major cytosolic Ca\(^{2+}\) buffers, calbindin-28 and calretilin, was substantially decreased in the hippocampus, but not in the cerebellum and cortex of aged rats\(^{48,49}\) and aged rabbits\(^{50}\); calbindin-28 concentrations were also reduced in retinal preparations of aged rats\(^{49}\). Similarly, the number of calbindin-immunoreactive neurones was significantly decreased in the hippocampus of aged rats\(^{51,52}\). In contrast, in the medial septum and striatum, the calbindin expression remained either unchanged or slightly reduced, whereas the number of neurones positive for parvalbumin, another Ca\(^{2+}\)-binding protein, were markedly decreased\(^{53}\). These intracellular Ca\(^{2+}\) buffers were also reduced in peripheral adrenergic nerves of the aged rats\(^{54}\). Therefore, ageing is associated with a significant, albeit region-specific, decrease in neuronal Ca\(^{2+}\)-buffer capacity.

Ca\(^{2+}\) extrusion and intracellular-Ca\(^{2+}\) accumulation

The clearance of Ca\(^{2+}\) loads after neuronal activity and maintenance of a low resting [Ca\(^{2+}\)]\(_{cyt}\), is performed by several plasmalemmal and intracellular systems. The extrusion of Ca\(^{2+}\) to the extracellular space can be mediated by either plasmalemmal Ca\(^{2+}\)-ATPase (PMCA) or an electrochemically driven Na\(^{+}\)/Ca\(^{2+}\) exchanger. In addition, Ca\(^{2+}\) is removed from the cytoplasm by accumulation into endoplasmic reticulum, via SERCAS, or into another capacious Ca\(^{2+}\) pool, the mitochondria. Several studies have demonstrated a reduced Ca\(^{2+}\) efflux from synaptosomes: this could have arisen from a decreased performance of either the PMCA or the Na\(^{+}\)/Ca\(^{2+}\) exchanger, or both; however, the system that is primarily affected remains unclear\(^{55,56}\). In addition, the decay of depolarization-induced [Ca\(^{2+}\)]\(_{cyt}\) transients was significantly prolonged in aged peripheral and central neurones\(^{57,58}\), indicating an impairment of the Ca\(^{2+}\)-extrusion systems or intracellular Ca\(^{2+}\) buffers, or both. Little is known about the activity of SERCA-dependent Ca\(^{2+}\) transport; however, some indirect data provide an indication that it might also be impaired\(^{57,58}\). Finally, mitochondrial Ca\(^{2+}\) uptake was decreased in synaptosomes prepared from the brain of several strains of aged rats\(^{49}\).

The concentration of intracellular Ca\(^{2+}\) in senescent neurones

The experimental data discussed previously suggest that in neuronal preparations from aged rats there are...
changes in various [Ca^{2+}] regulatory mechanisms (Fig. 1). Because most of the reports have analysed one or other of the Ca^{2+}-regulatory systems in isolation, it is not clear whether or not the described changes would result in age-dependent abnormalities of either [Ca^{2+}] homeostasis or stimulus-evoked Ca^{2+} signalling; this requires direct measurements of [Ca^{2+}] in aged neurons, and there are very few such studies. As shown in Table 2, the most consistent findings demonstrate an increase in resting [Ca^{2+}] in aged neurons, and, after stimulation, a prolongation of [Ca^{2+}] recovery towards the resting concentration.

An important consideration when interpreting these data concerns the suitability of the neuronal preparations used. Knowledge of [Ca^{2+}] homeostasis in aged neurons has come from several neuronal preparations, including; [Ca^{2+}] recordings in synaptosomes, cuvette [Ca^{2+}] recordings in brain cell suspensions, [Ca^{2+}] measurements of isolated and cultured neurons, and [Ca^{2+}] measurements of brain-slice preparations. Because important differences exist between types of neurons, with respect to the generation of Ca^{2+} signals via their receptors and channels, the utility of pooling studies of neurons or neuronal fragments from different brain regions is debatable. Furthermore, whereas the use of synaptosomal preparations for the study of the biochemical properties of enzymes is appropriate, their use as a model of neuronal physiology is not. Also, tests such as staining with "vital dyes" do not provide much information about the functional state of neurons, and the functional viability of neurons in suspension that are used for cuvette [Ca^{2+}] measurements has not been checked directly. Indeed, experiments on cell suspensions show an unusually high resting [Ca^{2+}] (300–600 nm) that might indicate profound cell damage (although the presence of glial contamination might complicate the results). The use of freshly isolated or cultured neurons avoids these disadvantages. Because the isolation of neurons from aged tissues requires stronger and longer enzymatic treatment, both the viability and survival in culture of freshly isolated neurons is limited. Thus, the preparation of choice for estimation of [Ca^{2+}] dynamics in aged neurons, as well as for the investigation of their electrophysiology, is the acutely isolated brain-slice preparation. A few groups have used such a preparation; one group has studied [Ca^{2+}], by using a ratiometric dye, and others have used fluo-3 as a Ca^{2+} indicator; however, the latter does not provide a value for [Ca^{2+}].

**New perspectives on the Ca^{2+} hypothesis of neuronal ageing**

In summarizing the current status of the Ca^{2+} hypothesis of neuronal ageing, it can be seen that, although the experimental data suggest an age-dependent impairment of [Ca^{2+}]-regulatory systems, the lack of direct [Ca^{2+}] measurements does not allow full confirmation of the hypothesis. The alterations in [Ca^{2+}] homeostatic machinery that are detected in aged neurons are not dramatic. Indeed, it is possible that the numerous feedback systems that allow the regulation of [Ca^{2+}], to be adaptable could, in the long-term, overcome the subtle impairment of one or other of the [Ca^{2+}]-homeostatic mechanisms. Thus, convincing support for the Ca^{2+} hypothesis of neuronal ageing requires a substantial increase in our knowledge of [Ca^{2+}], regulation in aged neurons. As previously emphasized, it is important that new information about [Ca^{2+}], homeostasis in aged neurons is obtained using appropriate preparations and experimental techniques. Assuming that the overall [Ca^{2+}], homeostasis is impaired in the aged brain, an important question
remains unanswered: do these changes cause the age-dependent decline in neuronal performance, as the 'calcium hypothesis of ageing' implies, or are they secondary changes that accompany other processes? It is possible that the age-dependent changes in [Ca$^{2+}$], regulation might be part of a neuronal adaptive reaction. From a wider perspective on neuronal physiology that takes into account the general mechanisms involved in neuronal development and maturation, a moderate increase of [Ca$^{2+}$], can have beneficial effects: for example, it is well known that sympathetic (PNS) or cerebellar granule (CNS) neurones require, in the absence of growth factors, depolarizing concentrations of K$^{+}$ for normal survival and maturation. These conditions are associated with increases in the resting [Ca$^{2+}$], and maximal survival is obtained at a [Ca$^{2+}$], level of around 250 nM (Refs 66,67). What is important under these conditions is the relative level of [Ca$^{2+}$], and these observations led to the proposal of the 'Ca$^{2+}$ set-point hypothesis' of neuronal survival$^{18}$. According to this hypothesis, levels of [Ca$^{2+}$], outside the low and high Ca$^{2+}$ set-points result in neuronal death. Between these two extremes, the value of resting [Ca$^{2+}$], is inversely proportional to the concentration of growth factors present in the extracellular space. The supportive role of [Ca$^{2+}$], under these conditions is mediated through its multiple effects on various nuclear signalling cascades that regulate gene expression$^{19}$. It is conceivable that in ageing neurones an increased resting [Ca$^{2+}$], might, initially, be involved in a 'survival' reaction to yet unidentified ageing factors. Currently, the behaviour of [Ca$^{2+}$], in the early stages of neuronal senescence is not known: recent studies have reported modifications in Ca$^{2+}$-homeostatic mechanisms, suggesting that the compensatory mechanisms might have become overloaded, resulting in movement of the concentration of [Ca$^{2+}$], towards harmful levels. To address these possibilities it is necessary to study various aspects of Ca$^{2+}$ signalling and homeostasis in multiple age groups that have been monitored at different stages of development, starting as early as young adulthood. No such reports are currently available.

Another issue in need of further scrutiny is that of the differences between the pathological (neurodegenerative diseases) and the physiological changes appearing during ageing. The cellular death observed in AD and other related diseases could involve an acute deterioration in [Ca$^{2+}$], homeostatic mechanisms. Whether or not the impairment of [Ca$^{2+}$], regulation contributes to the loss of cognitive function in normal brain-ageing remains unclear. Certainly, it can be speculated that an age-associated deterioration in the mechanisms controlling [Ca$^{2+}$], might alter synaptic transmission and neuronal plasticity, and the information currently available does implicate age-dependent abnormalities of Ca$^{2+}$-extrusion systems: a diminished capacity for the clearing of Ca$^{2+}$ loads following neuronal activity could underlie the increased vulnerability of aged neurones to excitotoxic insults. Indeed, increased vulnerability to a variety of factors is widely accepted as a common property of aged nerve-cells$^{20}$. Furthermore, a down-regulated capacity for Ca$^{2+}$ extrusion might be a major factor in the situation where physiological neuronal activity becomes excitotoxic: this is especially important for synaptic transmission that depends on [Ca$^{2+}$], fluctuations in synaptic regions. In the aged brain there is a considerable decrease in the number of functional synapses that coincides with an impairment of various forms of neuronal plasticity, including long-term potentiation$^{21,22}$. It is possible that in conditions of impaired Ca$^{2+}$-clearing capacity, local excitatory events in the synaptic area could become local excitotoxic events, thus leading to the loss of the most active synapses. Similarly, changes in presynaptic and intracellular Ca$^{2+}$-channel density could influence the time course and amplitude of Ca$^{2+}$ signals, as well as the spatial distribution of their 'microdomains' of action$^{22}$, and this could affect intracellular signal-processing. This seems to be particularly important for hippocampal neurones, where an increase in the Ca$^{2+}$ channel density was shown in acutely isolated brain-slice preparations$^{20,31}$. Moreover, chronic treatment with a neurotropic Ca$^{2+}$ antagonist, nimodipine, which presumably acts by reducing Ca$^{2+}$ entry, had beneficial effects on learning

<table>
<thead>
<tr>
<th>Source</th>
<th>Preparation</th>
<th>Method of measurement</th>
<th>Resting [Ca$^{2+}$]</th>
<th>Ca$^{2+}$ signals</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat: whole brain</td>
<td>Synaptosomes</td>
<td>Quin2, Fura-2</td>
<td>Increased</td>
<td>Increased</td>
<td>23</td>
</tr>
<tr>
<td>Rat: cortex cerebrum</td>
<td>Synaptosomes</td>
<td>Quin2</td>
<td>Increased (NS)</td>
<td>Increased and prolonged decay</td>
<td>24</td>
</tr>
<tr>
<td>Rat: hippocampus and cerebral cortex</td>
<td>Synaptosomes</td>
<td>Fluo-3</td>
<td>Increased$^a$</td>
<td>Increased</td>
<td>60</td>
</tr>
<tr>
<td>Rat: hippocampus and cerebral cortex</td>
<td>Synaptosomes and cell suspension</td>
<td>Fluo-3</td>
<td>Increased$^a$</td>
<td>Increased and prolonged decay</td>
<td>60</td>
</tr>
<tr>
<td>Rat, mouse: whole brain</td>
<td>Cell suspension</td>
<td>Fura-2</td>
<td>Decreased$^d$</td>
<td>Decreased</td>
<td>61</td>
</tr>
<tr>
<td>Rat: cortex, hippocampus, striatum, cerebellum</td>
<td>Cell suspension</td>
<td>Fura-2</td>
<td>Decreased in hippocampus and cortex$^d$</td>
<td>Decreased</td>
<td>62</td>
</tr>
<tr>
<td>Rat: DRG</td>
<td>Culture</td>
<td>Fura-2</td>
<td>Increased</td>
<td>Decreased and prolonged decay</td>
<td>63</td>
</tr>
<tr>
<td>Rat: DRG, hippocampus, neocortex</td>
<td>Freshly isolated</td>
<td>Indo-1</td>
<td>Increased</td>
<td>Decreased and prolonged decay</td>
<td>57</td>
</tr>
<tr>
<td>Mouse: cerebellar granule neurones</td>
<td>Slice</td>
<td>Fura-2</td>
<td>Increased</td>
<td>Decreased and prolonged decay</td>
<td>56</td>
</tr>
</tbody>
</table>

$^a$Fluo-3 does not permit accurate estimation of the concentration of intracellular Ca$^{2+}$. $^1$Unusually high resting concentration of intracellular Ca$^{2+}$ (300–600) nM. Abbreviation: NS, not significant.
performance in several animal models (for example, Refs 28,73). It is still not known how these mechanisms change in aged nerve-cells. However, before these speculations can take the shape of a well-defined theory, much more experimental background is needed.

Finally, when studying the cellular mechanisms of brain ageing, it should be borne in mind that the brain contains a huge population of glial cells that are responsible for the regulation of the brain microenvironment. They can also play an important role in the integrative function of neurons by controlling the concentrations of neurotransmitters, and neuropeptides, and thus affecting synaptic transmission. The possible involvement of glial cells in brain ageing remains unclear, although several reports have shown an increase in astrocytic proliferation in certain regions of aged brain. Glial cells, especially astrocytes, rely heavily on [Ca\textsuperscript{2+}] signalling that is involved in most of their responses to neurotransmitters. Indeed, propagating intercellular Ca\textsuperscript{2+} waves might be important for interglial and neuronal-glial interactions. At present, the age-dependent changes in glial physiology and the contribution of glial cells to brain ageing are totally unexplored. Thus, future investigation into [Ca\textsuperscript{2+}] homeostasis in aged brain requires the inclusion of experiments on glial cells.

Concluding remarks

The Ca\textsuperscript{2+} hypothesis of neuronal ageing is still an attractive one. However, there are important problems that need examination: the thorough evaluation of [Ca\textsuperscript{2+}] homeostasis and Ca\textsuperscript{2+} signalling in aged neurons using appropriate preparations, such as acutely isolated brain-slices; and the determination of whether or not altered [Ca\textsuperscript{2+}], regulation modulates neuronal plasticity and performance. The ultimate aim of the investigative effort is to understand whether the physiological process of ageing is determined by irreversible or functional changes in neuronal activity. This effort could have a tremendous impact therapeutically, because it might be possible to target functional changes directly. Whether or not this leads to a pharmacological treatment of [Ca\textsuperscript{2+}], regulatory mechanisms remains an intriguing and challenging question.

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