

COGNITIVE NEUROSCIENCE

COMMENTARY

Looking for answers to L-type calcium channels in the ageing brain (Commentary on Zanos *et al.*)



Juliane Proft and Norbert Weiss

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Alteration of intracellular calcium (Ca^{2+}) homeostasis has emerged as an underlying mechanism of neurodegenerative diseases (Marambaud *et al.*, 2009). Within neurons, calcium ions (Ca^{2+}) represent an essential signalling molecule, responsible for regulating a large number of diverse cellular functions including membrane excitability, synaptic transmission, gene expression, synaptogenesis, cell death and survival, but also cellular processes underlying learning and memory (Berridge, 1998). To make use of and to regulate the amplitude, duration and subcellular localization of the Ca^{2+} signal, nerve cells have developed an extremely complex machinery, the so-called ' Ca^{2+} signaling toolkit' (Berridge *et al.*, 2000). It includes ion channels, pumps and exchangers both in the plasma membrane and in the membranes of intracellular organelles, but also Ca^{2+} binding proteins and transcriptional factors that together coordinate neuronal Ca^{2+} signalling and homeostasis (Brini *et al.*, 2014). Whereas the molecular mechanisms responsible for alterations of neuronal Ca^{2+} signalling in the ageing brain are not clearly understood, numerous studies have reported age-related changes in the expression and/or activity of some of the key players of the Ca^{2+} machinery, often associated with decreased synaptic plasticity, and in some cases with progressive neuronal loss. Hence, increased activity of $\text{Ca}_v1.2$ L-type voltage-gated Ca^{2+} channels (VGCCs) has been reported in aged hippocampal neurons (Porter *et al.*, 1997; Thibault *et al.*, 2001). In parallel, increased ryanodine receptor-dependent Ca^{2+} release from internal stores has been observed (Liu *et al.*, 2014), possibly via enhanced Ca^{2+} -induced Ca^{2+} release (CICR) as a consequence of increased Ca^{2+} influx through L-type channels (Gant *et al.*, 2006). Additionally, decreased plasma membrane Ca^{2+} -ATPase activity and Ca^{2+} extrusion (Michaelis *et al.*, 1996), as well as diminished Ca^{2+} buffering capacity of the aged neuron has been reported (Murchison & Griffith, 1999; Murchison *et al.*, 2004). A study by Zanos *et al.* (2015), reported in this issue of *European Journal of Neuroscience*, examined the specific contribution of $\text{Ca}_v1.2$ channels in ageing-associated cognitive decline.

In the mammalian brain, $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ channels are the most prominent VGCCs accounting for the L-type Ca^{2+} current (Sinnegger-Brauns *et al.*, 2009), where they support activity-dependent gene transcription processes (Barbado *et al.*, 2009). The implication of L-type channels in brain functions is best exemplified by their contribution to various synaptic plasticity and learning and memory processes. In addition, extensive support for a role of L-type channels in age-related cognitive deficits arose from studies on both normal and pathological ageing using a variety of animal models and diverse experimental approaches (Berger & Bartsch, 2014). However, the lack of selective modulators of the most prominent L-type channel isoforms has hampered the elucidation of their respective contributions. Zanos *et al.* used quantitative polymerase chain reaction (qPCR) and cognitive behaviour analysis in young and aged mice to test the hypothesis that age-related cognitive decline may rely on alteration of $\text{Ca}_v1.2$ channel expression. To make such a study possible, the authors took advantage of *Cacna1C* haplo-insufficient mice that possess a genetic deletion of a single *Cacna1c* allele (the gene coding for $\text{Ca}_v1.2$). In turn, these mice present with 50% decreased $\text{Ca}_v1.2$ protein level and decreased L-type currents in CA1 hippocampal neurons. Using the novel object recognition, Y-maze, and passive avoidance cognitive tests, the authors found that ageing was associated with object recognition and contextual/emotional memory deficits. In addition, a correlation between age-related cognitive decline and increased $\text{Ca}_v1.2$ mRNA expression levels was observed in both male and female left hippocampus. In contrast, aged haplo-insufficient mice demonstrated normal cognitive behaviour and did not show age-related increases in $\text{Ca}_v1.2$ mRNA expression levels. However, this phenotype was observed specifically in male haplo-insufficient mice, suggesting a sex/hormone-dependent component in age-associated memory impairment.

Collectively, Zanos *et al.* elegantly highlighted a correlation between age-related cognitive decline and increased $\text{Ca}_v1.2$ mRNA expression levels in the left hippocampus, but stopped short of demonstrating alteration of $\text{Ca}_v1.2$ protein levels and L-type Ca^{2+} currents. Although increased L-type Ca^{2+} currents in aged hippocampal neurons has been nicely documented in various studies (Campbell *et al.*, 1996; Porter *et al.*, 1997; Thibault *et al.*, 2001; Wang & Mattson, 2014), whether this relies on an altered protein expression levels of $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ channels has been a matter of debate. Recently, the respective protein expression levels of L-type channel isoforms have been analysed in the hippocampus from young and aged rats. Surprisingly, while western blot analysis revealed an overall decrease in total expression levels of $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ channels, surface biotinylation and immunohistochemical analysis, in contrast, revealed increased surface expression of $\text{Ca}_v1.2$ channels in CA1 and CA3 regions, and increased $\text{Ca}_v1.3$ expression levels limited to the CA3 region (Núñez-Santana *et al.*, 2014). Although the molecular mechanisms underlying increased surface expression of L-type channels in aged hippocampus remain elusive, it is conceivable that age-dependent alteration of protein degradation may contribute to the phenotype (Martinez-Vicente *et al.*, 2005). Consistent with this idea, control of surface expression of VGCCs by the ubiquitin–proteasome pathway has been reported for $\text{Ca}_v1.2$ (Altier *et al.*, 2011), $\text{Ca}_v2.2$ (Waithe *et al.*, 2011) and more recently $\text{Ca}_v3.2$ channels (García-Caballero *et al.*, 2014). Whether ubiquitination of $\text{Ca}_v1.2$ channels is altered in the aged hippocampus and contributes to the increased surface expression of the channel remains an open question, one

certainly worthy of investigation. On the other hand, it was recently reported that $Ca_v1.2$ channels undergo a proteolytic cleavage resulting in channel fragments that remain on the plasma membrane, and in an attenuation of the L-type current (Michailidis *et al.*, 2014). Interestingly, silencing of $Ca_v1.2$ channels by proteolytic cleavage was shown to increase dramatically with ageing, suggesting that it may represent a homeostatic/neuroprotective mechanism to counterbalance an age-associated increase of $Ca_v1.2$ expression. However, it is also conceivable that age-dependent increased $Ca_v1.2$ proteolytic cleavage represents the primary neuronal alteration, and that increase of channel expression may constitute a compensatory mechanism to maintain sufficient functional channels and preserve neuronal functions. Although difficult to test experimentally, these aspects should be taken into consideration when designing L-type channel blockers for disorders such as Parkinson's disease or Alzheimer's disease, hypertension or cardiac arrhythmia. For instance, chronic administration of the L-type channel antagonist

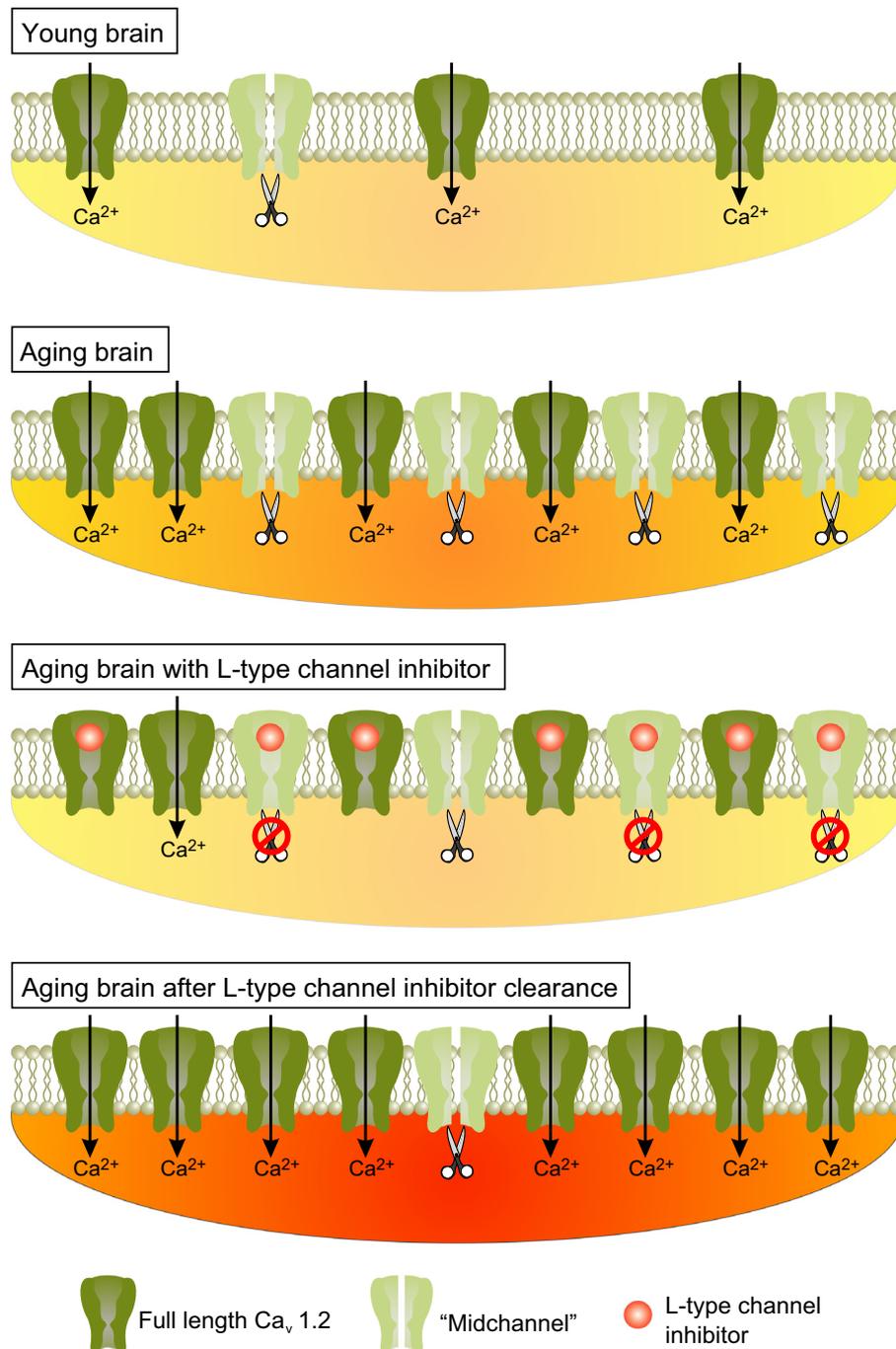


FIG. 1. Putative crosstalk between $Ca_v1.2$ channel expression, proteolytic cleavage and pharmacological inhibition with ageing. In the young brain (first panel), especially in the hippocampus, $Ca_v1.2$ expression is comparatively low and proteolytic cleavage of the channel is limited. Ageing is associated with an increased proteolytic cleavage of $Ca_v1.2$ channels ('midchannels'), partially silencing the concomitant increase of channel surface expression (second panel). Pharmacological inhibition of $Ca_v1.2$ channels in the aged brain not only inhibits $Ca_v1.2$ currents, but also decreases proteolytic cleavage of the channel, leading to an overall increased surface expression of full-length channels (third panel), and recovery of channel activity after clearance of L-type channel inhibitor leads to a significant increase of L-type currents and Ca^{2+} influx compared with the non-treated aged brain (fourth panel).

nimodipine has been shown to attenuate age-related decline of hippocampal-dependent memories (Sandin *et al.*, 1990; Quevedo *et al.*, 1998). However, considering that proteolytic cleavage of Ca_v1.2 channels is regulated by channel activity and can be prevented by pharmacological inhibition of the channel (Michailidis *et al.*, 2014), *in vivo* administration of L-type channel antagonists is expected to produce a dual effect (Fig. 1): (i) an acute inhibition of the L-type current, and (ii) preclude proteolytic cleavage of Ca_v1.2, which in turn may eventually lead to an increase of fully functional channels at the surface, especially after drug clearance, questioning the exact cellular mechanisms by which L-type channel antagonists prevent cognitive decline with ageing, but also the possible outcomes of long-term treatment. Further examinations will certainly provide essential information on how to therapeutically manipulate L-type channels for the management of age-related cognitive decline.

Overall, the findings of Zanos *et al.* provide novel insights into the contribution of L-type Ca²⁺ channels in ageing neurons, and establish Ca_v1.2 channels as key players in age-associated memory decline.

Conflict of interests

J.P. and N.W. declare no conflict of interest and derive no financial interest from this research.

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