Nuclear calcium signalling in the regulation of brain function

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Abstract | Synaptic activity initiates biochemical processes that have various outcomes, including the formation of memories, increases in neuronal survival and the development of chronic pain and addiction. Virtually all activity-induced, long-lasting adaptations of brain functions require a dialogue between synapses and the nucleus that results in changes in gene expression. Calcium signals that are induced by synaptic activity and propagate into the nucleus are a major route for synapse-to-nucleus communication. Recent findings indicate that diverse forms of neuroadaptation require calcium transients in the nucleus to switch on the necessary genomic programme. Deficits in nuclear calcium signalling as a result of a reduction in synaptic activity or increased extrasynaptic NMDA receptor signalling may underlie the aetiologies of various diseases, including neurodegeneration and cognitive dysfunction.

Calcium signals are used in virtually all cells to carry information and regulate numerous biochemical processes[1]. Life would be impossible without calcium ions making the heart beat, causing skeletal muscle to contract, activating the immune system for defence against pathogens or stimulating the release of neurotransmitters to mediate cognitive functions. Calcium signals can act locally near the site of calcium entry into the cytoplasm but are also often transmitted over long distances and can reach the cell nucleus[2].

Nuclear calcium has emerged as one of the most potent regulators of neuronal gene expression[3]. Synaptic stimuli that give rise to increased nuclear calcium levels in a neuron activate gene programmes that can induce structural and functional alterations in the same cell and in the network to which it belongs. These changes include the build-up of a neuroprotective shield[4] or a persistent increase in the efficacy of synaptic transmission, which is considered to be a cellular correlate of behavioural adaptations, such as learning, memory or addiction[5–8]. Several years ago it was proposed that both activity-driven changes in functional properties and the generation of nuclear calcium transients are causally linked and that nuclear calcium is the signal that switches on the genomic components needed for the long-term implementation of neuroadaptations[9]. This 'nuclear calcium hypothesis' (REF. 9) predicted that persistent adaptations — both at the cellular and the behavioural level — take place when calcium enters the cell nucleus to activate transcription.

In this Review, I summarize recent findings supporting this hypothesis and outline the role of nuclear calcium as an evolutionarily conserved signal that is used in many cell types and organisms to control signal-induced, long-lasting adaptive responses. A general overview of the various other mechanisms by which signals can be relayed to the cell nucleus in neurons can be found in REFS 10,11.

Synapse-to-nucleus signalling

Adaptation is a characteristic of life. Virtually all organisms adjust to changes in their environment, and this may be advantageous or even essential to cope with new conditions. For such changes to be long lasting, the cells' gene expression patterns must be reprogrammed appropriately. One example of a transcription-dependent adaptation in the nervous system is the sensitization of the gill withdrawal reflex in the sea slug *Aplysia californica*[12,13]. In this non-associative learning paradigm, the pairing of a touch to the siphon with a brief electrical shock to the head results in an exaggerated gill withdrawal response. If the paired stimulation is applied repeatedly, this change in the behaviour of the animal persists for days. This long-lasting form of sensitization was found to be dependent on gene transcription and new protein synthesis[14]. This was a landmark discovery showing that the process of 'learning' and the resulting long-term adaptation requires a dialogue between synapses and the cell nucleus. Subsequent studies revealed that signalling to the nucleus and the activation of genomic responses

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doi:10.1038/nrn3531
Published online
14 August 2013

are also required for olfactory associative learning in the fruitfly *Drosophila melanogaster*[15,16], learning of spatial cues in rodents[17] and for late-phase long-term potentiation or depression of synaptic efficacy, which may underlie learning[18,19].

Addiction and chronic pain are also adaptive responses of the nervous system, in which the relevant stimulus (an addictive drug or the prolonged irritation of sensory neurons) induces changes in synaptic transmission that functionally alter the participating neuronal network and lead to pathological behaviour[6,8,20]. As with classical learning-associated events, signalling to the nucleus and the resulting genomic responses are crucial for maintaining the new—in this case pathological—state[21,22]. Further examples of adaptive responses associated with the activation of nuclear signalling pathways and signal-regulated transcription include activity-dependent neuronal survival[3,23–25], light-induced changes in circadian rhythmicity[26,27], neural repair after injury[28,29] and neuronal activity-induced neurogenesis[30]. Calcium has a central role as the mediator of the intracellular information flow in virtually all forms of neuroadaptation. Within the nucleus, calcium signals integrate synapse-to-nucleus communication and translate particular activity patterns into altered gene transcription.

Why calcium and why nuclear calcium?

There are several reasons for the selection of calcium as a key second messenger during evolution, and these have been reviewed in detail elsewhere[31,32]. Briefly, as a divalent cation, calcium can generate more stable complexes with other compounds than can monovalent ions such as sodium, potassium or chloride. Compared with the divalent magnesium ion, calcium has a larger radius and a more complex electron shell configuration. This provides the flexibility that allows it to readily form bonds with coordination sites that have irregular geometry, such as those characteristic of biological macromolecules. In addition, proteins can form compact binding cavities matching the dimensions of calcium, which is more difficult to achieve for the smaller magnesium ion owing to the limited structural flexibility of proteins. Thus, owing to its size and particular coordination chemistry (also see REFS 31,32), calcium is far better suited than other ions that are commonly present in a biological environment for the specific, high-affinity interactions with proteins that can cause the conformation of the binding partner to change from one state to another.

Because the presence or absence of calcium cannot be regulated through its synthesis or destruction, cells have developed an efficient mechanism to keep its cytosolic levels very low. Calcium is eliminated from the cytosol by sarcoendoplasmic reticulum calcium ATPase (SERCA) pumps localized in the endoplasmic reticulum (ER) membrane, an intracellular calcium store that extends throughout neurons, as well as by plasma membrane calcium ATPases and sodium–calcium exchangers (NCXs). This creates a steep concentration gradient across the cell membrane, which provides the driving force for calcium entry into the cytosol through calcium channels embedded in the plasma membrane and the ER membrane.

The evolutionary emergence of voltage-sensitive gating in plasma membrane calcium channels was probably also important for the selection of calcium as a neuronal signal transducer. This biophysical feature provides the mechanistic basis for a direct coupling between the main function of neurons (that is, the depolarization of their membranes and the firing of action potentials) with a rise in intracellular calcium levels. Thus, without the need for any intermediate enzymatic reaction, an intracellular signal that can mirror the neurons' electrical activity state is generated. Calcium transients caused by calcium entry from the extracellular space can be further enhanced by ryanodine receptor-mediated, calcium-induced calcium release from the ER or the Golgi apparatus[33,34]. A second exit route for calcium from the intracellular stores (ER and Golgi apparatus) is through the inositol trisphosphate (IP3) receptor[33,34]. The opening of IP3 receptors may be associated with synaptic activation if the neurotransmitter involved stimulates G protein-coupled receptors (GPCRs) that activate phospholipase C, the enzyme that cleaves phosphatidylinositol-4,5-biphosphate to generate IP3 as well as diacyglycerol, which stimulates protein kinase C[35].

Downstream of the initial activity-induced calcium transient, signal processing can diversify and engage other molecules. For example, increases in cyclic AMP (cAMP) and nitric oxide can be generated through calcium-sensitive adenylyl cyclases and nitric oxide synthases, respectively[36–39]. Calcium signalling also feeds into classical receptor tyrosine kinase-regulated pathways, including the RAS–extracellular signal-regulated kinase (ERK)/mitogen-activated protein (MAP) kinase cascade[40,41], which calcium activates by stimulating neuronal RAS-specific guanine nucleotide-releasing factor (RASGRF; also known as Ras guanine nucleotide exchange factor)[42] and by inhibiting a synaptic RAS GTPase-activating protein[43]. However, the spread of these secondary calcium signalling events is generally rather limited because they are primarily mediated by diffusion and are subject to rapid enzymatic inactivation[44]. Although calcium signals also face elimination by calcium uptake and other clearance systems, they can, within milliseconds, bridge long distances of up to several hundred micrometres to reach the signalling end point, the cell nucleus[45–50]. The nuclear envelope is not a measurable diffusion barrier for calcium, which can freely enter and exit the nucleus via the nuclear pore complexes[51,52]. By bypassing the need for an energy-dependent nuclear import mechanism (required for large, protein-based signal transducers), signal propagation is simplified and the chances for failure and possible loss of information are reduced.

Calcium imaging indicates that the generation of nuclear calcium transients strongly correlates with the number of spikes evoked by high-frequency or theta-burst stimulation[50]. Thus, nuclear calcium can provide a fingerprint of the electrical activity of neurons; it integrates spike patterns and is ideally suited to rapidly, efficiently and faithfully relay stimulation conditions to the nucleus for the initiation of appropriate genomic responses.

Long-term potentiation
A long-lasting (hours or days) increase in synaptic efficacy that is most commonly measured as the response of neurons to stimulation of their presynaptic afferents after a brief patterned stimulus (for example, a 1-s, 100-Hz stimulus).

Nuclear envelope
Two membranes (outer and inner) surrounding the cell nucleus; the outer membrane is continuous with the endoplasmic reticulum. The outer nuclear membrane is connected to the inner nuclear membrane at the nuclear pore complexes.

Nuclear pore complexes
Large multiprotein complexes that form channels in the nuclear envelope of a eukaryotic cell. Nuclear pore complexes span the inner and outer nuclear membranes and allow the exchange of ions, metabolites and macromolecules between the nucleus and the cytoplasm.

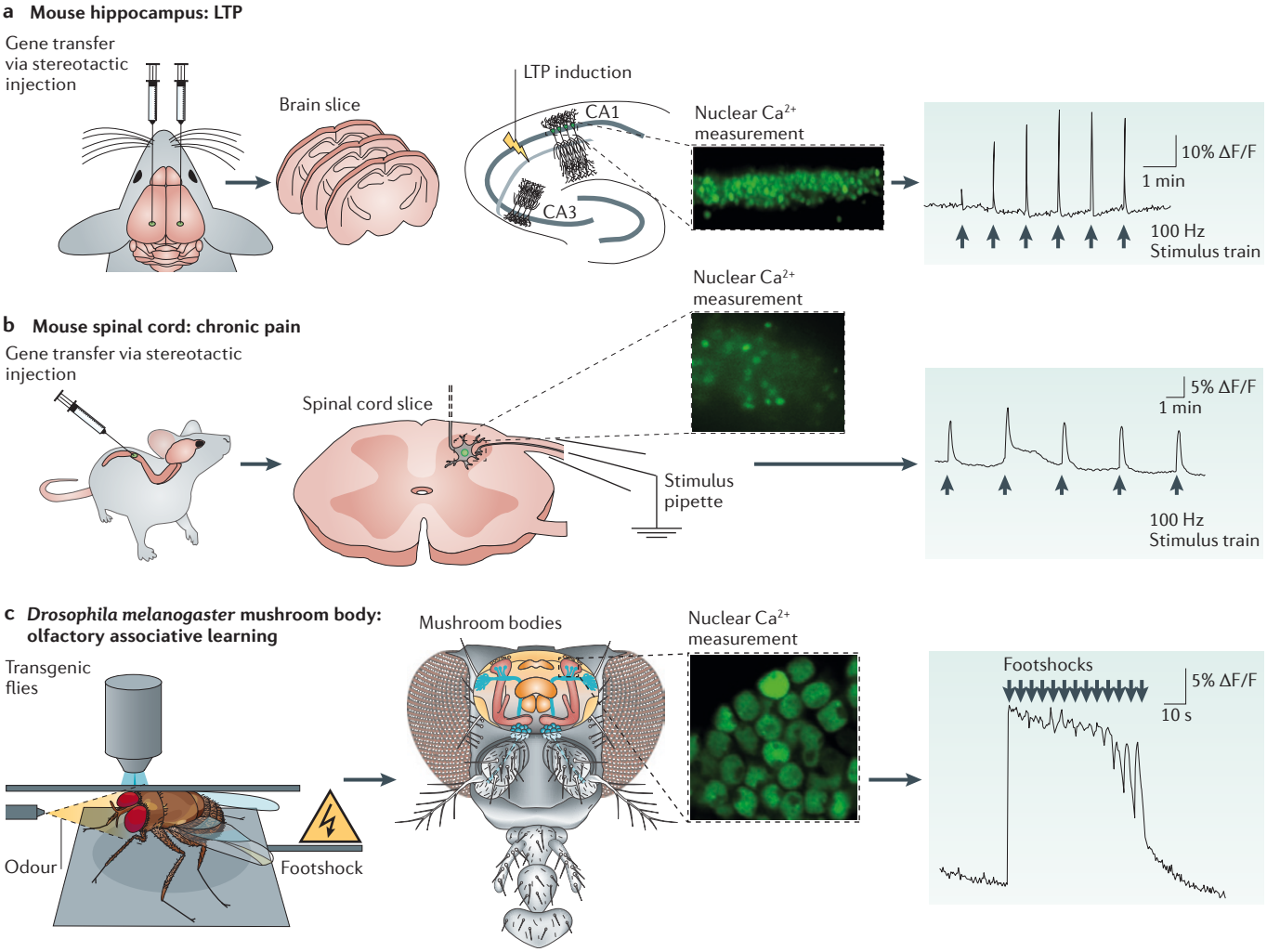


Figure 1 | **Detection of nuclear calcium signals in models of neuronal plasticity, learning and memory, and pain.** Recombinant, nuclear-targeted calcium sensors were expressed in the mouse hippocampus (panel **a**) or spinal cord (panel **b**) using viral vector-mediated gene transfer and in the brain of *Drosophila melanogaster* (panel **c**) using transgenic technology. The panels show a schematic illustration of the detection of nuclear calcium signals in hippocampal CA1 pyramidal neurons upon long-term potentiation (LTP)-inducing, high-frequency stimulation of the Schaffer collaterals (panel **a**), and in spinal cord neurons after stimulation of nociceptive-like activity (panel **b**). Nuclear calcium transients are also detected in the intact living fly brain during olfactory associative learning, in which a fly learns to associate a particular odour with a footshock (panel **c**). Nuclear calcium signals are detected as changes in the fluorescence of the imaged cells, and these measurements are used to create the traces shown on the right. Illustrations are based on the imaging experiments described in REFS 50,53,54.

Generating nuclear calcium transients

The first unambiguous demonstration of synaptic activity-induced calcium rises in the cell nucleus was made possible with the advent of recombinant calcium sensors that can be targeted to this specific subcellular compartment[50]. More recently, a nuclear-targeted sensor was used to detect nuclear calcium signals in mouse spinal cord slices during stimulation of nociceptive-like activity[53] and in the intact living brain of *D. melanogaster* during olfactory associative learning[54] (FIG. 1).

Upon excitatory glutamatergic synaptic stimulation, calcium signals can reach the nucleus by two possible routes (FIG. 2). The principal pathway is triggered by the activation of synaptic NMDA-type glutamate receptors (NMDARs) and AMPA- and kainate-type glutamate receptors causing an excitatory postsynaptic potential, which, if it is above threshold at the axon hillock, initiates action potentials. These propagate down the axon and retrogradely into the dendritic tree[55], causing plasma membrane depolarization and the opening of L-type voltage-gated calcium channels that are highly expressed in the somatic and perisomatic area[56,57] and functionally coupled to transcription[58–61].

Calcium in the cell soma can enter the cell nucleus via the nuclear pore complexes[51,52]. Both the amplitude and kinetics of nuclear calcium increases may be influenced by ER calcium uptake and release systems. SERCA pumps, which facilitate calcium clearance, may shield

Excitatory postsynaptic potential
The depolarizing voltage response of a postsynaptic neuron to a neurotransmitter released by one or more afferent presynaptic terminals that moves the membrane potential towards the action potential threshold.

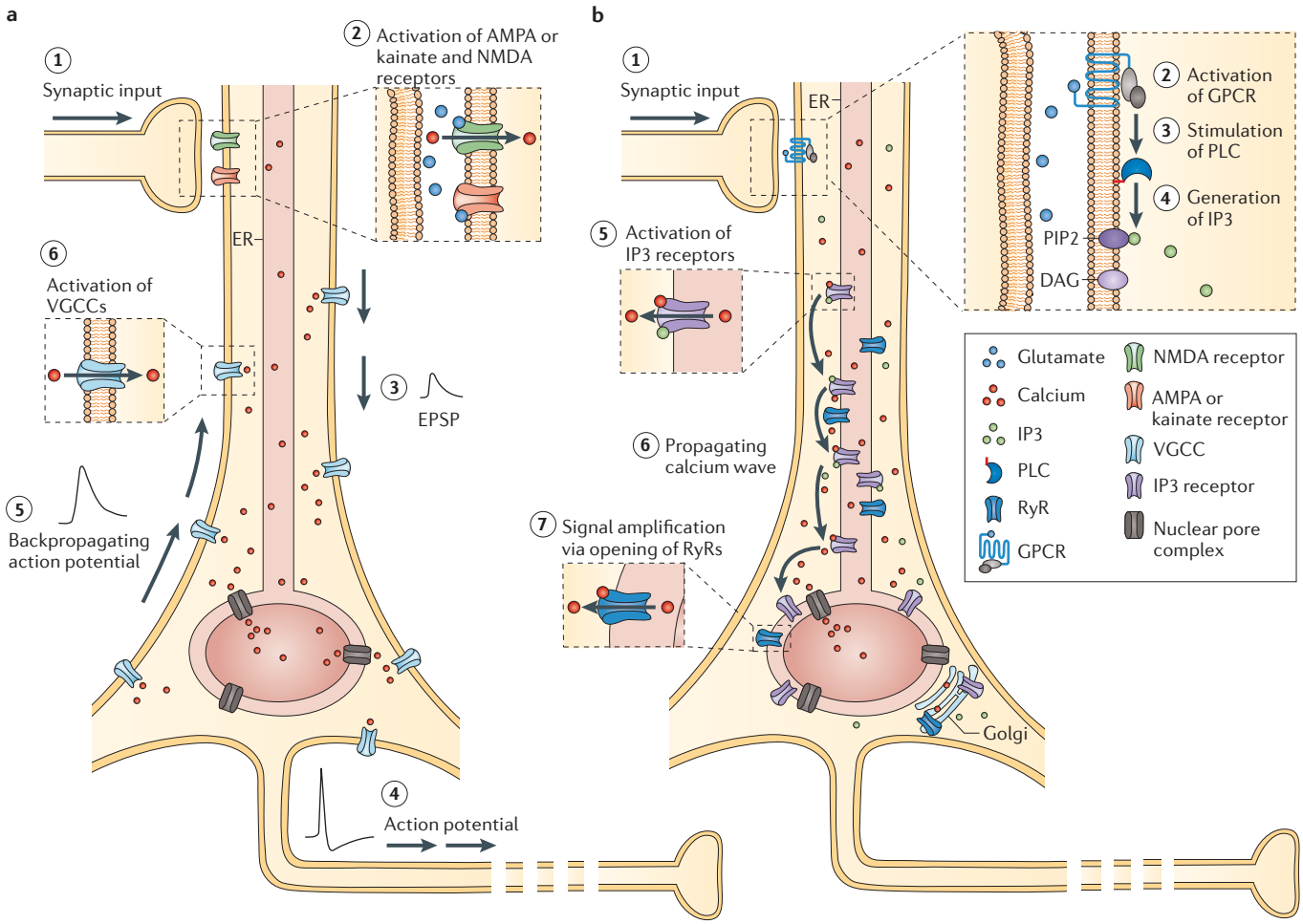


Figure 2 | **Mechanisms by which synaptic activity can generate nuclear calcium transients.** Schematic illustrations of the two principal pathways through which increases in the nuclear calcium concentration are generated. **a** | Synaptic inputs (step 1) activating AMPA, kainate and NMDA receptors (step 2) evoke excitatory postsynaptic potentials (EPSPs) (step 3), which trigger action potentials (step 4) that backpropagate into the dendrites (step 5), causing membrane depolarization and calcium entry through voltage-gated calcium channels (VGCCs) (step 6). Synaptic NMDA receptor-mediated depolarization can boost somatic calcium entry through VGCCs[50,236] (not shown) and in addition may amplify calcium increases by calcium release from the endoplasmic reticulum (ER) or the Golgi apparatus[33,34] via ryanodine receptors (RyRs) or inositol triphosphate (IP3) receptors (see part **b**). **b** | Synaptic inputs (step 1) lead to stimulation of G protein-coupled receptors (GPCRs) (step2) that, via the activation of phospholipase C (PLC) (step 3), lead to the production of IP3 (step 4). The activation of IP3 receptors (step 5) causes the release of calcium from intracellular stores, which initiates a propagating intracellular calcium wave (step 6). Calcium-induced calcium release from the ER or the Golgi apparatus via RyRs may amplify the calcium increases (step 7). Mitochondria (not included in the illustration) are an additional source and sink for calcium that can further shape intracellular calcium dynamics. DAG, diacylglycerol; PIP2, phosphatidylinositol-4,5-bisphosphate.

the nucleus against calcium invasions, whereas ryanodine receptors and IP3 receptors are capable of amplifying nuclear calcium signals[33]. Whether the ER acts as a source or sink for calcium depends on the calcium content in the ER lumen. Under resting conditions, the ER may only be partially filled[62], which may be one reason why calcium release from intracellular stores was found to play a minor part in the generation of nuclear calcium transients in CA1 neurons from acute hippocampal slices upon stimulation of the Schaffer collaterals[50,63]. To act as a calcium source, the ER lumen may need first to be stocked up with calcium that enters the neurons from the extracellular space — for example, during membrane depolarization induced by backpropagating action potentials[64–67]. Thus, by progressively accumulating calcium, the ER is capable of generating a memory trace of the neuron's recent synaptic activity history, which can subsequently be read out via IP3 receptor- or ryanodine receptor-mediated calcium release[33].

The second mode of calcium signal transduction to the nucleus involves IP3 receptors. Because of the characteristic bell-shaped dependence of IP3 receptor activation on cytosolic calcium concentrations[68], increases in IP3 production can initiate a calcium wave that propagates along the dendrite, and this propagation is sustained through the sequential activation of

neighbouring IP3 receptors (see REF. 33 for a review) (FIG. 2). Provided that the readily releasable ER calcium store is sufficiently loaded (see above), such regenerative calcium waves, which can travel to the soma and invade the nucleus[46–49], can be triggered by various agonists of GPCRs or receptor tyrosine kinases. These include the neurotransmitters glutamate and acetylcholine acting on group I metabotropic glutamate receptors and muscarinic acetylcholine receptors, respectively[46–49,64,69–73], but also many hormones, growth factors, and pro-inflammatory compounds[1]. Whether physiological synaptic activity patterns trigger calcium waves *in vivo* remains to be investigated.

The existence of a third possible mode for calcium signal transduction to the nucleus has been proposed based on theoretical considerations[74]. In this model, an electrotonic signal initiated by synaptic activity spreads intracellularly along the ER membrane and, upon arrival at the nucleus, depolarizes the nuclear envelope, leading to the opening of putative voltage-gated calcium channels residing in the outer and/or inner nuclear envelope[75,76].

Except for the mechanism described in the third model, nuclear calcium transients occur — with a slight delay — together with increases in the cytosolic calcium concentration. However, the possibility that the nucleus can generate calcium signals independently of the cytosol is frequently raised (BOX 1).

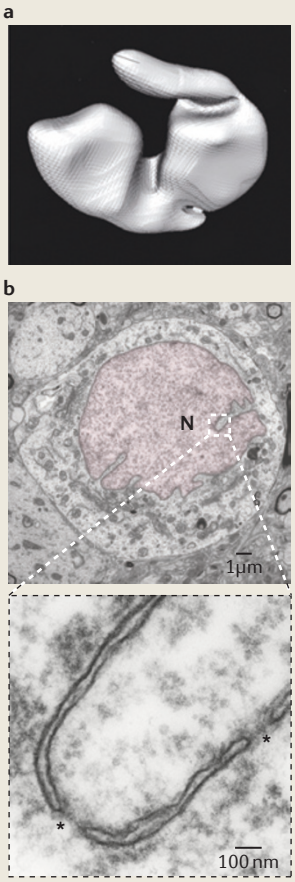
Nuclear calcium regulates transcription

In 1997, an experiment with the mouse pituitary cell line AtT20 unearthed the functional importance of nuclear calcium in gene regulation[77], a concept that had been speculated upon since signal-induced nuclear calcium transients were first observed[78,79]. In this experiment,

Box 1 | **Are nuclei autonomous calcium signalling units?**

Because the endoplasmic reticulum (ER) is continuous with the nuclear envelope, it has been suggested that calcium may directly enter the nucleoplasm from the ER lumen, thereby bypassing the cytosol. The presence of the phosphoinositide–phospholipase C signalling system (which produces inositol triphosphate (IP3)) in the nucleus[212–214] offers a possible mechanism by which this might occur. However, in order for this mechanism to function independently of the cytosol, IP3 receptors would have to be located in the inner leaflet of the nuclear envelope. Mechanistically, it is unclear, particularly in postmitotic neurons, how IP3 receptors or any other large calcium release channel with several transmembrane regions would get past the nuclear pore complex while trafficking from the ER and outer nuclear envelope to the inner nuclear envelope. Although biochemical and electrophysiological data are consistent with the presence of IP3 receptors on the inner nuclear envelope[215–219], an incisive electron microscopy localization analysis of IP3 receptors in any cell type is lacking. Moreover, in neurons and other animal cells, conditions in which calcium transients occur in the nucleus but not in the cytosol have not been identified so far. Thus, an autonomous generation of nuclear calcium transients is currently merely a conceivable scenario.

There is also controversy about the existence of an intranuclear calcium reservoir. Using light microscopy, large extensions of the ER containing IP3 receptors have been detected in the nuclei of a human liver adenocarcinoma cell line[220]. Similar indentations of the nuclear envelope have also been observed in neurons and retinal ganglion cells[221–227], and in plant cells[228]. These structures, if they are formed by the inner nuclear envelope and contain calcium release channels (see above), could function as a reticular network of calcium stores and a rich source from which calcium could flow directly into the nucleoplasm. However, electron microscopy studies have revealed that — at least in hippocampal neurons — such invaginations are lined by two membranes: that is, the inner and the outer nuclear envelope [229]. Therefore, rather than containing intranuclear cisterns forming a nucleoplasmic reticulum, many nuclei of hippocampal neurons are highly infolded, and form suborganelle compartments and signalling microdomains[229,230]. The figure (part **a**) shows a three-dimensional reconstruction of an infolded nucleus of a cultured rat hippocampal neuron and an electron micrograph picture (part **b**) of an infolded nucleus (N, shaded in pink) of a neuron from the CA1 pyramidal cell layer of a hippocampal slice taken from an adult rat. The close-up (inset of part **b**) shows that the invaginations are lined by both the inner and outer nuclear membranes and contain nuclear pore complexes (indicated by asterisks). Nuclear infoldings are dynamic, signal-regulated structures: they can form in response to synaptic activity and are lost after the stimulation of death signalling pathways after extrasynaptic NMDA receptor activation[229]. The volume of infolded nuclei is similar to that of nuclei with a near-spherical shape, but their surface area is significantly larger, and diffusion distances between cytosolic and nuclear locations are shorter. Infolded nuclei also contain more nuclear pore complexes, the principal nuclear entry sites of calcium[51,52]. These properties facilitate calcium transfer into and out of the nucleus and enhance its ability to temporally resolve oscillating signals[229]. Thus, the geometry of the nucleus — itself regulated by synaptic activity — can influence calcium signal transduction. Part **a** image courtesy of G. Queisser, Goethe-Center for Scientific Computing, Goethe University Frankfurt am Main, Germany, and part **b** images courtesy of A. Hellwig, University of Heidelberg, Germany.



Electrotonic signal
A passively propagating electrical impulse. It differs from an action potential in that its spread of charge along a cellular membrane does not involve the activation of voltage-dependent transmembrane currents.

the calcium chelator BAPTA coupled to 70-kDa dextran was microinjected into the nucleus of AtT20 cells. As the 70-kDa dextran-coupled BAPTA is too large to rapidly diffuse out of the nucleus through the nuclear pore complexes, this manipulation selectively attenuated nuclear calcium transients but did not affect cytosolic calcium signals evoked by membrane depolarization, which causes influx of calcium through voltage-gated calcium channels. Under these conditions, induction of gene expression mediated by the transcription factor cAMP-responsive element-binding protein (CREB) was completely blocked[77] (FIG. 3). By contrast, transcriptional activation mediated by a different calcium-responsive DNA regulatory element, the serum response element (SRE), was unaffected[77]. The SRE is a target of the ERK/MAP kinase pathway[80,81], which is activated in hippocampal neurons by an increase in the calcium concentration in the submembranous space in the vicinity of calcium entry channels[40,61,82]. An example of an SRE-driven gene is *EGR1* (also known as *ZIF-268*, *NGFIA* or *KROX-24*)[83–85]. As expected, *EGR1* is not part of the nuclear calcium-regulated gene pool[3], although its expression is robustly induced by synaptic activity[3].

Nuclear calcium signalling targets CREB[77,86] but also stimulates the activity of the CREB co-activator, CREB-binding protein (CBP), which is critical for CREB-dependent transcription[87,88]. Because CBP interacts with many DNA-binding proteins[89], it can confer nuclear calcium inducibility to other transcription factors, such as c-Jun[90]. Thus, CBP regulation by nuclear calcium signalling provides a mechanism through which synaptic activity can control several transcriptional regulators and a broad array of target genes that have functions in various neuronal processes[9].

The main signalling intermediate in synaptic activity-driven, CREB–CBP-dependent transcription is calcium/calmodulin-dependent protein kinase IV (CaMKIV), a serine/threonine kinase predominantly located in the cell nucleus[91–93]. Upon activation by nuclear calcium/ calmodulin, CaMKIV phosphorylates CREB on its activator site serine 133, allowing CREB to interact with CBP. This phosphorylation event, which can be catalysed by a number of different protein kinases[94,95], is frequently used as a 'marker' for ongoing CREB-dependent gene expression. However, the phosphorylation of CREB on serine 133 alone is not sufficient to stimulate transcription[87,96]. A second regulatory step required for the initiation of transcription is the activation of CBP by nuclear calcium and CaMKIV[87,97]. Besides nuclear calcium–CaMKIV signalling, an increase in cAMP, leading to the stimulation of cAMP-dependent protein kinase, is the only other pathway known to be sufficient to provide both activation events for CREB–CBP-dependent transcription[87,94,95].

CBP has intrinsic histone acetyltransferase activity, suggesting that synaptic activity-driven transcription results from increases in the acetylation of histones bound to target genes. These chromatin changes, which enhance the ability of polymerase II to transcribe the DNA[98,99], are further promoted by the nuclear export of class IIa histone deacetylases (HDACs), which is triggered by synaptic activity and is mediated by a nuclear calcium-dependent mechanism[100–102] (FIG. 3). Changes in the methylation patterns of DNA represent a second chromatin remodelling process associated with synaptic activity[103–108]. Although traditionally thought to be associated with gene silencing[109,110], recent findings also link DNA methylation to gene activation[111,112]. One mechanism through which synaptic activity leads to increases in DNA methylation involves the robust transcriptional induction — partly controlled by nuclear calcium signalling — of the *de novo* DNA methyltransferase, *Dnmt3a2* (also known as *Dnmt3a*)[113]. *Dnmt3a2* is associated with actively transcribed euchromatin (many other DNA methyltransferases generally localize to heterochromatin[114]) and is required for a full transcriptional response of the plasticity-related genes *Arc* (which encodes activity-regulated cytoskeleton-associated protein) and *Bdnf* (which encodes brain-derived neurotrophic factor) in synaptically activated hippocampal neurons[113]. In aged mice, *Dnmt3a2* expression decreases, and this decrease is causally linked to the animals' inability to form long-term memories[113]. The rescue of *Dnmt3a2* levels reverses these memory deficits, suggesting a gating function for *Dnmt3a2* and DNA methylation in cognitive abilities[113].

The decoding of methylation patterns by the transcription regulating machinery involves methyl-CpG-binding protein 2 (MECP2), a widely expressed, methylated DNA-binding protein[115]. In the nervous system, MECP2 has an important role in neuronal circuit formation[116]. Mutations in *MECP2* are associated with Rett Syndrome, a neuropsychiatric disorder that is thought to be caused by synapse dysfunction and malformation of neuronal networks[117]. MECP2 may function as a classical transcription factor regulating specific genes[118], although it is more likely that it acts in a histone-like fashion to modulate transcription more globally through chromatin remodelling processes[119,120]. Several MECP2 phosphorylation sites have been identified[118,121,122]. At least one of these, serine 421, is crucial for proper MECP2 function and is phosphorylated in response to synaptic activity in a nuclear calcium-dependent manner[123]. Unlike CREB–CBP activation, this function of nuclear calcium is mediated by nuclear CaMKII[118,123]. Thus, within the nucleus, calcium signals regulate the expression of specific target genes via CaMKIV and, in a more widespread manner, modulate chromatin structure via CaMKII–MECP2 signalling (FIG. 3).

Two additional mechanisms contribute to the pathways through which nuclear calcium modulates genomic events. One involves the downstream regulatory element (DRE) antagonist modulator (DREAM; also known as calsenilin and KChIP3)[124–126]. DREAM is a multifunctional protein that contains several EF hand calcium-binding domains[124–126]. At low nuclear calcium concentrations, DREAM can repress transcription through its interaction with the DRE that is present in many genes[127]. Binding of calcium to the EF hands of DREAM causes it to dissociate from the DNA and allows transcription to take place. The second mechanism, which is less well understood, involves the modulation of the nucleocytoplasmic shuttling of FOXO3A (also known as FOXO3), a forkhead transcription factor with a known role in cell death processes[128,129]. In hippocampal neurons, death

Histone acetyltransferase (HAT). An enzyme that catalyses the addition of an acetyl group to specific lysine residues in histones. In general, increased levels of histone acetylation are associated with the activation of gene expression. Many HATs function as transcriptional co-activators.

Histone deacetylases (HDACs). Enzymes that remove the acetyl groups from lysine residues that are located at the amino termini of histones. In general, decreased levels of histone acetylation are associated with the repression of gene expression.

EF hand calcium-binding domain
A highly conserved calcium-binding domain, comprising two helices (E and F after the fifth and sixth helices of parvalbumin) that are linked by a short acidic calcium-binding loop that coordinates the calcium ion in a pentagonal bipyramidal arrangement. EF hands are found in many calcium-binding proteins, including calmodulin.

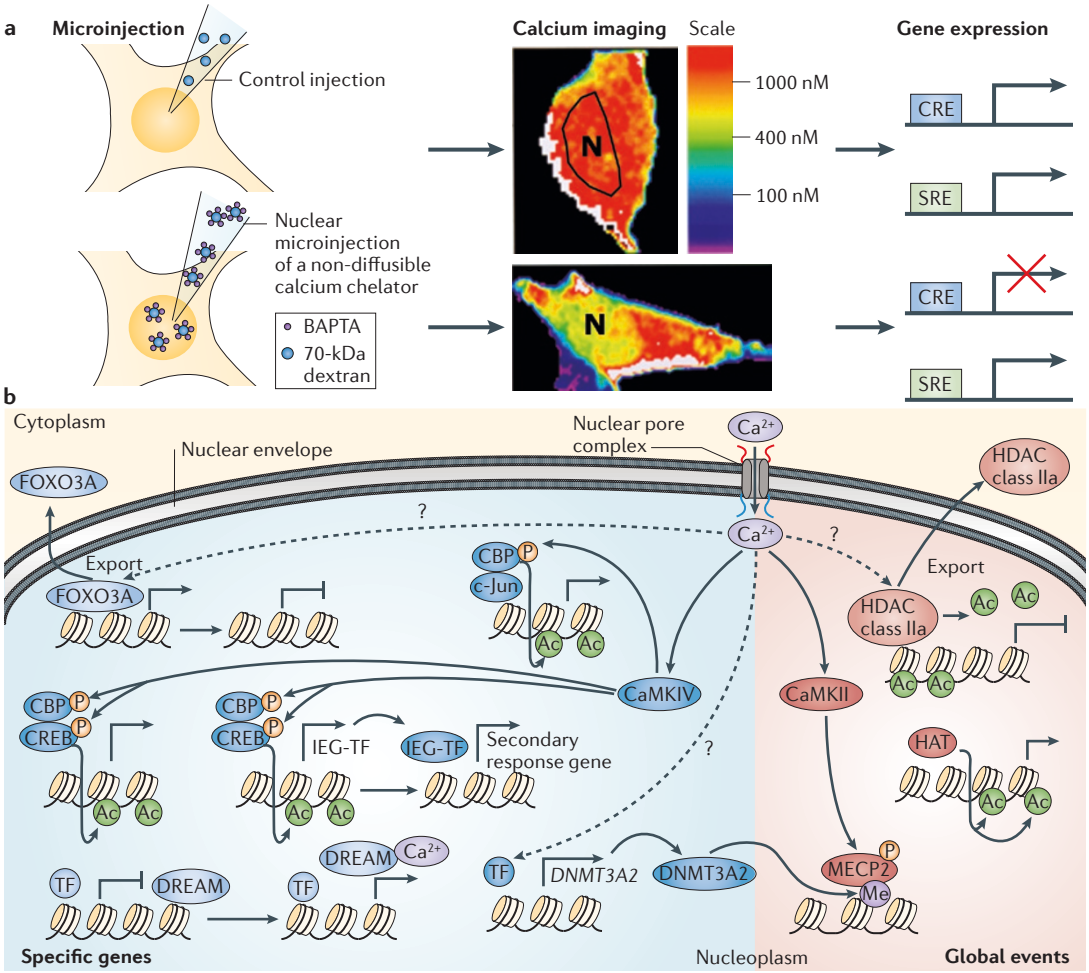


Figure 3 | **Transcriptional regulation by nuclear calcium signals.** **a** | Functional analysis of the cyclic AMP (cAMP) response element (CRE) and the serum response element (SRE) — two DNA regulatory elements that are present in the promoter of the *c-FOS* gene — unearthed their distinct roles in calcium-mediated regulation of gene expression[77]. The calcium imaging experiments illustrate in pseudocolours (scale bars indicate corresponding calcium concentration) increases in the intracellular calcium concentration in a mouse pituitary cell line exposed to 50 mM extracellular potassium, which triggers calcium entry through voltage-gated calcium channels. N marks the position of the cell nucleus. A control-injected cell shows a global increase in the calcium concentration (upper panel). In a cell in which the nucleus has been microinjected with 70-kDa dextran-coupled BAPTA (a 'non-diffusible' calcium chelator that is too large to rapidly diffuse out of the nucleus through the nuclear pore complexes), nuclear calcium transients are selectively attenuated (lower panel). Analysis of calcium-regulated gene expression using reporter gene constructs containing either a CRE or an SRE in their promoter revealed that control cells activate transcription via both the CRE and the SRE (upper panel). By contrast, in cells in which the generation of nuclear calcium transients is inhibited by the nuclear microinjection of 70-kDa dextran-coupled BAPTA, transcription mediated by the CRE is blocked (lower panel). **b** | Calcium flux from the cytoplasm into the nucleus via the nuclear pore complexes activates several signalling molecules that can both initiate global gene regulatory events and trigger transcriptional induction or repression of specific target genes. Global events include the nuclear export of class IIa histone deactylases (HDACs). HDACs antagonize the action of histone actetyltranferases (HATs) by removing acetyl (Ac) residues from histones, rendering chromatin less permissive for transcription. Nuclear export of class IIa HDACs thus promotes gene transcription. A second global event is the phosphorylation of methyl-CpG-binding protein 2 (MECP2) by nuclear calcium/calmodulin-dependent protein kinase II (CaMKII). The regulation of expression of specific genes by nuclear calcium signalling involves CaMKIV-mediated activation of cAMP-responsive element-binding protein (CREB)-binding protein (CBP) that is recruited to specific promoters by CREB as well as by other DNA-binding proteins such as c-Jun. The CREB–CBP interaction requires CREB phosphorylation on serine 133, which can be catalysed by CaMKIV. CBP stimulates transcription through its intrinsic HAT activity. Several CREB– CBP-regulated immediate-early genes (IEGs) encode transcription factors (IEG-TFs), which stimulate or repress the expression of secondary response genes. Nuclear calcium promotes nuclear export of the pro-apoptotic transcription factor FOXO3A and binds to the transcriptional repressor downstream regulatory element antagonist modulator (DREAM), which then dissociates from the DNA, allowing transcription of target genes to take place. It also stimulates remodelling of chromatin through the induction of expression of the *de novo* DNA methyltransferase DNMT3A2. The solid and dashed lines indicate mechanistically resolved and mechanistically still unresolved signaling pathways, respectively. Images of calcium signals in part **a** are reproduced, with permission, from REF. 77 © (1997) Macmillan Publishers Ltd. All rights reserved.

Transcriptome
The complete set of RNA molecules produced by a cell or a population of cells at a given time point.

Acquired neuroprotection
A synaptic activity-driven and gene transcription-dependent enhancement of the ability of neurons to survive harmful conditions.

Memory consolidation
A molecular mechanism by which memories are converted into an enduring form. This process typically lasts for a few hours after learning and requires new protein synthesis.

signalling pathways induced by the activation of extrasynaptic NMDARs cause the translocation of FOXO3A from the cytosol to the nucleus[130]. A nuclear calcium–CaMKIV-mediated process triggered by synaptic activity can protect neurons against this death signal-associated relocation of FOXO3A[130]. Thus, nuclear calcium signalling can modify the availability of transcription factors in the nucleus (FIG. 3).

Functional roles of nuclear calcium

Nuclear calcium is one of the most potent regulators of neuronal gene transcription[3] and has become the prime candidate for a universal 'on-switch' for genomic programmes that convert adaptive changes in neurons, networks and animal behaviour from labile into stable forms. Evidence supporting this concept comes from studies of a broad spectrum of neuroadaptations in different species and also from transcriptome analyses that uncovered the nuclear calcium dependency of the expression of many genes that had been linked to behavioural alterations (FIG. 4). Studies of the functional importance of nuclear calcium required a means to selectively interfere with these signals. Protein-based nuclear calcium buffers and the design of a recombinant inhibitor of nuclear calcium signalling that can be expressed in the nuclei of large populations of neurons *in vitro* and *in vivo* provided powerful tools for determining the role of nuclear calcium signalling in complex brain functions[3,25,53,102,113,123,130–134]. The inhibitor is comprised of multiple copies of the M13 peptide derived from myosin light chain kinase and contains a nuclear localization signal. It binds with high specificity to the calcium/calmodulin complex in the cell nucleus and blocks, in a competitive manner, the activation of nuclear calcium/calmodulin-dependent processes[135].

Nuclear calcium in acquired neuroprotection. Acquired neuroprotection is an increase in neurons' ability to survive harmful conditions. In hippocampal neurons, the build-up of a neuroprotective 'shield' is induced by synaptic activity and guards against both classical apoptotic cell death and excitotoxic damage. This process requires nuclear calcium signalling[3,25,132]. Nearly a dozen nuclear calcium-regulated genes have been identified that can protect hippocampal and cortical neurons from various forms of cell death, including stroke-induced cerebral brain damage[3,25,136]. This group of genes is collectively referred to as activity-regulated inhibitor of death (AID) genes[3] and primarily includes transcriptional regulators and secreted factors. A common end point in neuroprotection afforded by nuclear calcium signalling is the strengthening of mitochondria against toxic insults and cellular stress[137] (BOX 2).

Nuclear calcium in memory formation. The evidence for a role of nuclear calcium signalling in memory is twofold. First, transgenic mice expressing either a recombinant inhibitor of nuclear calcium signalling or a dominant interfering mutant of CaMKIV in forebrain neurons exhibit impaired memory consolidation[131,138]. Second, in *D. melanogaster*, blockade of nuclear calcium signalling during classical olfactory associative learning abolishes the transcription-dependent formation of long-term memories, whereas all other forms of memory that are independent of transcription are not affected[54].

The nuclear calcium-regulated gene pool responsible for memory consolidation in rodents includes vascular endothelial growth factor D (VEGFD)[134]. In the mouse brain, VEGFD expression is dependent on basal synaptic activity and nuclear calcium–CaMKIV signalling and is required for the maintenance of total dendrite length and arborization[134]. Downregulation of VEGFD using RNAi technology in the hippocampus *in vivo* impairs the ability of mice to form long-term memories in spatial learning tasks and contextual fear conditioning[134]. In addition to its role in regulating dendrite geometry, nuclear calcium is also important for spine morphogenesis. Interfering with nuclear calcium or CaMKIV signalling in hippocampal neurons causes a reduction in both dendritic spine density and spine size[134,139]. These structural alterations may be mediated by the secreted complement C1q subcomponent subunit C (C1QC), the expression of which increases under conditions in which

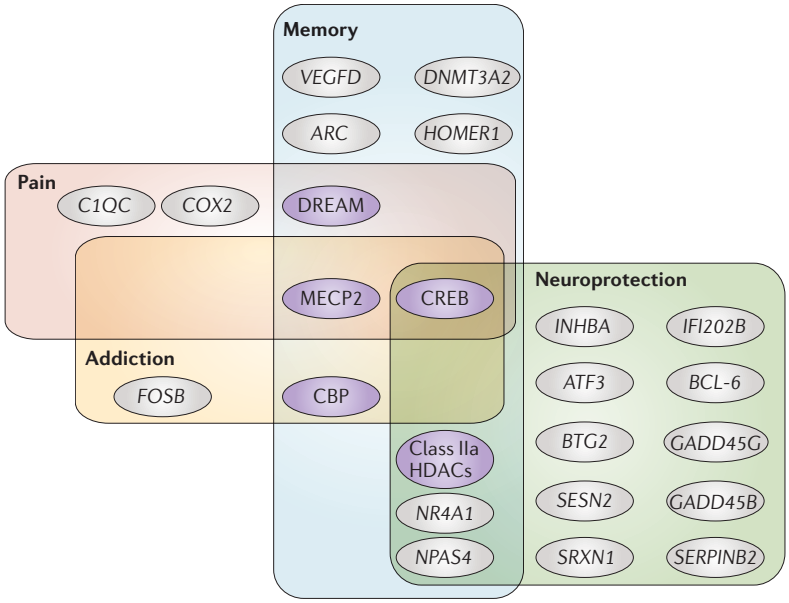


Figure 4 | **Nuclear calcium-regulated transcriptional regulators control diverse forms of neuroadaptation.** A schematic showing transcriptional regulators (purple) and target genes (grey) — many of which also have gene regulatory functions — that are controlled by nuclear calcium signalling and are involved in adaptations linked to memory, pain, addiction and neuroprotection according to current literature[3,25,53,101,102,113,115–117,123,134,136,140–145,151–154,156–163,231,232,237–239]. The transcriptional regulators are shared among different adaptations, whereas individual components of nuclear calcium-regulated target gene pool tend to have adaptation-specific functions. *ARC*, activity-regulated cytoskeleton-associated protein; *ATF3*, activating transcription factor 3; *BCL-6*, B-cell lymphoma-6; *BTG2*, BTG family member 2; *C1QC*, complement component C1q, C chain; CBP, CREB-binding protein; *COX2*, cyclooxygenase 2; CREB, cyclic AMP-responsive element-binding protein; DREAM, downstream regulatory element antagonist modulator; *GADD45B*, growth arrest and DNA damage-inducible protein 45-β; *GADD45G*, growth arrest and DNA damage-inducible protein 45-γ; HDACs, histone deactylases; *IFI202B*, interferon-activated gene 202 B; *INHBA*, inhibin-β A; MECP2, methyl-CpG-binding protein 2; *NPAS4*, neuronal PAS domain protein 4; *NR4A1*, nuclear receptor subfamily 4 group A member 1; *SERPINB2*, serpin peptidase inhibitor, clade B (ovalbumin), member 2; *SESN2*, sestrin 2; *SRXN1*, sulphiredoxin 1; *VEGFD*, vascular endothelial growth factor D.

Box 2 | **Mitochondria in neuroprotection: a 'neuronal Warburg effect'**

The activity of several activity-regulated inhibitor of death (AID) genes[3] can render mitochondria more resistant to an NMDA-induced collapse of the mitochondrial membrane potential, an early event in excitotoxic cell death[3,133]. One mechanism through which this can occur involves the synaptic activity-induced, neuronal PAS domain protein 4 (NPAS4)-mediated downregulation of expression of the mitochondrial uniporter MCU[231]. In addition, the nuclear calcium-regulated genes sestrin 2 (*SESN2*) and sulphiredoxin 1 (*SRXN1*)[3] that are part of the synaptic activity-regulated genomic programme controlling the thioredoxin–peroxiredoxin system can boost the neurons' antioxidant defences, which inactivate damage-causing reactive oxygen species (ROS)[232]. Another possible path to reduce oxidative stress could involve a reduction in mitochondrial oxidative respiration in favour of less efficient ATP production via glucose fermentation. Such a shift in energy metabolism has been observed in cancer cells and is known as the 'Warburg effect' (REF. 233). It seems counterintuitive that highly metabolically active neurons would switch to an inefficient anaerobic process for the conversion of glucose to cellular energy. Such a transition would, however, have the benefit of reducing ROS accumulation. Consistent with this hypothesis, transcriptome studies have revealed that synaptically activated hippocampal neurons increase the expression of both the neuronal glucose transporter *GLUT3* (also known as *SLC2A3*) to facilitate glucose uptake and the enzyme pyruvate dehydrogenase kinase 3 (REFS 3,25). Phosphorylation of pyruvate dehydrogenase by pyruvate dehydrogenase kinases inhibits the conversion of pyruvate, an end-product of glycolysis, to acetyl coenzyme A[234], causing a reduction in mitochondrial oxidative respiration and ROS production. In addition, given the stimulating effects of calcium in the mitochondrial matrix on oxidative phosphorylation[235], the nuclear calcium–NPAS4-dependent transcriptional repression of MCU[231] may further reduce potentially harmful mitochondrial energy conversion. Thus, analogous to what has been observed for cancer cells, a 'neuronal Warburg effect' could enhance the survival potential of neurons, albeit at the expense of a lower rate of ATP yield in glucose metabolism.

nuclear calcium signalling is blocked[53]. When added to the media of cultured hippocampal or spinal cord neurons, the C1q complex is sufficient to induce spine loss[53]. Additional nuclear calcium-regulated genes that function in memory consolidation include *DNMT3A2*, *ARC*, *HOMER1*, neuronal PAS domain protein 4 (*NPAS4*) and nuclear receptor subfamily 4 group A member 1 (*NR4A1*)[3,113,140–145]. Although the identification of many components of the memory-relevant gene pool has not revealed precisely how memories are laid down, it has underscored the importance of particular cell biological processes and structural features of neurons required for this process.

Nuclear calcium in chronic pain and addiction. Chronic pain and drug addiction are considered pathological manifestations of neuronal plasticity in central nociceptive pathways and brain reward systems, respectively[6,8,146–149]. Similar to adaptations to physiological stimulants, behavioural changes in animal models of chronic pain or addiction are accompanied by persistent structural and functional alterations of synapses in the relevant anatomical regions[146–150].

Another feature common to both adaptive and maladaptive responses is the requirement for gene transcription for their long-term implementation. In inflammatory pain pathogenesis, this process is controlled by nuclear calcium[53]. Nociceptive-like activity evokes nuclear calcium transients in mouse dorsal horn neurons in slices taken from the spinal cord[53] and triggers early nuclear calcium signalling events, including the phosphorylation of CREB and MECP2 on serine 133 and serine 421, respectively[151–153]. Inhibition of nuclear calcium signalling blocks the transition from acute to long-term nociceptive sensitization in a mouse model of chronic inflammatory pain[53]. Cyclooxygenase 2 (*COX2;* also known as *PTGS2*), a gene well known for its role in pain pathogenesis[154], is part of the nuclear calcium-regulated gene pool that controls the development of long-lasting nociceptive hypersensitivity[3,53]. A second important neuronal target of nuclear calcium signalling is *C1QC*, which can suppress the persistent pain state through a mechanism that involves the elimination of dendritic spines[53].

A role for nuclear calcium in addiction has not yet been established. However, NMDARs and calcium signalling are activated by drugs of abuse, and, similar to chronic pain, transcription-regulating events controlled by synaptic activity and nuclear calcium signalling have been causally implicated in addictive behaviour[155–161]. In addition, ΔFOSB, a component of the AP1 transcription factor complex and an important molecular switch for the initiation and maintenance of addiction[162,163], is a product of the *FOSB* gene, whose regulation by synaptic activity, at least in hippocampal neurons, is dependent on nuclear calcium signalling[3].

Thus, nuclear calcium may be an important signalling intermediate through which peripheral tissue injury or inflammation and possibly also drugs of abuse hijack mechanisms of neuronal plasticity normally used in learning and memory to remodel connectivity within pain or reward circuitries in a way that leads to persistent behavioural abnormalities.

A generic adaptive gene programme? The genomic responses initiated by synaptic activity-induced nuclear calcium transients may be largely invariable across brain regions if one assumes that different types of neurons have a comparable composition of the relevant signal-processing machinery in the nucleus and that their target gene chromatin structures are similarly permissive for signal-regulated transcription. The existence of such a generic adaptive gene programme is supported by several observations. First, the pool of nuclear calcium-regulated genes is large and contains subsets of genes involved in virtually all aspects of neuronal function, including survival, synaptic transmission, structural plasticity, excitability and energy metabolism[3]. In cultured hippocampal neurons, a set of 185 genes representing 43% of all synaptic activity-regulated genes, require nuclear calcium for their full activation or repression[3]. This number would be even larger if one considered a lower threshold in the gene chip-based transcription analysis used for the selection of activity-regulated genes. Thus, the nuclear calcium-induced genomic response offers a comprehensive portfolio of opportunities for adjustments of neuronal properties from which neurons can choose depending on circumstances and needs.

Second, transcriptome data revealed a large overlap in the pool of synaptic activity-regulated genes identified in different brain regions[3,25,164–173], even though the functional relevance of many genes may

Contextual fear conditioning
A hippocampus-dependent form of Pavlovian conditioning in which rodents learn to associate a specific spatial context with the administration of an aversive stimulus: for example, an electrical footshock. When re-exposed to the same environment, animals will demonstrate a fear response: for example, freezing.

be limited to a subset of neurons or circuits. This may also apply to genes targeted by nuclear calcium signalling, although there is currently only limited information available on the nuclear calcium-dependency of activity-regulated genes from regions other than the hippocampus and the spinal cord. Thus, the expression of many genes appears to be induced or repressed irrespectively of whether they contribute to the desired adaptive response. One example is *COX2*, which has an important function in the development of neuroinflammatory pain[154]. *COX2* is part of a pain-associated, nuclear calcium-regulated gene programme in spinal dorsal horn neurons[53]. It is also robustly induced in a nuclear calcium-dependent manner in the hippocampus[3], where it does not appear to be an essential component of adaptations typically seen in this brain area. Similarly, *FOSB* expression increases with synaptic activity in the nucleus accumbens, where its stable splice variant product ΔFOSB is critical for the development of addictive behaviour[162,163]. *FOSB* is also a target of nuclear calcium signalling in the hippocampus and the spinal cord[3,53], even though no essential function has been assigned to FOSB and/or ΔFOSB in those brain areas. To stereotypically activate a large genomic programme when only a subset of genes is required may appear a waste of resources and energy. However, such a cellular strategy offers the advantage of being simple and robust, and may ultimately be more economical than establishing and maintaining complicated, fail-safe regulatory mechanisms to 'individualize' transcriptional responses.

'Co-acquisition' of adaptations. The broad range of functions covered by the nuclear calcium-induced gene programme suggests that neurons may undergo more than one adaptation at a time. For example, hippocampal neurons that are synaptically activated during a learning task will not only induce genes required for memory formation but also those that mediate acquired neuroprotection. It seems inevitable that multiple adaptations are implemented simultaneously; in particular, the survival-promoting effects of synaptic activity may be always co-opted, which could explain the common wisdom that 'an active brain lives longer'. This property of neurons may have emerged as a result of evolution to preserve those networks that have gained additional features and are responsible for newly acquired cognitive abilities. When unwanted adaptations are considered, however, co-acquisition may seem to come at a price. For example, it is less obvious why a circuit within the reward system that is responsible for addictive behaviour after having gone through a drug-induced alteration should have a survival advantage.

Nuclear calcium signalling dysfunction

Nuclear calcium signalling is strongly antagonized by transcriptional shut-off pathways that are activated by extrasynaptic NMDARs (e-NMDARs)[4,24]. One of the main targets of these pathways is CREB: in the first phase after e-NMDAR stimulation CREB is functionally inactivated through the rapid dephosphorylation of its activator site serine 133 (REF. 24), after which it is degraded[174]. Signalling events initiated by e-NMDARs also repress transcription on a global scale by causing the nuclear accumulation of class IIa HDACs[100]. Moreover, e-NMDAR activation is directly linked to cell death by processes that culminate in the nuclear translocation of the pro-apoptotic transcription factor FOXO3A[130] and, most importantly, by the rapid breakdown of the mitochondrial membrane potential, an early step in excitotoxic cell death[24]. As calcium entry sites into neurons, e-NMDARs have the potential to generate calcium signals that invade the nucleus. Indeed, such rises in the nuclear calcium concentration are readily detectable in cultured hippocampal neurons after stimulation of e-NMDARs by bath-applied glutamate or NMDA[88,175]. Although the cell nucleus is unlikely to be able to distinguish calcium transients induced by synaptic activity from those induced by e-NMDARs, the stimulation of e-NMDARs fails to generate the typical nuclear calcium-regulated genomic responses because transcription-promoting activities are overridden by the simultaneous induction of transcription shut-off pathways and mitochondrial dysfunction[3,24,25].

E-NMDARs are activated primarily under pathological conditions. These include the classical *in vitro* models for excitotoxic cell death, in which e-NMDARs are stimulated either by treatment of cultured neurons with NMDA or glutamate, or by oxygen–glucose deprivation[4], which causes uncontrolled glutamate release into the extracellular space owing to the reverse operation of glutamate uptake systems[176]. *In vivo*, e-NMDAR activation can result from spill-over of synaptically released glutamate after seizure-like activity or may occur as a consequence of hypoxic and/or ischaemic conditions associated with a stroke. A physiological function of e-NMDARs is not obvious, although given that glial cells can both take up and release glutamate, they may play a part in glia-to-neuron communication[177–182].

'Nuclear calciopathy'. Because of its central role in brain function, nuclear calcium signalling or its deregulation is inevitably involved in both neurodegenerative and neuropsychiatric disorders. The disease phenotype reflects where in the brain the generation of normal nuclear calcium transients is distorted or the antagonism of this signal by e-NMDARs is increased. For example, in Huntington's disease mouse models, the degeneration of striatal neurons involves an enhancement of e-NMDAR signalling caused by a redistribution of NMDARs from synaptic to extrasynaptic sites[183–186]. The pathogenesis of Alzheimer's disease has also been linked to e-NMDAR signalling[187,188], which can increase the production of amyloidogenic amyloid precursor protein[189] and cause DNA damage in the form of DNA double-strand breaks[190] that could compromise gene regulatory events required for cognitive functions and/or neuronal survival. Moreover, synapse loss, a characteristic early event in Alzheimer's disease[191–193], and impaired synaptic plasticity appear to result from the stimulation of e-NMDARs by

Oxygen–glucose deprivation
An *in vitro* model of cerebral stroke in which cultured neurons or brain slices are exposed to media containing insufficient amounts of glucose and oxygen, which leads to cell death.

β-amyloid-induced glutamate release from glial cells and/or neurons[187,188,194,195]. Having fewer synaptic contacts, Alzheimer's disease-affected neurons may receive fewer excitatory inputs and, consequently, may not sufficiently stimulate nuclear calcium signalling. Decreased nuclear calcium signalling together with an increase in e-NMDAR activity would not only reduce CREB–CBP-mediated gene expression[24,86,87], but would also actively repress transcription by causing a nuclear accumulation of class IIa HDACs[100,102]. This chain of events could compromise the expression of target gene programmes required for maintaining neuronal health and structural and functional integrity, thereby perpetuating the disease process, causing neurons to degenerate and cognitive abilities to decline (FIG. 5).

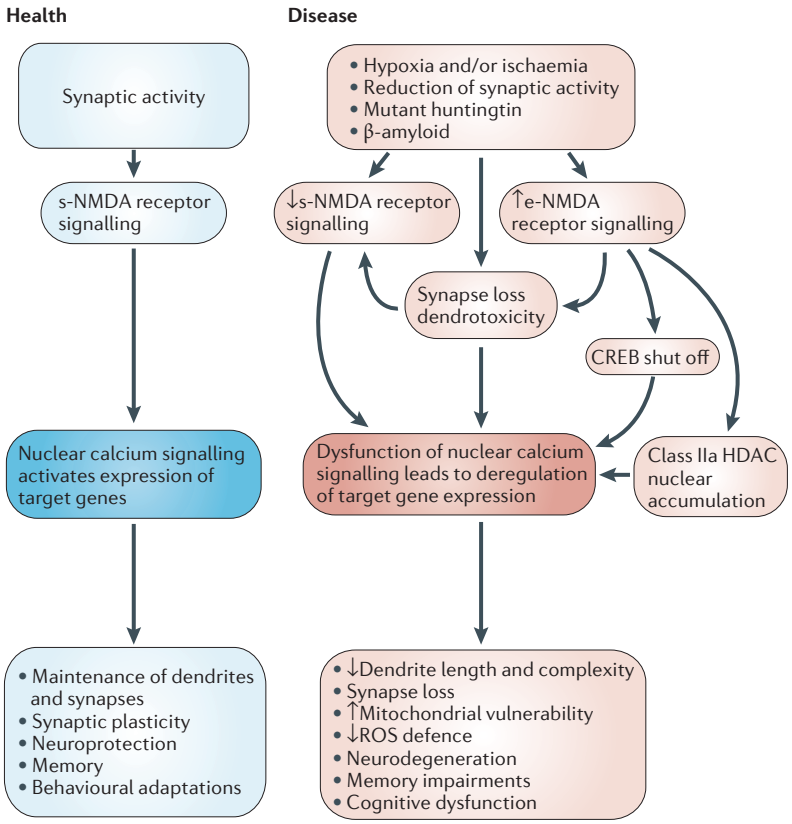


Figure 5 | **'Nuclear calciopathy' as a common factor in the aetiology of neurodegenerative and cognitive disorders.** Nuclear calcium signalling induced by synaptic activity stimulating synaptic NMDA (s-NMDA) receptors and regulating specific target gene expression is important for neuronal health and essential for the maintenance and functional integrity of synapses and dendrites (left panel). Toxic molecules, genetic defects or harmful conditions (such as β-amyloid, mutant huntingtin, deprivation of synaptic activity or hypoxia and/or ischaemia) and possibly also ageing can lead to perturbations in the balance between s-NMDA receptor and extrasynaptic NMDA (e-NMDA) receptor signalling (right panel). An increase in the number or activity of e-NMDA receptors and/or a decrease in s-NMDA receptor function owing to synapse loss or dendrotoxicity can lead to dysfunctioning of nuclear calcium signalling, which includes the shut-off of cyclic AMP-responsive element-binding protein (CREB) function and nuclear accumulation of class IIa histone deactylases (HDACs). The resulting deficits in the expression of nuclear calcium target genes may increase mitochondrial vulnerability, decrease the neurons' antioxidant defence systems and perpetuate the disintegration of dendrites and the loss of synapses, leading to neurodegeneration and cognitive decline. ROS, reactive oxygen species.

In addition to synapse loss, several other cellular defects could conceivably impair nuclear calcium signalling. These include changes in excitability and neurotransmitter release, the presence of defective sites for calcium entry or release from intracellular stores and altered calcium buffering capacities. Each fault could impair the regulation of CREB–CBP, MECP2 and class IIa HDACs, leading to the deregulation of AID genes[3] and other nuclear calcium targets, such as *VEGFD* (also known as *FIGF*), *DNMT3A2*, *C1QC*, *SESN2* (which encodes sestrin 2) and *SRXN1* (which encodes sulphiredoxin 1). This would not only weaken the neurons' neuroprotective shield by increasing mitochondrial vulnerability and reducing resistance to oxidative stress, but it could also compromise memory formation and other cognitive abilities by preventing affected neurons from developing and/or maintaining the level of dendritic complexity and synaptic density required for proper network function (FIG. 5). This may be relevant for autism spectrum disorders in which several components of the 'synaptic activity–nuclear calcium signalling network' are altered[196].

Thus, the deregulation of nuclear calcium signalling, referred to as a 'nuclear calciopathy' (REF. 136), represents a hub for various disease aetiologies (FIG. 5). This conceptually rather simple and unifying framework for understanding brain dysfunction suggests two general strategies for the development of treatments. Neuronal protection and improved cognitive function may be afforded, on the one hand, by promoting synaptic activity via a stimulating environment or, if necessary, using pharmacological means. This applies to all stages in a person's lifetime, particularly old age, but also early childhood, when neuronal networks are shaped by synaptic activity and nuclear calcium signalling impinging, for example, on *NPAS4* and MECP2, two key regulators of neural circuit development[115,144,197,198]. On the other hand, it is equally important to constrain e-NMDAR signalling, for example in Alzheimer's disease and stroke, and to rebalance the system when, as in Huntington's disease, NMDARs redistribute from synaptic to extrasynaptic sites[185,186].

A promising approach involves the use of the NMDAR blocker memantine[199]. At low doses, memantine preferentially blocks e-NMDARs and has been proven to be beneficial in animal models of Huntington's disease and in Alzheimer's disease[185,186,200–203]. Selective blockade of e-NMDARs may also be possible by attaching an NMDAR blocker to a nanoparticle[204]. If the size of the nanoparticle exceeds that of the synaptic cleft, the blocker can only target e-NMDARs while sparing synaptically localized NMDARs. The design of compounds that specifically boost nuclear calcium signalling is more difficult but may be realized by developing strategies for altering the clearance of calcium from the nucleus or by changing nuclear calcium buffering. Modulators of nuclear calcium signalling may also find their way into treatments for maladaptive processes, particularly chronic pain and addiction, where, however, the aim would be to disrupt rather than enhance nuclear calcium-regulated processes in selected brain and spinal cord regions.

Conclusion and future challenges

Because adaptations are essential for an organism to adjust to and cope with a constantly changing environment, simple, but robust and effective, cellular mechanisms to instruct functional changes must have evolved to minimize the chances of failure. Nature's choice of calcium as a second messenger, together with the development of a cellular mechanism that tightly couples neuronal activity with changes in intracellular calcium levels, provides neurons with a straightforward and highly effective means for relaying extracellular stimuli to an intracellular signal transduction apparatus. Increases in cytosolic calcium are used locally near their site of generation to initiate functional changes in pre-existing molecules that mediate short-term plasticity, but they also trigger a process that conveys information to distant locations, particularly the cell nucleus. The nuclear invasion of calcium transients represents a signalling end point of synaptic activity that — according to the 'nuclear calcium hypothesis' (REF. 9) — is a common requirement in diverse forms of persistent neuroadaptations. The concept of nuclear calcium signalling offers a beautifully simple process to mirror stimulus strength in the nucleus and provides the basis for a reliable genomic switch for the progression of adaptations from labile to long-lasting forms.

In vivo *imaging of nuclear calcium*. Transgenic flies expressing a nuclear-targeted calcium sensor provided a first glimpse at nuclear calcium signalling *in vivo*[54]. Advances in microscopy techniques, such as two-photon imaging and fibre optics technology[205–207], in conjunction with viral vector-mediated delivery of recombinant calcium indicators to defined brain areas, have opened the door to monitoring calcium transients in the nucleus in the intact brains of mice or rats. Imaging studies using awake rodents may not only reveal learning-associated nuclear calcium transients but, if combined with electrical recordings, may allow us to determine precisely which activity patterns switch on nuclear calcium signalling and thus specify the transition from short-term to long-term adaptations. In addition, *in vivo* imaging is best suited to assess possible alterations in the dynamics of nuclear calcium transients due to ageing or neurodegenerative conditions.

Towards nuclear calcium signalling-based therapies. To facilitate the translation of the knowledge of nuclear calcium signalling into medical benefits, it is important to more precisely define functional modules within the nuclear calcium-regulated gene pool that mediate the implementation of specific adaptations in particular brain regions. This is likely to reveal new unexpected drug targets, as has already been achieved with the identification of VEGFD and DNMT3A2 as regulators of memory consolidation in the hippocampus[113,134] and C1QC as a mediator of chronic inflammatory pain in the spinal cord[53]. Pharmacological modulation of the nuclear calcium signal itself holds the potential for a wide range of clinical applications. Enhancers of nuclear calcium signalling are expected to promote neuronal survival and memory consolidation and may thus be used for the treatment of neurodegenerative and ageing-related neurological dysfunction. Such enhancers may also be beneficial in anxiety disorder therapies, in which fear memories are first retrieved and subsequently extinguished. Indeed, like memory consolidation, the extinction of memories requires experience-induced changes in gene expression. These may be mediated by the nuclear calcium signalling target CREB[208], raising the possibility that increasing nuclear calcium levels during fear extinction could help to erase distressing memories such as those underlying post-traumatic stress disorders. Pharmacological blockade of nuclear calcium signalling could be used to prevent the development of chronic pain.

Modulators of nuclear calcium signalling would ideally target the nuclear pore complex, the principal entry and exit route for calcium into neuronal nuclei. However, it may not be possible to selectively change the calcium-gating properties of nuclear pore complexes while preserving other functions. Alternative drug targets include perinuclear IP3 receptors, for which a role in nuclear calcium signalling in the heart has been reported[209], or proteins such as the NCX that may localize to the inner nuclear envelope and take part in the clearance of nuclear calcium transients[210]. Changes in the dynamics of nuclear calcium transients may also be achieved by developing means of increasing or decreasing the calcium buffering capacity in the nucleus. Finally, chemical modifiers of calmodulin designed to act specifically in the cell nucleus would be valuable tools to attenuate or boost signalling downstream from nuclear calcium.

Variations among individuals. It is tempting to speculate on the contribution of nuclear calcium signalling to inter-individual differences in human brain function. The ability of neurons to generate nuclear calcium signals is expected to vary among healthy individuals. These differences may translate into differences in cognitive abilities and the neurons' longevity, a hypothesis that can now be tested using induced pluripotent stem cell-derived human neurons that are thought to have functional and structural properties similar to those of the donors' neurons[211]. Although the establishment of such a link would bring us one step closer to understanding the brain, it would, at the same time, have far-reaching implications for the possible manipulation of mental processes and intellectual skills and, as such, raises serious ethical concerns.

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Acknowledgements
This Review is dedicated to my thesis advisor, W. Hasselbach and to E. Carafoli, both pioneers in the field of calcium signalling. I am very grateful to the many excellent young scientists who I have had the pleasure to work with over the years; without them it would not have been possible to establish the function of nuclear calcium in biological adaptations. I would like to thank the members of my laboratory for comments on the manuscript and in particular A. Hagenston-Hertle, B. Buchthal and C. P. Bengtson for their help with the design of the figures. The work in the author's laboratory is supported by the Deutsche Forschungsgemeinschaft, the Alexander von Humboldt Foundation and the European Research Council Advanced Grant.

Competing interests statement
The author declares no competing financial interests.