

Structural dynamics of the cell nucleus

Basis for morphology modulation of nuclear calcium signaling and gene transcription

Gillian Queisser,^{1,*} Simon Wiegert² and Hilmar Bading³

¹Goethe Center for Scientific Computing; Faculty of Computer Science and Mathematics; University Frankfurt am Main; Kettenhofweg, Frankfurt am Main;

²Friedrich Miescher Institute for Biomedical Research; Group Thomas Oertner; Basel; ³Interdisciplinary Center for Neurosciences; Department of Neurobiology; University Heidelberg; Heidelberg, Germany

Neuronal morphology plays an essential role in signal processing in the brain. Individual neurons can undergo use-dependent changes in their shape and connectivity, which affects how intracellular processes are regulated and how signals are transferred from one cell to another in a neuronal network. Calcium is one of the most important intracellular second messengers regulating cellular morphologies and functions. In neurons, intracellular calcium levels are controlled by ion channels in the plasma membrane such as NMDA receptors (NMDARs), voltage-gated calcium channels (VGCCs) and certain α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receptors) as well as by calcium exchange pathways between the cytosol and internal calcium stores including the endoplasmic reticulum and mitochondria. Synaptic activity and the subsequent opening of ligand and/or voltage-gated calcium channels can initiate cytosolic calcium transients which propagate towards the cell soma and enter the nucleus via its nuclear pore complexes (NPCs) embedded in the nuclear envelope. We recently described the discovery that in hippocampal neurons the morphology of the nucleus affects the calcium dynamics within the nucleus. Here we propose that nuclear infoldings determine whether a nucleus functions as an integrator or detector of oscillating calcium signals. We outline possible ties between nuclear morphology and transcriptional activity and discuss the importance of extending the approach to whole cell calcium signal

modeling in order to understand synapse-to-nucleus communication in healthy and dysfunctional neurons.

The MM of Nuclear Calcium Signaling

Similarly to other neuronal features such as the dendritic spine, the nucleus can undergo robust, synaptic activity-dependent changes in its shape and forms distinct nuclear infoldings that compartmentalize the inner nuclear domain. In order to investigate the influence of the nuclear morphology on calcium signals, we developed a computational model of calcium signaling in the nucleus, which takes into account the real nuclear morphology obtained from confocal images of cultured hippocampal neurons and reconstructed with a previously developed neuron reconstruction algorithm.^{6,22} By reconstructing a large set of nuclei (examples in Fig. 1), we were able to perform in silico experiments of calcium signals in nuclei that provide a direct link between calcium and morphology (example of simulation in Fig. 2). In unison to model findings, experiments using a combination of electrophysiology and imaging, confirmed that by altering its morphology, the nucleus can modulate calcium signals, a phenomenon we refer to as morphology modulation (MM) of nuclear calcium signals. In particular, nuclear infoldings are able to form nuclear compartments, which elicit distinct calcium patterns, differences in calcium amplitudes and frequency as well as local differences in histone H3 phosphorylation.

Key words: morphology modulation, nuclear envelope, infoldings, calcium signaling, histone H3 phosphorylation, ageing, neurodegenerative diseases, Alzheimer's Disease

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*Correspondence to: Gillian Queisser;
Email: gillian.queisser@gcsc.uni-frankfurt.de

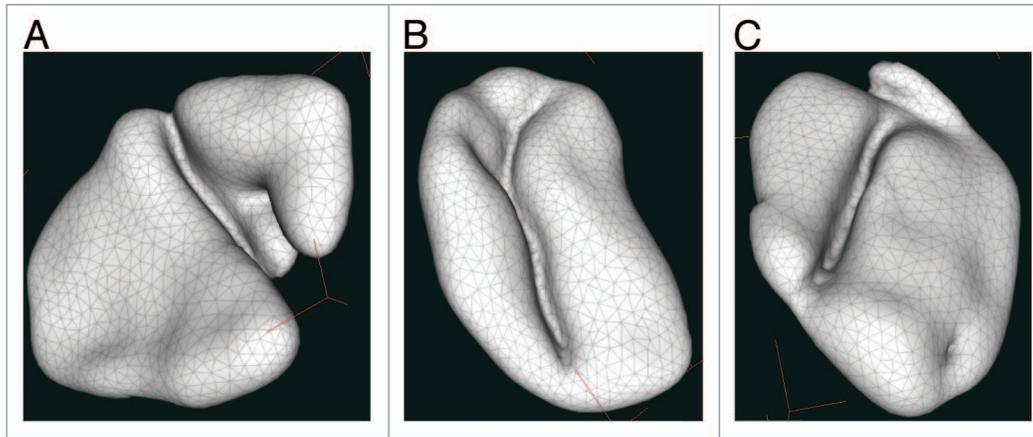


Figure 1. Reconstruction of three hippocampal nuclei (A–C). Using the automatic reconstruction tool NeuRA applied to confocal image stacks of the nucleus surface- and volume-grid of nuclei can be generated. This forms the morphologic basis for detailed three-dimensional modeling.

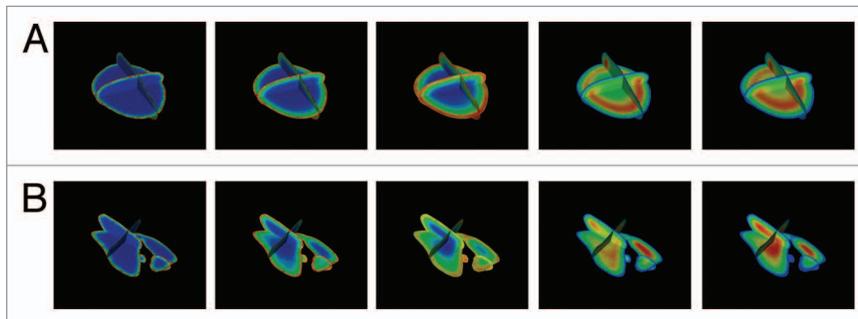


Figure 2. Simulations of a cytosolic calcium transient entering the nucleus and propagating in (A) a spherical nucleus and (B) an infolded nucleus. Depicted here are the calcium distributions inside the nuclei.

In analogy to amplitude modulation (AM) and frequency modulation (FM)¹⁸ of calcium signals, MM can act on the amplitude as well as the frequency of a nuclear calcium signal. One observation described in Wittmann et al. (2009) concerns the size of the nuclear envelope, which increases with the degree of nuclear infoldings. The increase in nuclear surface is proportional to an increase in NPCs immunoreactivity; an increased number of NPCs can accelerate calcium entry into infolded nuclei. Thus, nuclear morphology can regulate rise and decay times of nuclear calcium transients, which affects the kinetics and amplitudes of nuclear calcium signals.

The nuclear morphology also influences to what extent oscillating calcium signals can be relayed to the nucleus. Our computational model describes calcium signal propagation in the nucleus as the physical process of diffusion. This means

that the temporal dynamics of calcium signals are dependent on the *size of the volume* in which they are evolving in and the *diffusion distances* the signals have to travel. With an increase of nuclear infoldings diffusion distances decrease. Signals reach centered sites faster and calcium is cleared from the nucleus more quickly. In other words “nuclear inertia” is reduced by membrane infoldings. To study the consequence of MM on signal frequency we here introduce a measure termed *nuclear activity* A_{nuc} . There are indications that calcium concentration thresholds play an important role in nuclear calcium signaling. We therefore introduced the parameter A_{nuc} in our studies as the volume within the nucleus that lies above a given threshold (V_{th}) for a downstream signaling event at a given time relative to the total nuclear volume (V_{total}), $A_{nuc} = V_{th}/V_{total}$. A_{nuc} calculates the state of activity a given nucleus is in and more importantly the influence of

the nuclear morphology on the underlying nuclear calcium distribution.

As has been reported, the nucleus reacts differently to a constant calcium signal, compared to an oscillating signal.²⁴ We therefore studied nuclei in different infolding states and compared their activity, and their reaction to different stimulus frequencies (Fig. 3 and 4). Figure 3 shows, that spherical and infolded nuclei are distinguishable in their activity. This effect is due to changes in diffusion distances; shorter diffusion distances allowing calcium waves from opposite sides to overlap quicker, hence raising the local calcium level above threshold (A_{nuc} increases). This effect becomes more and more prominent when the threshold is raised. Another interpretation of the threshold is the strength of the cytosolic calcium signal, meaning instead of changing the threshold one could change the amplitude of the cytosolic calcium signal. Reinterpreting the effect in Figure 3 thus shows that infolded nuclei are more proficient at recognizing small changes in the cytosolic calcium dynamics and that small compartments are more adept at this than large compartments. Nuclear membrane infolding therefore increases the sensitivity of the nucleus to fluctuations in the cytosolic calcium dynamics.

Signal Integrators vs. Signal Detectors

What happens under different stimulation frequencies? Infolded nuclei are

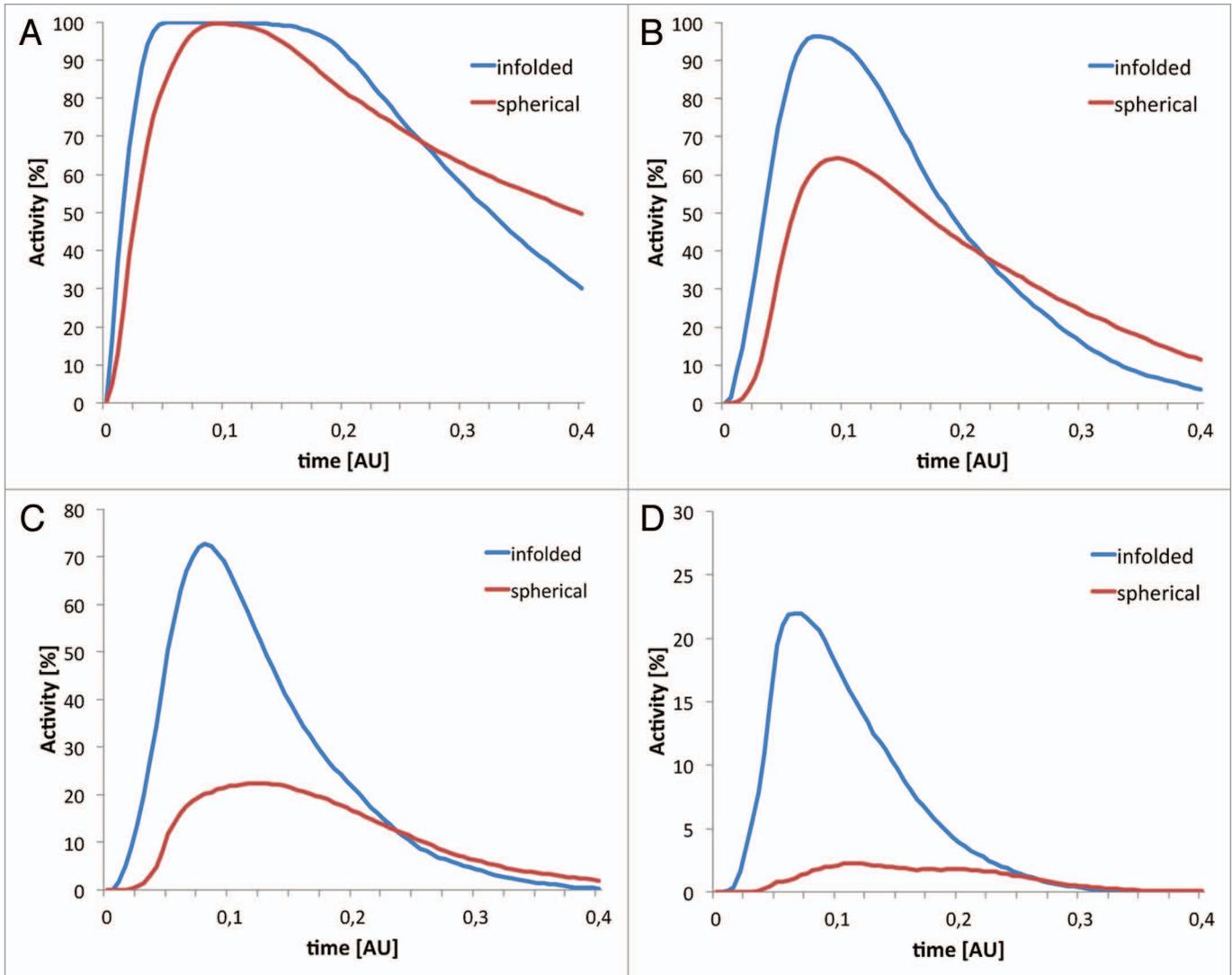


Figure 3. Comparing the nuclear activity A_{nuc} of a spherical and infolded nucleus at increasing threshold values $T_A - T_D$ (A–D). The thresholds are defined as a percentage of the cytosolic calcium amplitude A_{cyt} ($T_A = 20\% A_{cyt}$, $T_B = 40\% A_{cyt}$, $T_C = 60\% A_{cyt}$, $T_D = 80\% A_{cyt}$). Nuclear activity A_{nuc} is defined as the percentage of nuclear volume that lies above a given threshold T . For higher thresholds infolded nuclei show higher activity than spherical ones. The reverse argument (high threshold equivalent to low amplitude signals) indicates that small cytosolic calcium signals can activate infolded nuclei better than spherical ones.

better at resolving high frequency stimuli.³ Here we show, that the measure A_{nuc} is also strongly affected by frequency (Fig. 4). While the two types of nuclei, infolded and spherical, react similarly at low frequencies, spherical nuclei go into a plateau-like state when subjected to a high-frequency stimulus. This does not happen to a small compartment of an infolded nucleus. At higher frequencies a spherical nucleus acts as a signal integrator, while infolded nuclei resolve high frequencies. This indicates that at high-frequency calcium signals, these nuclear types fall into separate functional groups.

The MM of signal frequency is schematically shown in Figure 5.

What is the Biological Relevance of the Nuclear Geometry and its Effect on Nuclear Calcium Signaling?

Alongside enhanced sensitivity of the nucleus to cytoplasmic calcium transients, changes in the nuclear morphology may be cause and consequence of elevated transcriptional activity. It has been well documented in the past that enhanced neuronal activity and subsequent nuclear

calcium signaling induce transcription of a large number of genes.^{1,2} Consequently, the activity of calcium-sensitive transcription factors, which reside in the nucleus such as CREB, will be directly affected by the frequency and amplitude of nuclear calcium signals.

Aside from the generation of signaling compartments, nuclear infoldings increase the surface-to-volume ratio of the nucleus. As mentioned above, in the study by Wittmann et al. (2009) we demonstrated that enlargement of membrane surface area is accompanied by increased immunoreactivity for NPCs.³ In their

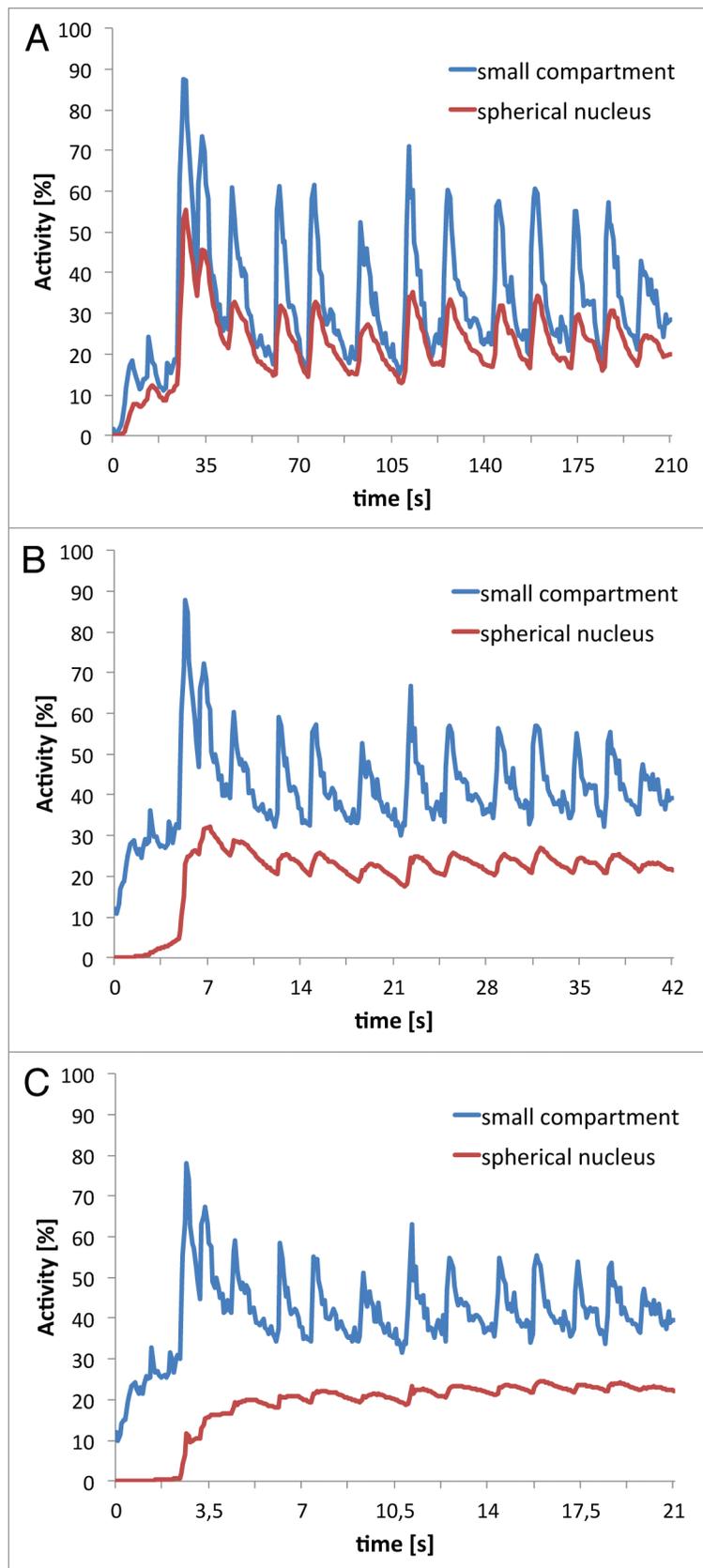


Figure 4. Comparison of the small compartment of an infolded nucleus with a spherical nucleus under increasing stimulation frequencies (A–C). With increasing cytosolic calcium frequency spherical nuclei go into a plateau-state, thus integrating the frequency of the calcium signal, whereas infolded nuclear compartments are able to resolve these frequencies. Based on this, neuronal nuclei may be categorized as signal integrators (spherical nuclei) and signal detectors (infolded nuclei).

function as ‘flood gates’ for nuclear calcium, an increased number of nuclear pore complexes will make the nuclear envelope more permeable.^{4,5} Notably, the generation of signaling microdomains in the nucleus in conjunction with increased surface expression of nuclear pores will not only facilitate nuclear signaling of calcium—as outlined above—but it will also augment nuclear signaling of second messengers in general^{3,6} (Fig. 6). How the insertion of nuclear pores is connected to growth of the nuclear envelope is less clear. It remains to be shown whether new pores are assembled in membrane areas of low pore density or whether newly formed pores may recruit lipids to enhance the membrane area.

A second link to nuclear infoldings exists at the level of ERK1/2-signaling and chromatin remodeling. Recent work has revealed a role for nuclear ERK1/2 signaling in chromatin remodeling, a mechanism for regulating gene transcription with widespread importance for LTP, memory formation, epilepsy, drug addiction, depression and neurodegenerative diseases. For example, in striatal or hippocampal neurons, phosphorylation of histone H3 at serine 10 requires nuclear signaling of ERK1/2.⁷⁻¹⁰ This histone modification is sufficient to allow specialized enzymatic protein complexes to bind to and uncondense chromatin which in turn induces transcription from promoters of immediate early genes such as c-Jun or c-Fos.¹¹ Chromatin remodeling identified by histone H3 phosphorylation was more pronounced in nuclei displaying more complex infoldings.³ Moreover, structural changes of the nuclear envelope were dependent on ERK1/2 signaling. Increased NMDAR-dependent synaptic activity induces large sets of genes and

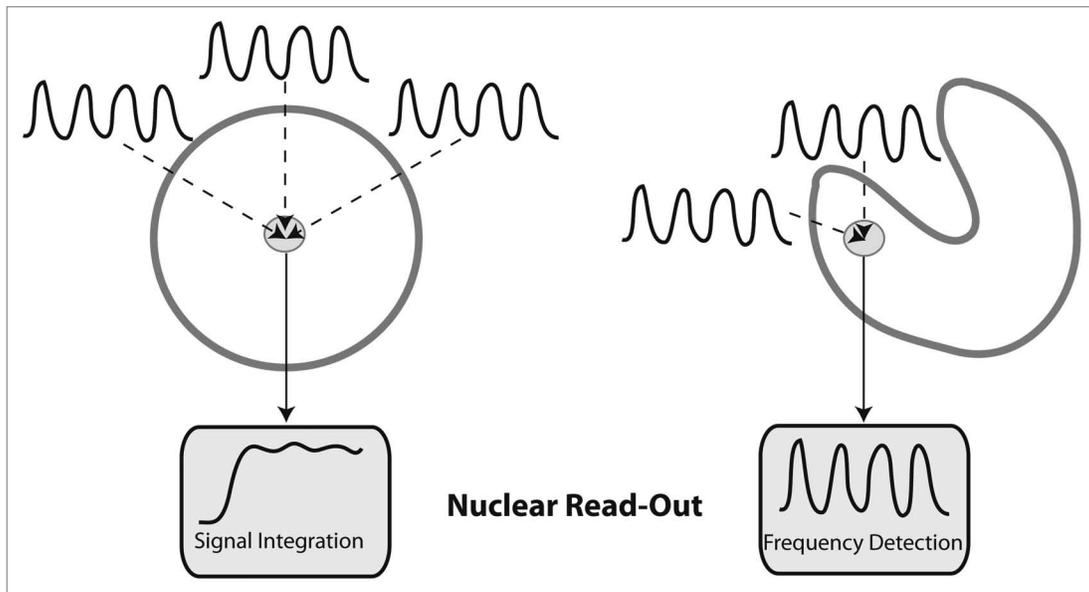


Figure 5. Illustration of the difference between spherical and infolded nuclei when resolving signal frequencies. Shortened diffusion distances in infolded nuclei allow calcium signals to reach their destination more quickly; at the same time, calcium can diffuse back into the cytosol more rapidly which enables the infolded nucleus to detect the input frequency. In spherical nuclei, the input frequency of the cytosolic transients is lost and a plateau calcium signal is generated.

many of these genes require nuclear calcium and ERK1/2-activity for their transcription.^{1,2} Such a dramatic shift in the gene transcription profile may require chromatin remodeling at a large scale in the nucleus.

Two observations support the view that changes in nuclear geometry are important for gene transcription regulation. First, sites of active gene transcription appear to be localized to certain subnuclear territories¹² and hotspots of transcription are found in conjunction with NPCs.¹³⁻¹⁵ Notably, their involvement in gene transcription regulation assigns a further, important role to NPCs aside from controlling nucleo-cytoplasmic shuttling. An increased number of NPCs in infolded nuclei could provide more anchoring sites for transcriptionally active promoter regions to bind to NPCs providing the cell with a means to adequately respond to the increased demand for gene transcription (Fig. 6). Second, A-type lamins, which are major components of the nuclear lamina—a protein network controlling nuclear shape, have also been implicated in the regulation of gene transcription and chromatin organization.¹⁶ Pre-existing c-Fos can be sequestered at the nuclear envelope by lamin A/C¹⁷ and

their direct phosphorylation by ERK1/2 liberates c-Fos from the nuclear envelope. Released c-Fos, in turn, can form dimeric complexes with c-Jun and thereby activate AP-1-dependent gene transcription in the nucleoplasm.^{16,17} Thus, deep infoldings may reflect structural remodeling of the cell nucleus due to increased transcriptional activity. At the same time this form of plasticity provides the basis for enhanced and refined nuclear calcium signaling (Fig. 6).

Outlook

To determine the precise relationship between nuclear infoldings and gene transcription regulation requires further research. In the future it will be important to unravel the pathway through which ERK1/2 acts on nuclear morphology and how insertion of NPCs and membrane expansion are linked. Moreover, the direct functional consequences of enhanced nuclear calcium signaling on neuronal function need to be resolved in more detail. MM shown to affect nuclear calcium signals should be extended to the level of cellular calcium dynamics. Thus an extension of the modeling and experimental focus of MM to the cellular scale is

needed. Our mathematical model, which is based on partial differential equations derived from continuum mechanics theory, took into account only the nucleus. As a boundary condition for the model equations we used experimentally recorded cytosolic calcium transients (CCTs). This coupled the cytosol with the nucleus in our model. Studying the nucleus-cytosol interface is a new and exciting frontier. The previously measured CCTs for the computational model raise several questions: How are CCTs generated and regulated? And what could affect the transfer of information from the synapse to the nucleus? Novel insights in the field of cellular calcium signaling are found in ageing- and Alzheimer Disease (AD)-related topics. In these conditions, several aspects of calcium homeostasis may be altered; this includes the density and distribution of IP₃-receptors on the ER membrane,¹⁹ the function of ryanodine receptors²³ and SERCA pumps²⁰ or the loss of synaptic connections,²¹ all of which could cause alterations in the generation of CCTs and their propagation towards and into the nucleus. In order to understand when and how synaptic activity-induced calcium transients propagate to the nucleus and activate vital genomic responses, it is

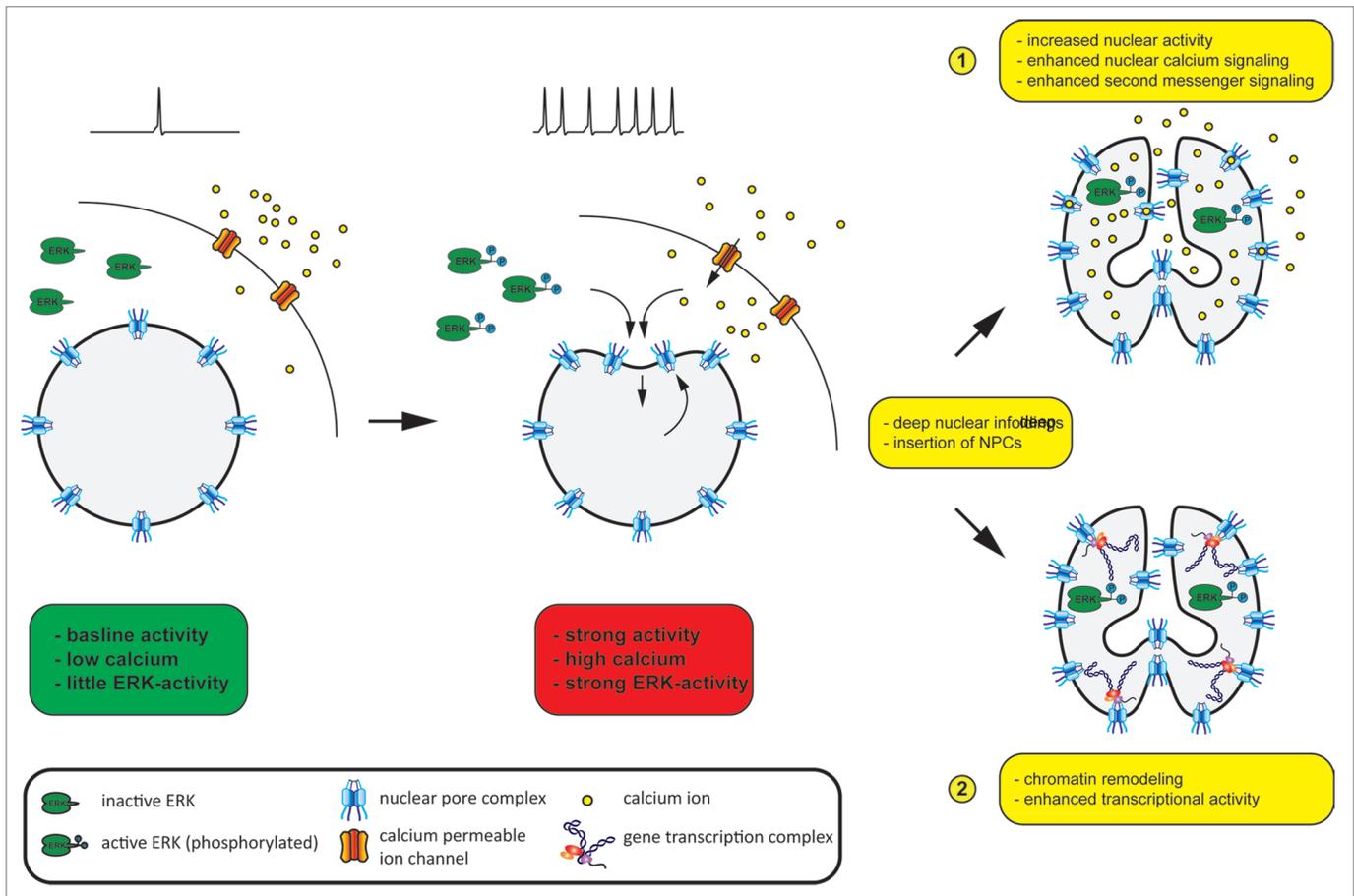


Figure 6. Activity-dependent plasticity of nuclear geometry in hippocampal neurons—biological implications: During baseline synaptic activity neuronal nuclei display no or only minor infoldings. Calcium signaling is at rest and most ERK1/2 is in its inactive (i.e., unphosphorylated). Upon initiation of strong electrical activity and subsequent calcium influx deep nuclear infoldings appear in an ERK1/2-dependent manner. This process is accompanied by the insertion of nuclear pore complexes (NPCs) into the nuclear membrane. The direct consequences of nuclear geometry changes are: larger surface area with more nuclear pore complexes resulting in (1) deeper penetration of calcium and second messengers and (2) enhanced chromatin remodeling along with increased NPC-associated transcriptional events.

important to take the current model of nuclear signaling to the next level, i.e., the entire cell. An appropriate mathematical model for studying signal-regulated cellular calcium dynamics and their modulation by morphological features should be based on real three-dimensional geometries of entire neurons and should take into account the detailed biophysics of calcium signal generation and propagation.

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