

MINIREVIEW

Action Potentials: To the Nucleus and Beyond

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The neuronal nucleus is now widely accepted as playing a vital role in maintaining long-term changes in synaptic effectiveness. To act, however, the nucleus must be appropriately relayed with information regarding the latest round of synaptic plasticity. Several constraints of doing so in a neuron pertain to the often significant spatial distance of synapses from the nucleus and the number of synapses required for such a signal to reach functional levels in the nucleus. Largely based on the sensitivity of transcriptional responses to NMDA receptor antagonists, it has been postulated that the signals are physically relayed by biochemical messengers from the synapse to the nucleus. Alternatively, a second, less often considered but equally viable method of signal transduction may be initiated by action potentials generated proximal to the nucleus, wherefrom the signal can be relayed directly by calcium or indirectly by biochemical second messengers. We consider action potential-dependent signaling to the nucleus to have its own computational advantages over the synapse-to-nucleus signal for some functions. This minireview summarizes the logic and experimental support for these two modes of signaling and attempts to validate the action potential model as playing an important role in transcriptional regulation relating specifically to long-term synaptic plasticity. *Exp Biol Med* 233:385–393, 2008

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Introduction

“The road stretches ahead. How will this little man get to the end of it?” [*Curious George* by H. A. Rey]

In his lift-the-flap books for children, Hans Augusto Rey wants his readers to figure out the best route to ‘the end of the road’ based on a set of clues. This idea of taking the best route is not limited to books for children, but applies equally to the biology of signal transduction where a signal may be required to travel fast, while taking the shortest route possible in order to mediate timely cellular responses. In laboratories, answers about the best path are not found under flaps or at backs of books, but are often hidden and sometimes counterintuitive. For instance, it is now well recognized that synaptic activity-induced long-term alterations in synaptic strength (synaptic plasticity) is heavily contingent on signaling from synapses to the nucleus and subsequent transcriptional events within the nucleus (1–3). The mechanism of traversing this long road from distally located synapses to a nucleus, however, remains unknown. How does a relatively weak signal generated in a faraway synapse find its way to the nucleus to inform it that plasticity had occurred? Two possibilities have been intellectually entertained by those in the field. The signal can either ramify biochemically through a repertoire of cytoplasmic signaling messengers after being initiated by synaptic glutamate receptors (Synapse-to-nucleus *biochemical* model) (4) or alternatively, it can be relayed by membrane depolarization, action potentials, and calcium (*Action potential* model) (5, 6). Both these models of neuronal signal transduction have their share of theoretical advantages, disadvantages, and experimental support. For various reasons, they are not expected to be mutually exclusive.

Rapid Nuclear Response—Why and How?

Repetitive signaling at glutamatergic synapses can produce increases and decreases in strength of synapses

under different conditions. These changes, known as Long-Term Potentiation (LTP) and Long-Term Depression (LTD), are typically induced with high-frequency (~100 Hz) or low frequency (1–3 Hz) afferent stimulation, respectively (7, 8). While both types of plasticity are thought to be *expressed* as changes in AMPA type receptor-mediated transmission, both LTP and LTD are *induced* with the activation of NMDA type glutamate receptors and influx of calcium into the postsynaptic neuron (9). It is now understood that LTD-inducing stimulation induces postsynaptic levels of calcium that predominately activate protein phosphatases (10), whereas LTP-inducing stimulation leads to even higher levels of calcium increases resulting in protein kinase activation (11). It is to be noted that at some intermediate frequencies, with the accompanying intermediate levels of calcium influx, no apparent change takes place in the synaptic response (8). While it is unknown exactly what kinase and phosphatase substrates mediate the changes in synaptic strength, the bulk of the evidence now points in favor of the idea that reversible protein phosphorylation mediates synaptic strengthening and weakening by regulating trafficking of AMPA type glutamate receptors into and out of the postsynaptic membrane (12).

In some cases a synapse is engaged with only these types of local biochemical changes that ensure short-term changes in the synapse (early-LTP; 1–2 hours). With slightly longer periods of synaptic activation, longer-term changes are made in addition to temporary ones by engaging protein synthesis locally in the dendrites (13), or for even more persistent potentiation, the nucleus (late-LTP; greater than 3 hours) (14). Because plasticity such as LTP has been shown to occur only at active synapses, and gene expression changes have the potential to impact synapses on the entire neuron, it has been proposed that induction of LTP sets a 'synaptic tag' at the synapse that aids in local sequestering of plasticity-related mRNAs or proteins to mediate enduring changes (14, 15). These transient tags remain active 1–2 hours after synaptic activation, and thus the onus to provide these synapses with *de novo* mRNA and proteins within that narrow window of time lies with a rapid nuclear response. This in turn demands a quick and efficient signaling to the nucleus from the synapses.

Other evidence of temporal constraints on nucleus signaling is available from studies involving pharmacological blockade of late phase LTP. Late-LTP is sensitive to transcription inhibitors, but the inhibitors are only effective if they are applied to the tissue prior to or during the act of stimulation. Importantly, these inhibitors fail to negate late-LTP when applied even after a very short critical time window after stimulation, suggesting that the stimulation-induced transcription occurs almost immediately after the trigger (14, 16). Considering the lack of information regarding the time taken by these inhibitors to diffuse into the nucleus, however, it is not possible to put a precise number to this time window. Because the mRNA encoding

activity-induced immediate early genes (IEGs) have been detected in as early as 2 minutes of electroconvulsive shock treatment (17), a model for learning and memory, it may be presumed that this critical time window could be as little as 2 minutes. Interestingly one of these genes, *arc*, is known to critically mediate late-LTP and memory consolidation (18). How is the nucleus signaled in the specific case of synaptic plasticity? Means to meet demands for such temporal frugality has triggered eager investigation in the field and the synopsis of our understanding in this regard is delineated below.

The Synapse-to-Nucleus Biochemical Model

The synapse-to-nucleus model for synaptic plasticity was initially based on the intuition that simple neuronal cell firing would not be sufficient to specifically signal LTP-related nuclear events, but two types of observations have largely supported the idea. Firstly, evidence that NMDA receptor antagonists attenuate neuronal activity-dependent gene transcription (19) strongly suggested that biochemical signals triggered by NMDA receptors were primarily responsible for employment of the nucleus. The NMDA receptors' permeability to calcium is known to trigger cascades of biochemical events locally that can impact proteins in the nucleus. For instance, pools of calcium (microdomains) near the NMDA receptors have been found to be critical for activation of the extracellular signal-regulated kinase (ERK), the kinase acting upstream of the cyclicAMP response element binding protein (CREB) (20, 21), which is known to regulate transcription of synaptic plasticity related genes (22). Although the studies using NMDA receptor antagonists seemed to provide solid support for the synapse-to-nucleus idea, the effects of NMDA receptor antagonists on action potential firing had not been considered in this context until recently (discussed in the following section). Interestingly, the very same debate on whether NMDA receptor antagonists inhibited action potential firing took place several years ago in the context of ocular dominance plasticity in the visual cortex (23, 24), but it failed to impact the field in the context of nuclear signaling.

A second, more recent line of evidence supporting the synapse-to-nucleus model is that several signaling messengers have now been identified '*en route*' to the nucleus in response to synaptic stimulation. Examples include messenger kinases like ERK (2), modulators like calmodulin (25), transcription factors like NF- κ B (26), and importins, the proteins that escort larger molecules through the nuclear pore complex (27). Recently, AIDA-1d, a component of the post-synaptic density (a protein cornucopia in synapses that is composed of several regulators of synaptic function) was found to translocate to the nucleus upon NMDA receptor stimulation (28), providing the best evidence that molecules at the very site of synaptic NMDA receptors may be

transported to nuclei. We will talk more about AIDA-1d soon.

Taken together, these observations have supported the notion that post-synaptic receptor activation and/or synaptic plasticity at synapses initiates the spatial movement of signaling moieties to the nucleus to consolidate synaptic changes. In essence, this hypothesis is an extended version of signal transduction in any non-neuronal cell, where receptors exhibited on the plasma membrane are located at a distance from the nucleus close enough to satisfy temporal requirements of a rapid nuclear response. However, in neurons, synapses can be located at significant distances from the nucleus, often hundreds of microns away. Therefore, the synapse-to-nucleus model, under such circumstances, appears to be limited on a spatial scale if speed of signaling is at a premium.

How fast is fast enough? We mentioned earlier that the 'synaptic tag' lasts only 1–2 hours, but is this period of time adequate enough to accommodate the physical flow of signaling elements from the synapse to the nucleus? When neurons were constituted with a GFP-tagged form of the synapse-to-nucleus transcription factor NF- κ B p65, it took the signal 20–30 min following reduction in distal dendritic GFP-p65 fluorescence to populate the nucleus after stimulation by depolarization, glutamate, or NMDA (26). In order to account for up-regulation of genes within 2 minutes, a signal would be required to travel faster than that. Are there mechanisms to handle such fast transport?

Recently, a clue was offered by AIDA-1d (28). When expressed in dissociated primary hippocampal neurons, eGFP-AIDA-1d was found to be chiefly localized in post-synaptic densities of dendritic spines and translocated to the nucleus within 3–5 minutes after stimulation with NMDA (28). However, because AIDA-1d's translocation to the nucleus was calcium-independent, this particular molecule probably signals the nucleus about synaptic glutamate release, and not plasticity *per se*, which would be the primary advantage of this model over the action potential model if such a feature were important for LTP-related signaling. Interestingly, AIDA-1d is not involved in transcription, but instead may have other functions in the Cajal bodies (28). Nevertheless AIDA-1d's rapid nuclear translocation does prove the presence of 'railroads' capable of quickly delivering yet unidentified immediate early gene-regulating 'cargo' to the nucleus within matter of minutes. Whether such a transported signal could be speedy enough to commence transcription of a gene like *arc*, detected within 2 minutes, however, is less likely.

In addition to concerns about timing, the synapse-to-nucleus hypothesis also faces certain stoichiometrical challenges. It has been previously proposed that any protein traveling from a single synapse could be diluted several thousand times due to larger size of the nucleus in comparison to the synapse (5). Such extreme dilution is expected to make transcriptional signaling by a synaptic molecule difficult unless hundreds of synapses were to act

co-operatively. Therefore, the task of activating a gene response by molecules from a few synapses appears to present certain physiological challenges. Although classically, LTP induction *has* been shown to require afferent co-operativity to achieve postsynaptic depolarization sufficient for NMDA receptor channel opening (29), modern ideas on spike-timing dependent plasticity (30) and other observations (31) support the notion that LTP can occur at single synapses. The mechanism underlying consolidation of such plasticity at single synapses through nuclear output remains unknown.

Considering all of these factors, the synapse-to-nucleus model appears to be better suited to mediate nuclear functions not requiring a rapid gene response and those requiring information from several concurrently activated synapses. Thus, like classical LTP induction, the nucleus would require the cooperation of signals from many synapses to commence transcription. In summary, this type of signal, where a small corps of protein(s) is transported from activated synapses, may not require induction of plasticity, but likely requires involvement of multiple synapses, and can be independent of the firing of the postsynaptic neuron.

The Action Potential Model

NMDA receptors require, in addition to glutamate, post-synaptic depolarization to open and allow flux of ions (32). Therefore action potentials appear to be important for LTP induction in some preparations, primarily in order to achieve sufficient depolarization for optimal NMDA receptor activation (29, 33). Further, when action potentials travel in a retrograde fashion back into the dendrites (called "back-propagating" action potentials) coupled with sub-threshold synaptic depolarization, calcium influx increases supralinearly (34, 35). Interestingly, the timing of glutamate release from a synaptic terminal with respect to the occurrence of a postsynaptic action potential is critical to the direction of plasticity that will occur (called "spike timing-dependent plasticity", STDP) (30). In addition to the role of inducing synaptic plasticity, we propose that action potentials also play a critical role in inducing transcription relating to plasticity consolidation.

Inspired by the fact that action potentials can signal gene transcription in dorsal root ganglion neurons without involvement of synapses (36), the action potential model posits that repetitive firing of action potentials can provide information to the nucleus relating to synaptic plasticity without direct involvement of transported biochemical signals from synapses. We propose that whenever a neuron is fired repetitively in a natural setting, LTP, LTD, or both, are likely to occur at one or more of the approximately 10,000 synapses on a cell, and that the nucleus would not necessarily require further information on the specific direction of plasticity. In fact, recent experiments have demonstrated that late-LTD at one set of synapses can

rescue an early-LTP from decay, and vice versa in a phenomenon called “cross-tagging” (37). Thus because the occurrence of LTD can substitute equally well for LTP in signaling the nucleus for production of proteins necessary for consolidating LTP, specific plasticity-induced signals from the synapse appear unnecessary. Also, because synapses are ‘tagged’ for occurrence of plasticity, the synapse-to-nucleus signal offers no targeting advantage over the action potential model. For these reasons, signaling at the nucleus can be thought of as being allowed to take place entirely independent of synaptic biochemistry. Furthermore, action potentials can encode additional information in their frequency and pattern (38) and are therefore well-suited to deliver specific messages about the animal’s behavioral state to neuronal nuclei.

It is important to remember that the remarkable specificity of signal transduction is often the result of specialized intra-cellular localization of signaling messengers (39, 40). This may be more so in neurons, because some molecules (for example ERK or CaMKII) may be present in somatic cytosol as well as near synapses and can mediate distinct function in each region (41). Synaptic activity may activate these molecules at both these locations; ones near synapses being activated by NMDA receptor-dependent processes, while those in soma and throughout the cell could be activated by action potentials (20, 42). Similarly, an entire neuron experiences calcium elevation as both NMDA receptor and action potential dependent calcium sources are recruited. The action potential model envisions the activation of these molecules in and around the soma by the action potentials, which would subsequently relay the information to the nucleus (5). In this case, these molecules do not have to travel all the way from distal synapses but need only to traverse a short distance between the somatic membrane and the nucleus like any other non-neuronal cell. Alternatively, calcium may relay the information directly to the nucleus (43, 44). This way, the temporal constraints imposed by distance between synapses and the nucleus is easily negotiated.

With both modes of nuclear signaling in mind, are there any experimental evidences to determine whether a synapse-to-nucleus signal is necessary for plasticity? Indeed direct evidence in favor of the action potential model for a role in consolidating synaptic plasticity was found when neurons were stimulated artificially, without activating synapses. When axons are stimulated, action potentials can propagate in both orthodromic (away from the cell body) and antidromic (toward the cell body) fashions. Using this concept of backward propagation by action potentials into the cell body, and that synapses can be “tagged”, such action potentials were found to be able to convert a decaying form of LTP (early-LTP) into a long-lasting form (late-LTP) (42, 45). The same type of antidromic stimulation of action potentials was also found to induce phosphorylation of important LTP-related signaling molecules like ERK and CREB and also induced IEG *zif268* expression (42). These

observations show that action potentials are sufficient to induce nuclear signaling and importantly demonstrate that signals from synapses are redundant for LTP consolidation during the time-frame investigated.

The above study brings us to an important question. If action potentials are sufficient in nuclear signaling, then why does blocking NMDA receptors impair transcription? The synapse-to-nucleus model relies on the fact that NMDA receptors initiate one or more biochemical pathways at synaptic locales. NMDA receptors, however, being ion channels, also contribute to the postsynaptic membrane depolarization in response to synaptic activity and thus could facilitate generation of action potentials. When this concept was tested, NMDA receptors were found to be important for action potential generation induced by synaptic stimulation; application of specific NMDA receptor antagonists, APV (D, L-2-amino-5-phosphonovalerate), prevents generation of action potentials in neurons from hippocampus (46). Importantly, activation of a kinase (ERK) in the nucleus still occurred in the presence of APV as long as action potentials were preserved by a pattern of stimulation that induced APV resistant action potentials or application of a GABA_A receptor antagonist, which blocks the inhibitory effect of GABAergic channels (46). This observation gains importance in light of the fact that ERK remained inactive in stimulation protocols where APV blocked generation of action potentials. Taken together, these experiments show that NMDA receptors have a significant role in generating action potentials, and this role confounds the interpretation of any experiments in which NMDA receptor antagonists were used to block gene transcription.

A genuine concern that could be raised relates to the possibility that spontaneous action potentials may generate these nuclear signals. Along these lines, we envision that only repetitive action potential firing rises above a threshold for triggering the nuclear signaling. For example, 60–80 electrical pulses to the afferent synapses resulting in an estimated 10–20 action potentials appear to be required for phosphorylation and subsequent activation of ERK (47), suggesting that a critical number of action potentials is required for nuclear signaling by this enzyme. Importantly, this threshold number is very close to being the same number of pulses required for induction of late-LTP (unpublished observation). Frequency of stimulation was also important, as stimulation at less than 3 Hz was ineffective at consistently activating ERK (47). So in contrasting the two different models, one has the nucleus adding up the number of active/modified synapses, and the other has it counting the number and frequency of action potentials (Fig. 1).

Taken together, action potentials qualify as messengers that can quickly and efficiently relay synaptic information to the nucleus. As action potentials only travel across the membrane, something must next relay the change in electrical potential to a biochemical signal. As we have

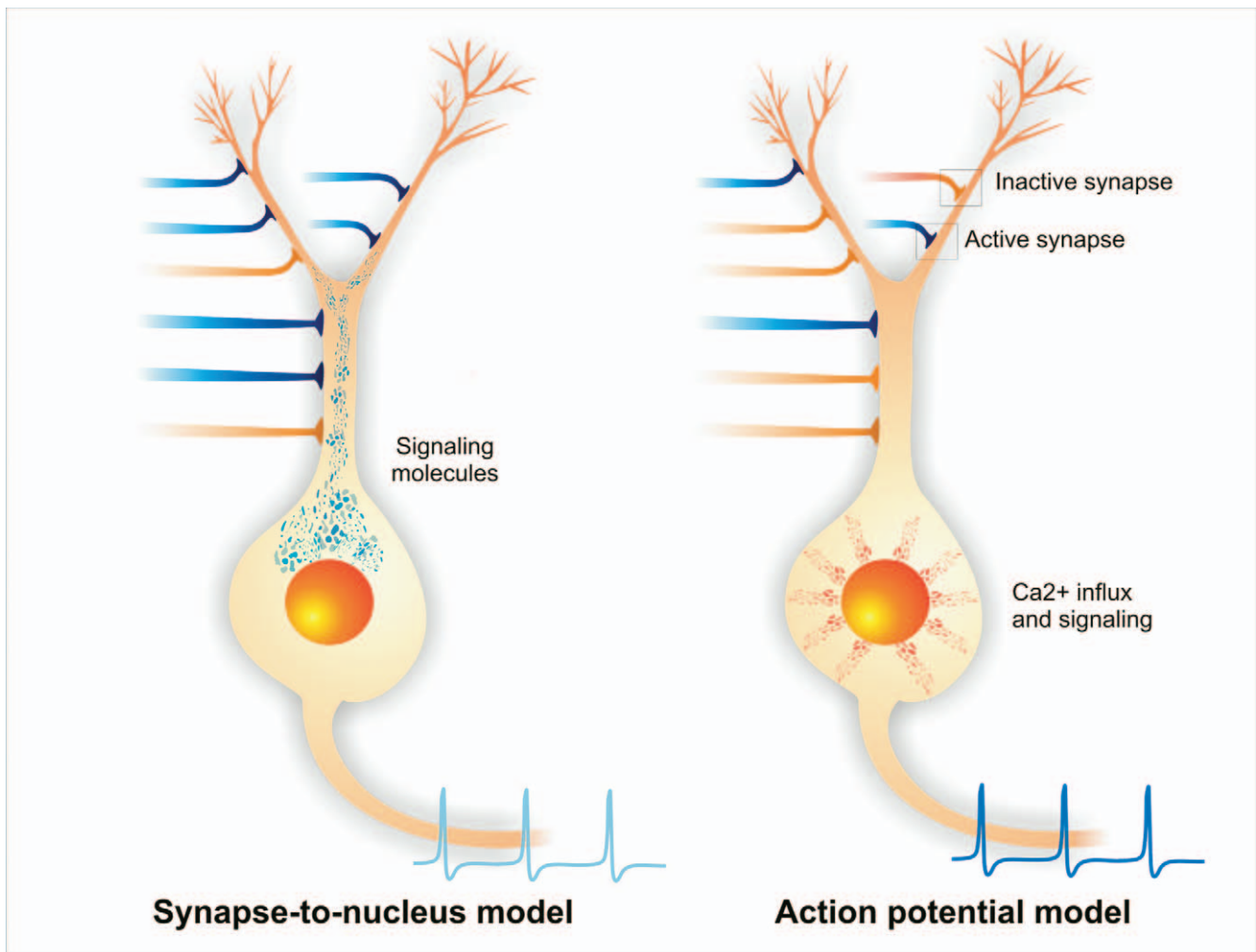


Figure 1. Two methods of signaling the nucleus in neurons. In the Synapse-to-Nucleus model, transport of molecules (blue dots) from active synapses (blue axons) is required for the signal to reach the nucleus. In this model, the nucleus adds up the signals from the many synapses where glutamate release and/or where plasticity had taken place. The transcriptional response, therefore, reflects the number of synapses active or plastic above a threshold number. In the Action Potential model, calcium (red dots) influx through voltage-gated ion channels and NMDA receptors signals the nucleus. In this case the nucleus is adding up the number and frequency of action potentials rather than the number of active synapses. The transcriptional response in this case is indirectly assessing the likelihood that plasticity had occurred. A color figure is available in the online version of the article.

mentioned earlier, calcium is the most likely candidate to mediate such a role.

Calcium: the Messenger of Choice

During synaptic activity, calcium enters post-synaptic dendrites through calcium-permeable channels like NMDA receptors and voltage-gated calcium channels. Using calcium-imaging techniques and calcium chelators, several groups have now proved beyond doubt that a rise in intracellular calcium is a primary event during onset of various forms of long-term plasticity (48, 49). Now appreciated, however, is that calcium can act in cellular locations far removed from the synapses, including at the nucleus. Such signaling to the nucleus can be thought of as taking place in two very different strategies. In the first case, calcium may act indirectly through calcium-sensing signal-

ing molecules at the membrane or in the cytosol. The second strategy involves direct activity of calcium itself within the nucleus.

Calcium influx through NMDA and voltage-gated calcium channels, at the synapse or in the cell body, can activate specific subsets of calcium-sensing signaling molecules, like, synaptotagmin and calmodulin, in the vicinity of the site of entry within the cytosol. Once activated, these molecules may then convey a signal to the nucleus through signaling cascades in a calcium-independent way. The ERK MAP kinase pathway is an example of such a signaling cascade. In several instances, the precise route of calcium entry into the neuron is initially responsible for setting up the cellular response by differentially activating signaling molecules that are either physical components or associates of channels or are found very near the site of calcium entry. For example, the C-terminus

fragment of the L-type calcium channel $\text{Ca}_v1.2$ serves as a transcription factor in the nucleus (50) and CASK, tethered to N-type calcium channels, can translocate to nuclei to regulate transcription (51, 52). The evidence that calcium influx acts close to the membrane and not deeper into the cytosol to initiate nuclear signals was found using a low affinity calcium chelator. By allowing calcium to rise at the membrane but not in the rest of the cell, the authors demonstrated that signals initiated at the membrane could propagate to the nucleus to induce CREB phosphorylation entirely independent of calcium in the whole cell (21). Thus calcium entry through specific sites can trigger unique membrane proximal signaling events which are potent enough to regulate transcription in the nucleus.

In addition to the route of entry, the amplitude and duration of the influx plays a major role in shaping the neuronal response. For example, at synapses, comparatively higher and rapid calcium influx into post-synaptic bodies results in kinase activation and leads to LTP while prolonged and quantitatively limited influxes result in activation of phosphatases and lead to LTD (48, 53, 54). Several such parameters have been proposed to integrate into a complex 'calcium code' for nuclear signaling (55) which renders the same calcium ion to bring about various long-term phenotypes. Similar differential activation of calcium-dependent kinases and phosphatases are important in nuclear signaling, as in the case of the transcription factor MEF2 (56), but whether the kinase/phosphatase balance is regulated in a way similar to that at synapses is unknown.

In addition to its indirect effects, evidence exists to suggest that calcium can bring about direct activation of enzymes within the nucleus. It is now known that transient calcium in the nucleus is sufficient for activation of CREB and the histone acetyltransferase CBP (43, 57, 58). In a cleverly designed experiment, mild-detergent-treated nuclear remains of hippocampal neurons demonstrated increased phosphorylation of CREB when the concentration of calcium was increased in the solution (43). Because the detergent treatment had dissolved any non-nuclear structures of the cell, this experiment conclusively demonstrated that nuclear calcium alone is a suitable trigger for activation of plasticity-associated transcription pathways. Moreover, sequestering of nuclear Ca^{2+} with injection of calcium-chelator BAPTA in the nucleus led to significant drop in depolarization-induced expression of *c-fos* (59). *In vivo*, further evidence that calcium can play a direct role in plasticity-related nuclear functions was shown in a mouse expressing a dominant inhibitor of calmodulin selectively in nuclei; these mice had impaired CREB phosphorylation, *fos* gene expression, LTP, and long-term memory (44). Together, these observations demonstrate that calcium in the nucleus plays a role of its own in mediating transcription related to long-term synaptic plasticity. Interestingly, this role is significantly different from that of the cytosolic calcium transients. Cytosolic transients have been shown to activate genes through serum response elements, whereas

risers in nuclear calcium led to gene activation through the cAMP-response element (59).

Membrane depolarization can activate a variety of voltage-gated ion channels, including several that conduct calcium. Are there particular types of voltage-gated calcium channels that are linked to nuclear signaling? Indeed multiple lines of evidence support the idea that the L-type voltage-gated calcium channels play a major role in nuclear signaling generally and in plasticity-related transcription specifically (60–62). For example, while both N- and L-type channels are tethered to various intracellular signaling molecules (63), only L-type bound calmodulin has been found to be adept at transcription-related calcium sensing (61). Calmodulin acts as a local sensor for calcium entering the cytoplasmic micro-niche around the channel, and calcium-calmodulin complexes bound to C-terminus of the activated channels bring about a conformational change leading to the inactivation of the channel along with simultaneous activation of the Ras-MAPK pathway, which can subsequently signal to the nucleus (61). Also as noted previously, a fragment of the L-channel itself has been found to be able to serve as a transcription factor within the nucleus (50). With regard to synaptic plasticity, one experiment showed that an inhibitor of L-type channels prevented the antidromically-induced action potential-dependent CREB and ERK phosphorylation in hippocampal neurons (42). This observation suggests that opening of ion channels can be finely tuned to the specific kinetics and amplitude of the depolarization, though, the role of action potentials in natural L-channel activation has been questioned (62). Subsequently, however, the depolarization from action potentials has been demonstrated to be very effective at opening these channels, and in fact, they can be even more effective than the depolarization from excitatory synapses (64). In summary, signaling cascades such as those important for CREB phosphorylation can be transduced by calcium in both direct (43) and indirect manners, and L-type calcium channels play a privileged role in this signaling. Whether both these processes occur simultaneously in nature, and whether there is any difference in subsets of targeted genes, is open for further investigation.

Beyond Two Mechanisms—Computations by the Nucleus

From the perspective of the nucleus, the synapse-to-nucleus and the action potential models provide opportunities for two distinct computations of signaling inputs which may result in independent patterns of gene expression. Considering several possible permutations and combinations amongst various aspects of these two models, the computational scopes for the nucleus are many.

The strength of synapse-to-nucleus or action potential mediated signaling is primarily delimited by two aspects: number of active synapses in the circuit and signal enrichment by amplification. Signal amplification is one of

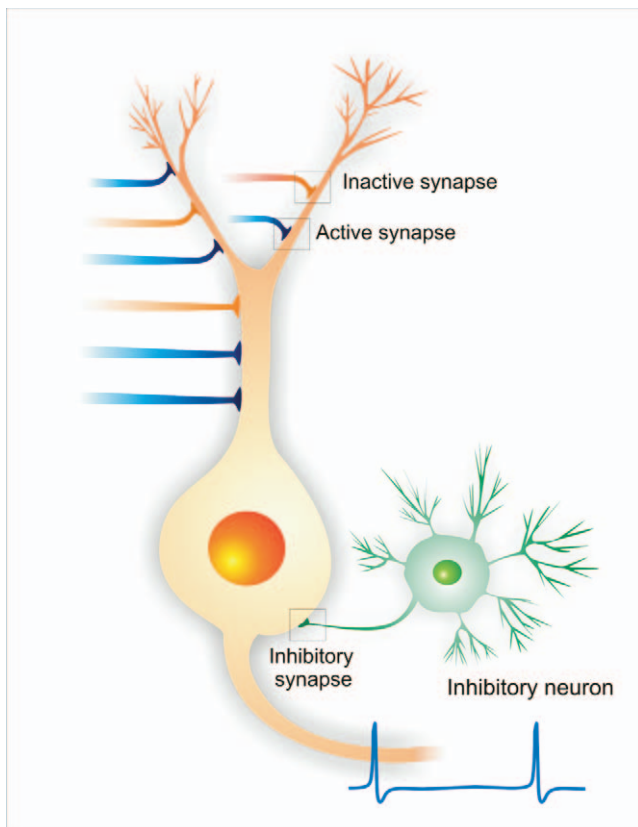


Figure 2. Regulating action potentials and nuclear function in the brain. Neurons in the brain are highly innervated with inhibitory (GABAergic) synapses (green), which can profoundly influence how and when a cell will fire action potentials. Thus, even if the principal neuron receives many excitatory signals (blue axons), it may not fire if the inhibitory inputs are not permissive. Nuclear function, therefore, may be directly impacted by the behavioral state of an animal and the resulting neuromodulatory and inhibitory influences on action potential firing. A color figure is available in the online version of the article.

the basic tenets of signal transduction in any cell type and must be considered in neurons as well. For instance, a signal originating at any one synapse could be amplified by a cascade phenomenon, such as calcium release from intracellular stores, or an amplifying cascade of kinase activity such as is the case for adenylyl cyclase, cAMP and PKA activation. However, the final signal perceived by the nucleus will be a complex outcome of such amplification times the number of active synapses in play, specially, in synapse-to-nucleus signaling. The concept of co-operativity (cooperation between several active synapses) becomes critical in the case of small transported signals. In other words, several synapses must post similar signals in order to be interpreted by the nucleus in a timely fashion. Alternatively, another hypothetical computational feature of this model would be that signals from many synapses could be added over several tens of minutes (vs. over milliseconds in the case of action potential-dependent signaling).

The action potential model is comparatively less reliant

on the quantity of active synapses. Although the number of active excitatory synapses typically correlate with the firing of postsynaptic neurons, brain rhythms shaped by inhibitory (GABAergic) circuitry, intrinsic excitability of the neurons, and recurrent excitatory synapses could contribute to action potential firing patterns that might differ considerably from ones arising out of simple algorithms of excitatory input and/or synaptic plasticity (65) (Fig. 2). Thus the presence of these parameters will offer various nuclear computational scopes for different neurons concurrently or the same neuron at different times.

We hypothesize that messages arriving in the nucleus through the synapse-to-nucleus pathway will generate patterns of gene expression somewhat different from those that are reported by action potentials. This is possible as these two models offer signals on a different spatial and temporal basis. The nucleus is probably served first by signals arising rapidly from action potentials. The time range for this is expected to be in seconds to few minutes. For example, elevation of nuclear calmodulin, which is severely contingent on Ca^{2+} entry through L-type channels, has been recorded to occur within 15 seconds of stimulation (66). In due course, the synapse-to-nucleus messengers are expected to arrive in matter of minutes to hours. Thus, there is a probability that these two sets of signals overlap leading to the expression of a specific subset of genes. However, when considering these scenarios, a reminder is warranted that action potential firing alone appears to be sufficient for rescue of late-LTP (42).

Many questions remain to be answered regarding synapse-to-nucleus signaling. For example, are there distinct signals representing the specific cases of LTP and LTD? Are there any signals that represent the specific cases of synaptic activity in absence of plasticity? This situation may arise due to presence of an inhibitory parameter (e.g., GABA_Aergic influence) in the network, or as a result of presynaptic activity in an intermediate frequency range (above the threshold for LTD, but below the threshold for LTP). A plasticity-independent signal has been recently identified; AIDA-1d translocation to the nucleus does not require calcium (28), and so it follows that it does not require synaptic plasticity for initiating its function. Finally, can the nucleus respond in a graded response to weak (dilute) synapse-to-nucleus messages? Even though most transcription is thought to require stoichiometrically favorable concentrations of factors in the nucleus, we do not rule out the possibility of a potent signal from a few synapses that could localize to specific nuclear addresses, like transcription factories (67). Localization of AIDA-1d in the relatively small-sized Cajal bodies makes a strong case that such a yet unidentified messenger could exist (28), but we favor the idea that the co-operativity of synapse-to-nucleus translocation may be well-suited to regulate transcription of the genes important for homeostatic function (68) or for turnover of synaptic proteins.

Concluding Remarks

We think that the nucleus is adept at 'decoding' messages induced by action potentials. This is demonstrated in studies where nuclei intercept and interpret messages from somatic action potentials evoked in neurons without synapses or by axonal backfiring without involvement of synapses (36, 42). Even though the nucleus is capable of reading signals from synapses via synapse-to-nucleus messengers, these computations do not appear to be necessary for the specific case of LTP; action potentials are sufficient to convert a decremting form of LTP into a stable one (42). When inhibitory (GABAergic) networks and intrinsic excitability are incorporated into the equation, it is clear that excitatory synaptic input is only one factor in determining whether a cell will fire repeatedly, thus making excitatory synaptic input and action potentials capable of mediating two distinct types of computations at the nucleus. That a GABA_A receptor antagonist can reestablish action potentials and subsequent cell-wide kinase activation in presence of NMDA receptor antagonists illuminates the importance of inhibitory regulation in action potential-dependent biochemical signaling (46). In addition to the fact that action potential-mediated calcium increases are both rapid and large, because the action potential model takes into consideration an animal's behavioral context during the time a neuron's synapses are undergoing plasticity, we find this model to better fit the requirements of a 'memory' signal to the nucleus to compute whether such synaptic changes will be consolidated with products of transcription. We look forward to definitive identification of the specific genes important in plasticity consolidation, as we are confident that action potentials will play an important role in their regulation.

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