Signaling from synapse to nucleus: the logic behind the mechanisms
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Signaling from synapse to nucleus is vital for activity-dependent control of neuronal gene expression and represents a sophisticated form of neural computation. The nature of specific signal initiators, nuclear translocators and effectors has become increasingly clear, and supports the idea that the nucleus is able to make sense of a surprising amount of fast synaptic information through intricate biochemical mechanisms. Information transfer to the nucleus can be conveyed by physical translocation of messengers at various stages within the multiple signal transduction cascades that are set in motion by a Ca\(^{2+}\) rise near the surface membrane. The key role of synapse-to-nucleus signaling in circadian rhythms, long-term memory, and neuronal survival sheds light on the logical underpinning of these signaling mechanisms.

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Abbreviations
BDNF brain-derived neurotrophic factor
CaM calmodulin
CaMK calmodulin-dependent protein kinase
CREB Ca\(^{2+}\)/cAMP responsive element binding protein
DREAM downstream regulatory element antagonist modulator
NFAT nuclear factor of activated T-cells
NMDAR N-methyl-D-aspartate receptor
NPY neuropeptide-Y
PACAP pituitary adenyl cyclase activating peptide
PKA protein kinase A
SCN suprachiasmatic nucleus

Introduction
Neuroscientists have long appreciated the computations that ion channels perform at the surface membranes of neurons. More recently, it has emerged that the complexity of this electrical signal processing is rivaled by the intricacies of biochemical signal processing that occur below the membrane, within elaborate networks of cytoplasmic signaling proteins. However, only within the past few years has it become clear that even deeper within the neuron lies perhaps the most formidable engine of computation of all [1]. The nucleus of the mammalian neuron creates, shapes, links and responds to these electrical and biochemical communication streams by actively controlling the expression of tens of thousands of genes — a more diverse repertoire than that presented by any other known cell type.

Until recently, little was understood about the specific role of the nucleus, apart from the notion that it could be involved in the maintenance of long-term changes in neuronal function [2]; it was not at all obvious how synapses, as myriad sources of fast electrical signals, could communicate even a tiny fraction of their rich information stream to the distant and apparently slow nucleus. It has now become evident that the nucleus is able to make sense of a surprising amount of fast synaptic information through intricate biochemical mechanisms [3–5]. Furthermore, as we review here, there is now a much clearer understanding of the crucial role of synapse-to-nucleus signaling in certain key neuronal processes, namely circadian rhythms, long-term memory and neuronal survival. This functional and behavioral level of understanding now anchors our interpretation of the underlying logic of the nuclear signaling mechanisms employed. An emerging theme as we show here is that the complexity of neuronal biochemistry may be necessary to generate signal processing streams (from the rapid ion channel kinetics that initially shape electrical information flow to the slower biochemical effectors) that are specific enough to convey an information-laden signal to the nucleus.

Many signaling pathways to the nucleus
What signaling entity physically translocates to the nucleus to support the transfer of information from the neuronal cell membrane? In their pioneering paper on excitation–transcription coupling, Morgan and Curran [6] put forward a hypothesis for how Ca\(^{2+}\) entry through L-type Ca\(^{2+}\) channels might activate expression of \(c\)-fos, an immediate early gene (IEG; IEGs constitute the first wave of genes to be turned on, often within minutes, after a stimulus). They suggested that a calmodulin (CaM)-sensitive kinase would phosphorylate a transcription factor, initially positioned in the cytoplasm, causing it to move into the nucleus to activate gene expression. This first speculation was prescient in broad outline and provocative
in highlighting the following fundamental questions. First, what are the crucial and specific signal initiators? Second, what are the nuclear translocators, and why are they selected for the task? Third and finally, what is the basis for stimulus specificity and reliable information transfer?

By focusing on the pathways triggered by an intracellular Ca\(^{2+}\) rise downstream of synaptic activity, we now know that the signal from the cytoplasm to the nucleus can be conveyed in several different ways (Figure 1). The translocation can take place at various stages within signal transduction cascades that are set in motion by the Ca\(^{2+}\) rise. At one extreme, as hypothesized by Morgan and Curran, the transcription factor itself, the terminal factor in the biochemical cascades, can move to the nucleus upon changes in its phosphorylation state. The classic example is a member of the nuclear factor of activated T-cells (NFAT) family of transcription factors. Originally characterized in immune tissue, the NFATc group plays a crucial role in neuronal plasticity as well as vascular development and muscular hypertrophy [7]. NFATc4 is expressed within hippocampal neurons, and undergoes a striking translocation from the cytosol to the nucleus upon the opening of L-type Ca\(^{2+}\) channels [8]. The translocation step is dependent upon calcineurin-mediated dephosphorylation of NFATc4, which causes the unmasking of multiple nuclear localization signals and the active transport of the transcription factor through the nuclear pore complex.

At the other extreme, a mechanism may exist that transduces directly the free Ca\(^{2+}\) transient that can arise in the nucleus after neuronal excitation [9]. The transcription factor downstream regulatory element antagonist modulator (DREAM) [10], a Ca\(^{2+}\) binding protein that contains three active Ca\(^{2+}\) binding motifs (E-F hands) [11*], is abundant in the nucleus. It has been proposed that nuclear DREAM remains bound to a downstream regulatory element (DRE) that acts as a gene silencer when nuclear Ca\(^{2+}\) is low, but dissociates upon elevation of Ca\(^{2+}\) causing DRE derepression, activation of downstream genes such as that which encodes prodynorphin [10] and attenuation of pain signaling in vivo [12*]. It will be interesting to see if DREAM function can be formally linked to nuclear Ca\(^{2+}\) levels, and to understand why DREAM is present outside the nucleus as well as within it.

Are there particular advantages for the signaling mechanisms at either one or the other of these extremes? On the one hand, the nuclear Ca\(^{2+}\) mechanism that is exemplified by DREAM is straightforward, fast and simple, but it might be a suboptimal choice in general as the nucleus would suffer from a severe loss of specificity with regards to the source of Ca\(^{2+}\) and the nature of the initial membrane signal. On the other hand, using a highly processed nuclear signal like the transcription factor itself (as in NFAT translocation) allows for a high level of signaling specificity, but in placing the bulk of the signal amplification and processing outside of nucleus more pressure is placed on nuclear transport mechanisms. As electrophysiologists know, it is unwise to amplify a signal before passing it through a channel that can be saturated, as it could result in serious distortion of the signal.

Activation of DREAM and NFATc4 define the extremes of information transfer from the plasma membrane to the nucleus, whereas Ca\(^{2+}\)/cAMP responsive element binding protein (CREB) signaling mechanisms typically exemplify the middle ground. In this case, the translocating message is not conveyed by free Ca\(^{2+}\) moving from the ion channels to the nucleus, or by the transcription factor Creb, but by intermediate players in the pathway. In fact, CREB remains constitutively bound to CRE (Ca\(^{2+}\)/cAMP responsive element) regulatory sites controlling target genes, and becomes activated by phosphorylation on its Ser-133 residue (with modulatory influences exerted by other residues). A fast CaM-dependent kinase cascade and a slow MAP kinase cascade (likely to be cAMP-modulated) converge on Ser-133 following surface membrane depolarization and Ca\(^{2+}\) influx, and work together to promote CREB-dependent gene expression [3,13].

How are these two signals transduced? It is well established that in response to physiological synaptic activity in hippocampal pyramidal neurons, Ca\(^{2+}\) entry through both N-methyl-D-aspartate receptors (NMDARs) and L-type Ca\(^{2+}\) channels initiates the nuclear signaling to CREB [14–16]. Nevertheless, Ca\(^{2+}\) ions can be excluded as the...
Figure 1

(a) SURFACE/CYTOSOLIC EVENTS

Ca\(^{2+}\) rise → CaM → CaN → NFATc4 → Information transfer to the nucleus → CREB phosphorylation → Interaction with NFATn: NFAT-transcription

Ca\(^{2+}\) rise → CaM → CaMKK/CaMKIV → CREB phosphorylation

Ca\(^{2+}\) rise → CaM → MAPK/Rsk → CREB phosphorylation → Interaction with CBP: CRE-transcription

Ca\(^{2+}\) rise → CaM → AC → PKA → CREB phosphorylation

Ca\(^{2+}\) rise → CaM → Binding to DREAM → Removal from DNA: DREAM repression lifted

(b) Early stages of activity-dependent signaling to the nucleus

Ca\(^{2+}\) → L-type VGCC → CaM → CaN → NFATc4 → Information transfer to the nucleus → CREB phosphorylation → Interaction with NFATn: NFAT-transcription

CaMK IV → CREB → Interaction with CBP: CRE-transcription

AC → PKA → CREB phosphorylation

DREAM → Removal from DNA: DREAM repression lifted

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**Signalling mechanisms**

Typical nuclear messenger in these cells as CREB activation at Ser-133 shows marked Ca\(^{2+}\)-channel-type specificity. Working alongside Ca\(^{2+}\) stores, many Ca\(^{2+}\) influx channels (including L-type, N-type, and P/Q-type Ca\(^{2+}\) channels as well as NMDA- and AMPA-type glutamate receptors) can cause a marked elevation in the bulk cytoplasmic and nuclear Ca\(^{2+}\) levels, and can play important roles in the neuron [17]. However, most of these are ineffective at directly activating CREB, with the exception of the L-type channel (privileged among the voltage-gated channels [15,16,18**]) and the NMDA receptor [14–16]. Therefore, although nuclear Ca\(^{2+}\) elevations are seen during relatively strong neuronal activity [9] and may support the signaling in some cases [19,20], nuclear Ca\(^{2+}\) is not typically sufficient to activate CREB [15,18**]. Furthermore, it appears that nuclear Ca\(^{2+}\) elevations are not necessary, as suppression of global cytoplasmic and nuclear Ca\(^{2+}\) elevations with intracellular loading of the Ca\(^{2+}\) chelator EGTA generally does not block Ca\(^{2+}\)–activated CREB Ser-133 activation [4,14,21**]. These findings, together with results that demonstrate micrometer-scale localization of L-type Ca\(^{2+}\) channels within the plasma membrane [22**] and a CaM-binding motif on the L-type channel itself [18**] are both crucial for CREB phosphorylation, have revealed that Ca\(^{2+}\) typically exerts its effects on nuclear CREB activation by acting within a domain <1 μm in radius from its source of entry [14].

The privileged roles of the NMDA receptor and the L-type channel [14–16] may be attributable to their selective ability to recruit CaM signaling [15]. While both channel types can directly bind CaM, both are also specifically localized at or near synapses in which additional pools of CaM are present for recruitment. Either or both mechanisms may underlie the selective ability of these channels to initiate locally Ca\(^{2+}\)/CaM-activated MAP kinase signaling to the nucleus [13,18**,21**,23] and nuclear translocation of CaM [15,20,24,25**]. The translocation of CaM to the nucleus is dependent upon its ability to bind to a CaM kinase [20,25**,26]. This binding would stabilize Ca\(^{2+}\)–CaM, although locally mobilized CaM could also subsequently make use of Ca\(^{2+}\) from other sources [19,20], perhaps from nuclear Ca\(^{2+}\) stores or even cytoplasmic Ca\(^{2+}\) waves. Use of ‘supportive’ Ca\(^{2+}\) in this way would still preserve the specificity of information carried by the selective synaptic activation of the NMDA receptor and the L-type channel, as only these channel types would be able to mobilize the CaM necessary to utilize efficiently the available Ca\(^{2+}\). Despite the high concentrations of detectable CaM in all cellular compartments, free or available CaM is generally limiting in intracellular biochemical reactions [27]. The resulting increase in available nuclear CaM results in strong activation of nuclear CaM-dependent protein kinase kinase (CaMKK) and its target CaMKIV. These kinases are ultimately responsible for the early wave of Ser-133 CREB phosphorylation [13,28,29**,30,31].

With stronger stimulation, the slower MAPK-dependent signaling pathway comes into play [13]. In this case, Ca\(^{2+}\)/CaM leads to local activation of the small GTP binding protein Ras, stimulation and nuclear translocation of mitogen-activated protein kinase (MAPK) and/or pp90 ribosomal protein S6 kinase (Rsk) [3,18**,32], in some cases with cooperation of protein kinase A (PKA). In summary, both CaMK and MAPK pathways to CREB generally use a downstream Ca\(^{2+}\)-effect of the translocating nuclear messenger. This thereby preserves information about the specific Ca\(^{2+}\) source, while also leaving ample room for signal amplification in the nucleus — a combination of features that have advantages over the more extreme scenarios.

Clearly then, the transfer of information from the membrane surface of a neuron into the nucleus can be achieved by physical translocation at various points in the signal transduction pathway. Understanding the choice of strategy and the associated information processing trade-offs can best be attempted in the context of the functional output of the pathway. Therefore, next we will review recent work in intact animals describing the disruption of nuclear signaling pathways and their high-level behavioral consequences: that is, generation of circadian rhythms, formation of long-term fear memories, and survival of brain cells.

**Circadian rhythms**

The generation and entrainment of circadian rhythms, of nearly universal importance in biology, provides an outstanding example of the functional importance of synapse-to-nucleus signaling. Many aspects of circadian rhythm generation are hardwired, in that they occur without specifically requiring neuronal activity, and arise in a network of transcription factors that generate rhythmicity from negative feedback signaling. In the suprachiasmatic nucleus (SCN) of the mammalian hypothalamus, for example (Figure 2), multiple proteins are involved in the clockwork mechanism, including dimers involving the transcription factors of CLOCK and BMAL, and the period gene products mPer1, mPer2 and mPer3. This endogenous rhythm is robust but not precise, however, and numerous other circumstances, including seasonal and migratory changes in patterns of light and dark, demand that the cycle be entrainable. In fact, exposure to light before the end of a subjective night, a signal indicating that the rhythm needs adjustment, triggers rapid induction of the genes for mPer1 (within 15–30 min) and mPer2 (within 2 h), leading to an efficient realignment of the circadian rhythm.

How are these clock genes activated? In addition to E-box elements (regulated by the CLOCK/BMAL system), both mPer1 and mPer2 have activity-sensitive CRE promoter elements that are regulated by the CRE-binding protein CREB [33]. Evidence is mounting that CREB-mediated control of these genes refers circadian rhythm
cycling and entrainment. First, CREB activation by phosphorylation of the Ser-133 residue follows an active circadian rhythm (peaking near subjective dawn) and can be directly triggered by entraining stimuli, such as photic stimulation during darkness [16]. Second, expression of the CREB-dependent genes c-fos, mPer1 and mPer2 correlates with CREB activation, and is blocked when a key modulatory site (Ser-142) on CREB is mutated [34**]. Third and finally, this CREB mutation attenuates entrainment of the circadian oscillator with respect to either phase advances or phase delays [34**]. Taken together, these data show that activity-dependent control of a nuclear transcription factor (CREB) mediates a very important complex behavior (entrainment of circadian rhythm).

What are the key design constraints on this activity-dependent signaling pathway? First, to be useful, the pathway should be fast by comparison with the circadian cycle (minutes), although signaling on a scale of seconds may be unnecessary. It need not resolve closely spaced stimuli (after all, closely spaced flashes of daylight were hardly a factor in circadian evolution!). Second, in responding to electrophysiological events, the pathway would ideally be sensitive to synaptic input from afferents that carry sensory information from the retina, but not to the continually free-running spiking of SCN neurons, which is intrinsic even in isolated neurons. Third, as non-photic inputs such as feeding or locomotor activity modulate entrainment in some cases, the pathway must allow for the convergence of multiple stimuli, not just retinal glutamatergic afferents.

Every one of these design requirements seems to be fulfilled by aspects of synapse-to-nucleus signaling in SCN neurons. First, phosphorylation of CREB Ser-133 does occur within about 10 min of light flashes or glutamatergic stimulation of SCN neurons, followed by mPer1...
induction within 15–30 min \[34^-\]. This suggests the involvement of either the fast CaMKIV signaling pathway leading to phosphorylation of CREB on Ser-133 or the slower CaM kinase/MAP kinase-dependent pathway, both of which can be efficacious at 10 min \[13\]. In fact, inhibitors of either CaM kinase or MAP kinase block CREB activation in SCN neurons \[35\]; in the future, circadian rhythmicity and fast signaling to CREB might also be studied in the SCN of CaMKIV knockout mice. Second, with regards to selective coupling to particular electrical events at the membrane, certain pathways of nuclear signaling in hypothalamic neurons have been found to respond relatively selectively to membrane potentials generated by synaptic input in preference to action potentials alone \[36\], as in various ganglionic neurons \[37,38\] and hippocampal pyramidal neurons \[14,39\]. It has become increasingly clear how gene expression avoids indiscriminate activation by the continuous tonic spiking of these cells. CREB signaling in hypothalamic SCN neurons responds to retinal glutamatergic afferents through a pathway initiated by NMDA-type glutamate receptors and voltage-gated L-type Ca\(^{2+}\) channels. Activation of the NMDARs requires synaptic glutamate, and therefore needs more than just spikes to occur. Among the many voltage-gated Ca\(^{2+}\) channels, only the L-type channel has been found to couple to mPER1 \[40\]. If this remains true when the contributions of the other channels are rigorously examined \[15\], it suggests that there is a possibility that L-type channels help to ‘filter out’ brief single action potentials by virtue of their voltage- and time-dependent kinetics \[39\]. This selectivity would probably not be possible if the nuclear message were conveyed by Ca\(^{2+}\) ions for example, as strongly spike-responsive Ca\(^{2+}\) channels (N-type or P/Q-type) also generate large increases in bulk cytoplasmic and nuclear Ca\(^{2+}\). The use of CaM as a synapto-nucleus messenger \[15\] would avoid this difficulty, a possibility that needs to be tested in SCN neurons. Third, signaling in the SCN appears well equipped to integrate modulatory input from non-photic CNS systems, including that of neuropeptide-Y (NPY; provided by afferents from the thalamus), serotonin, and pituitary adenylyl cyclase activating peptide (PACAP). All three systems appear to mediate behavioral influences on circadian rhythmicity, and are known to use G protein signaling to the cAMP/PKA pathway as a means of exerting control over intracellular events (PACAP positively, NPY negatively, and serotonin being capable of both). Indeed, application of forskolin, a direct activator of the cAMP/PKA pathway, promotes mPER1 expression in SCN neurons \[41,42\], and expression of dominant negative PKA inhibits CRE-dependent gene expression in SCN neurons \[41\]. PKA could influence nuclear signaling in several different ways, for example, by direct phosphorylation of CREB residue Ser-133, by stimulating a CREB transcriptional coactivator such as CBP or p300 \[43\], or by potentiating MAPK signaling \[23\].

Equally intriguing is the possibility that PKA-mediated signaling could act through direct modulation of ion channels, thereby regulating the ability of L-type Ca\(^{2+}\) channels to respond to the tonic electrical activity of SCN neurons. This would support the convergence of diverse inputs and allow a non-photic input to recruit exactly the same signaling pathway as a light stimulus, even without retinally driven synaptic input. Indeed, it is known that the ability of L-type channels to filter out action potentials is conditional, in that direct potentiation of ion flux restores some responsiveness to brief depolarizations \[39\]. It is also likely that spike width broadening or high-frequency bursts of action potentials would be able to conditionally overcome this filter \[44\]. To test the validity of this idea in SCN circadian rhythmicity, it would be important to conduct electrophysiological studies of SCN action potentials and the resulting L-type currents at 37°C \[45\]. Testing at this temperature would avoid the opposing effects on spike width and channel gating kinetics that are present under even slightly lower temperatures, and under a range of waveform and modulation conditions. The potential modulation of action potential filtering could also be tested \textit{in vivo}, by using L-type antagonists and potentiators to see if direct manipulation of L-type channels alters the behavioral responses to non-photic inputs through NPY, serotonin or PACAP.

Learning and memory exemplified by fear conditioning

Memory formation is another vital high-level behavioral function that depends on nuclear events \[2,46,47\]. Again, knowledge of the behavioral output of the nuclear event can greatly illuminate our understanding of the logic that underlies the nuclear signaling. Much attention has been directed towards the hippocampus, which not only supports explicit memory formation in tasks like spatial learning but also contributes crucially to contextual fear conditioning, in which the animal learns to associate a complex, generally benign environment with an aversive stimulus (Figure 3). The amygdala mediates cued fear memories, formed when an aversive stimulus is associated with a single stimulus, such as an auditory tone. In both cases, subsequent presentation of the harmless stimulus alone will trigger fear behavior such as freezing. Neurons in both the amygdala and the hippocampus display robust activation of CREB during these learning tasks \[25^-\,48\], and in both structures, genetic interference with CREB function prevents storage of long-term fear memories \[49,50^-\]. Additionally, in either one or the other region, memory deficits associated with reduced CREB function can be partially overcome by spaced repeated training, whereas high-level CREB function can allow long-term memories to form with single training sessions \[50^-\,51,52^-\].

Given these similarities, several predictions can be made. First, that the hippocampus and amygdala would employ...
a common molecular mechanism of signaling to CREB. Second, that CREB should respond strongly to synaptic input that bears incoming processed sensory information in both cases. Third, that activity-dependent signaling events that lead to CREB activation should display considerable speed and temporal resolution, as temporal relationships among events are important in elucidating their causal relationship. Fourth and finally, that stimuli that are spaced and repeated, those most appropriate for associations by the brain should be particularly advantaged in activating CREB by comparison with single stimulus bouts.

In the hippocampus, CREB signaling triggered by physiological electrical activity is indeed selectively dependent on Ca$^{2+}$ entry through L-type calcium channels and
NMAD receptors [14–16]. This pattern of source-specificity confers selective responsiveness to synaptic activity for the reasons discussed above. The role of these pathways for Ca\(^{2+}\) entry in amygdalar CREB activation has not been tested yet, but L-type Ca\(^{2+}\) channels and NMDA receptors are both required for amygdalar formation of long-term fear memories [53,54], which are CREB-dependent. In both the hippocampus and the amygdala neuronal activity causes rapid nuclear translocation of CaM and CaMKIV-dependent CREB phosphorylation [15,25**,28,29**,30], further reinforcing the similarities in CREB signaling. Furthermore, it has been demonstrated in both structures that the CaM/CaMKIV pathway is required for establishing CREB-dependent fear memories [25**,29**]. As noted above, the employment of a downstream effector of Ca\(^{2+}\) as a nuclear messenger, rather than free Ca\(^{2+}\) itself, facilitates Ca\(^{2+}\) source discrimination by the nucleus [2,5,15,18**]. The use of CaM as the messenger, which is capable of significant nuclear translocation within 15 s [20], sacrifices little in the way of speed.

Interestingly, the CaM nuclear translocation pathway, widely employed by non-neuronal cell types as well as neurons [15,24,26,27,55–58], is now also known to be almost ubiquitous throughout the mammalian brain, including hippocampus, amygdala, somatosensory cortex, insular cortex, anterior cingulate cortex and cerebellum [15,20,25**]. However, an exception has been found, three groups have determined that there is little CaM translocation in a substructure of the hippocampal formation called the dentate gyrus [19,20,25**]. Although the dentate gyrus is anatomically positioned as the ‘gateway’ to the hippocampus proper, its precise role is unknown. Dentate granule neurons differ from hippocampal pyramidal cells in many ways, including their characteristics of development, regeneration, morphology and synaptic plasticity. It would be interesting to find out if the role of these neurons at the systems level were somehow linked to unusual features of their subcellular signaling.

What are the advantages of this pathway? Use of the CaM/CaMKIV pathway to CREB lends itself well not just to speed but also to implementing repetitive spaced stimulus responsiveness. Brief mild stimulation of hippocampal neurons that selectively activates the calmodulin/CaMKIV pathway to CREB [13], also creates a primed state in which subsequent similar stimuli are more potent and even faster in their effects on nuclear CREB [20]. The increased speed of CREB formation suggests that the priming effect might be attributable to the nuclear use of persistently translocated CaM. After nuclear translocation, return of CaM to the cytoplasm proceeds gradually over about 1 hour [15]. This extended time course fits reasonably well with behavioral data on spaced stimuli [51,52*].

As noted earlier, CREB responds to multiple converging signaling pathways in hippocampal neurons, including a slower but more prolonged MAPK-mediated activation pathway that overlaps with the calmodulin/CaMKIV pathway [3–5,13], and a calcineurin/protein phosphatase-1-mediated CREB deactivation pathway [31,59]. Flexible association of aversive stimuli with a wide range of environmental situations of different importance and temporal structure may require such complexity of signaling. The MAPK pathway to CREB has poorer temporal resolution than the CAMK pathway (slower in onset, and more persistent), but it has several interesting properties, including further prolongation by spaced repeated stimulation [23] or PKA activation [23,32,60], and selective responsiveness to stronger stimulation [13]. Indeed, activation of the MAPK pathway can be involved in forming both amygdalar [61] and hippocampal [62] fear memories. Similarly, the calcineurin/PP1 pathway responds to repeated stimuli by potentiating CREB function — in this case through activity-dependent inactivation of calcineurin [31,63]. Notably, direct calcineurin inhibition potentiates memory formation in the amygdala [64*] and the hippocampus [65*].

Taken together, these data collected in the amygdala and the hippocampus reveal a clear pattern — stimuli of greater behavioral significance are endowed with a more potent means of activating CREB. In this manner, the nuclear computer capitalizes on the diverse characteristics of its many incoming signaling pathways, including sensitivity and temporal resolution, to calculate the statistical significance of candidate associations for storage in long-term memory.

**Control of neuronal survival and death**

Neurons receive specific instructions to survive or to die, the correct choice being crucial for development and mature functioning of the brain. The decision to die is particularly interesting, as neurons reach this all-or-none choice after weighing a wide array of external inputs, including synaptic events. This is reminiscent of faster electrophysiological choices made between quiescence and firing an action potential. The nucleus plays a key role in the survival/death computation, largely through activity-dependent regulation of transcription factors such as CREB, MEF2 and NF-kB. An emerging theme is the highly conditional influence of these transcription factors, in that they promote survival in some circumstances but not in others.

It has been known for years that stimulation of electrical activity at mild to rapid rates (for example, 5Hz to 50Hz electrical stimuli) works through both NMDA receptors and L-type Ca\(^{2+}\) channels to activate nuclear CREB [14,15]. Accordingly, activation of CREB would not be expected to promote cell death in the normal brain. Indeed, CREB generally favors neuronal survival. Postnatal
genetic inactivation of CREB (along with cAMP response element modulator [CREM]) causes widespread apoptosis of CNS neurons [66]. Other studies have shown that a low level of activation of NMDA receptors or L-type Ca^{2+} channels promotes survival of cerebellar granule neurons, presumably through the activity of CaMKIV and CREB [67]. CREB also mediates the survival-promoting effects of brain-derived neurotrophic factor (BDNF) [68,69] and other trophic factors.

On the other hand, excitatory stimuli that are known to be highly toxic to mature neurons, such as steady prolonged application of glutamate, promote general cellular dysfunction and death [70–72]. Furthermore, it has been suggested that under conditions of very strong and long excitation, CREB can actually promote apoptosis by acting through a nuclear p53 cascade [73]. Perhaps this action of CREB is aimed at eliminating neurons whose function is deranged by excessive stimulation, thus maintaining the integrity of CNS function. This may be reminiscent of the other effects of p53, ‘guardian of the genome’, which prevents mitosis and potential tumorigenesis in the setting of DNA damage.

Transcription factors from the MEF2 family [74], especially MEF2A, -C and -D, also appear to promote survival of cerebellar granule cells and cerebral cortical neurons in response to NMDA application or mild depolarization [75–77]. MEF2 function can be elevated directly by calcineurin and indirectly by CaMKIV [74], once again underscoring the importance of CaM signaling to the nucleus [15,31]. Additional activity-dependent signals provided by p38 MAP kinases and NFAT converge to promote MEF2 function [74]. However, a pro-apoptotic role for modified MEF2 proteins has also been identified [75,77]. Cleavage of these transcription factors follows activation of caspases, generating MEF2 fragments capable of DNA binding but lacking transactivation potential. These dominant-negative factors then strongly promote apoptosis. Thus, as in the situation with CREB, the influence of the transcription factor in favoring or disfavoring survival depends on the cellular context.

The NF-κB family of transcription factors is strongly expressed in the CNS, especially in the hippocampus and the cerebellum. They are activated by growth factors and cytokines as well as by depolarization or glutamate application, which leads to nuclear translocation and in general, promotion of neuronal survival [78]. However, several studies have identified a pro-apoptotic role for NF-κB. Though considerable controversy remains, the net effect of NF-κB appears to depend on the type of stimulation, the degree of NF-κB activation, and the particular NF-κB subunits that are expressed. Interestingly, beyond simple nuclear translocation [6] of NF-κB [79] (Figure 1), the p65 subunit of NF-κB can be further activated by phosphorylation at Ser-276, a residue whose amino acid sequence environment is nearly identical to that of CREB Ser-133. It has been proposed [79] that the site can support modulation of NF-κB function by CaMKIV or Rsk kinases, leading to disparate effects on survival or death that depend on the profile of kinase activation. Here again, as with CREB and MEF2, we see an emerging role of nuclear computation in the integration of rich streams of incoming information to reach a crucial decision between cell survival and cell death.

Concluding remarks and future prospects
We have deliberately focused on mechanisms that convey an initial message from the neuronal surface to a nuclear transcription factor, but in which the final outcome in gene expression also depends on multiple additional signaling events, as illustrated below for the cases of CREB- and NFAT-dependent transcription. In the case of CREB, attainment of the Ser-133 activated state is further modulated by additional activity-dependent events, including calcineurin-regulated dephosphorylation of Ser-133 as well as the phosphorylation of Ser-142 discussed above. Stabilization of the CREB transcriptional complex is likely to be further affected by activity-dependent regulation of co-activators, including the CREB binding protein (CBP) and possibly the closely related p300 [43]. With respect to activation of NFAT-dependent transcription, required co-activators include activator protein-1 (AP1) (composed partly by the CREB-regulated factor c-fos) and MEF2 [80], which is CaM-regulated in its own right as noted above. Neuronal activity not only triggers nuclear translocation of NFATc4 but it additionally regulates NFATc4-dependent transcription by delaying nuclear export of NFAT through L-type channel mediated inhibition of glycogen synthase kinase 3β [8]. The result is prolonged NFATc4 activation and increased NFAT-dependent gene expression [8]; this is analogous to CRE-mediated gene expression when CREB activity is extended by activity-dependent inhibition of CREB dephosphorylation [31,63]. Judging by these examples of signaling complexity, we have only seen the tip of the iceberg with respect to the convergence or apposition of multiple mechanisms.

Intricate networks of downstream genes represent the output of nuclear processing. A large number of genes show activity modulation in neurons, and many of these have plausible behavioral roles. Within hippocampal neurons, activation of NFATc4 leads to an upregulation of inositol 1,4,5-triphosphate receptor type 1 (IP3R1) [8] and BDNF [81]; both proteins are known to influence learning and memory [82–84]. CREB likewise drives BDNF expression [69] as well as c-fos and other factors implicated in neuronal survival and memory formation. In circadian rhythm generation, the CREB-regulated mPer genes are well-characterized targets of retinal activity and have clear behavioral roles. But exactly how the wide array of activity-regulated genes generate meaningful
changes in the brain’s neural networks is still not understood. Though many aspects of nuclear computation remain shrouded in mystery, it is clear that a combination of in vitro analysis and behavioral investigation can begin to illuminate the rationale for the unique biochemical complexity of neurons, and the elegant logic behind the design of nuclear signaling pathways.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

10. Osaka et al. [11] and Cheng et al. [12] characterized key aspects of DREAM [10], a nuclear and cytoplasmic protein that can bind Ca\(^{2+}\) ions (Kd ~ 14 μM). Though nuclear Ca\(^{2+}\) levels probably do not reach high micromolar concentrations, one of the functions of DREAM is to act as a transcription factor, and it has also been hypothesized to act as a direct target of nuclear Ca\(^{2+}\) ions. Early demonstrations of the other two main mechanism paradigms in Ca\(^{2+}\)--dependent nuclear signaling in neurons were obtained for translocation of the transcription factor itself [8] and translocation of an intermediate messenger [15].
Signaling from synapse to nucleus

Deisseroth, Meremelstein, Xia and Tsien

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This paper provided the first direct causal evidence linking CREB modulation to the important behavioral process of circadian rhythm entrainment. A crucial aspect of this link may lie in channel biophysics. Luckman et al. [36], Meremelstein et al. [39] and Deisseroth et al. [14] demonstrated that in hypothalamic and hippocampal neurons, nuclear signaling can filter out action potentials and respond selectively to synaptic potentials. A biophysical explanation was provided [39] linked to the activation gating kinetics of L-type calcium channels, a property that may be crucial in the behavioral roles of CREB (Figure 2).


50. Kida S, Josselyn SA, de Ortiz SP, Kogan JH, Chevere I, Masushige S, Silva AJ: CREB required for the stability of new and reactivated fear memories. Nat Neurosci 2002, 5:348-355. This study, along with that of Kogan et al. and Josselyn et al. [51,52*], demonstrates the importance of CREB in fear memory formation in the amygdala and the hippocampus, as well as the use of spaced training protocols to overcome insufficient CREB activation and lead to robust memory formation in both structures.


60. Martin KC, Michael D, Rose JC, Barad M, Casadio A, Zhu H, Kandel ER: MAP kinase translocates into the nucleus of the...
Signalling mechanisms


64. Lin CH, Lee CC, Gean PW: Involvement of a calcineurin cascade in amygdala depotentiation and quenching of fear memory. Mol Pharmacol 2003, 63:44-52.


