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Regulation of neurogenesis by calcium signaling

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Abstract

Calcium (Ca^{2+}) signaling has essential roles in the development of the nervous system from neural induction to the proliferation, migration, and differentiation of neural cells. Ca^{2+} signaling pathways are shaped by interactions among metabotropic signaling cascades, intracellular Ca^{2+} stores, ion channels, and a multitude of downstream effector proteins that activate specific genetic programs. The temporal and spatial dynamics of Ca^{2+} signals are widely presumed to control the highly diverse yet specific genetic programs that establish the complex structures of the adult nervous system. Progress in the last two decades has led to significant advances in our understanding of the functional architecture of Ca^{2+} signaling networks involved in neurogenesis. In this review, we assess the literature on the molecular and functional organization of Ca^{2+} signaling networks in the developing nervous system and its impact on neural induction, gene expression, proliferation, migration, and differentiation. Particular emphasis is placed on the growing evidence for the involvement of store-operated Ca^{2+} release-activated Ca^{2+} (CRAC) channels in these processes.

Keywords

Calcium; Stem cells; Neural development; STIM1; Orai1; CRAC channel

1. Introduction

The development of the nervous system occurs through a series of carefully choreographed steps in which neural stem/progenitor cells (NSCs) proliferate, migrate considerable distances from the germinal centers to their destinations, and ultimately differentiate into billions of neurons and glia that populate the brain. As these events unfold, rhythmic bursts of Ca^{2+} signals in the developing cells direct specific cellular Ca^{2+} responses to influence each step of this process. Cellular Ca^{2+} signals regulate nearly every aspect of neural development including neural induction [1], proliferation [2,3], migration [4], and differentiation [5]. A multiplicity of Ca^{2+} signaling proteins expressed in the developing brain create diverse Ca^{2+} signals to accommodate these processes [6]. The prototypical components of this toolkit include G-protein-coupled (GPCRs) and tyrosine kinase-linked receptors (RTKs) that sense extracellular cues, and numerous Ca^{2+} -permeable channels that flux Ca^{2+} into the cell through the plasma membrane or that release Ca^{2+} from intracellular

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stores. Downstream of Ca^{2+} entry are Ca^{2+} -binding effector proteins such as calmodulin, and Ca^{2+} -sensitive transcription factors, enzymes, and ion channels which mediate effector functions. Finally, Ca^{2+} pumps and exchangers refill Ca^{2+} stores or extrude Ca^{2+} from the cell to maintain the resting intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$). The temporal and spatial characteristics of the Ca^{2+} signal created by the concerted actions of all these Ca^{2+} signaling components encode specific messages that control the types of cellular programs that are activated [7,8]. This review summarizes our current understanding of the major pathways of Ca^{2+} influx that orchestrate the key effector functions of neural development (Fig. 1). A particular focus of this review is on the organization and functional role of store-operated CRAC channels in these development processes.

2. The molecular and functional organization of Ca^{2+} signaling

2.1. Spatial organization of Ca^{2+} signaling

At rest, the cytosolic concentration ($[\text{Ca}^{2+}]_i$) in eukaryotic cells is maintained at very low levels (50–100 nM). The opening of plasma membrane or organelle Ca^{2+} channels results in a sudden rise in $[\text{Ca}^{2+}]_i$ which alters the conformation of numerous nearby proteins and enzymes to initiate biological responses [6]. The wide range of cellular functions regulated by cellular Ca^{2+} signals begets the question of how selectivity is encoded by such a promiscuous messenger. One solution to this problem, it appears, is surprisingly simple: the spatial profile of the Ca^{2+} signal delivers a targeted message to control the type of downstream response. Local Ca^{2+} signals, or Ca^{2+} microdomains, develop rapidly near open Ca^{2+} channels, creating spatial Ca^{2+} gradients of high $[\text{Ca}^{2+}]_i$ near the open pore which can reach tens of micromolar [9,10]. The subsequent diffusion of Ca^{2+} away from the source is limited by cytoplasmic buffers and membrane pumps such that $[\text{Ca}^{2+}]_i$ declines steeply with distance [9,11]. Hence, localization of Ca^{2+} signaling complexes and downstream effectors in close proximity to the Ca^{2+} channels provide a means for rapid and specific activation of Ca^{2+} -dependent responses. These concepts are supported not only by modeling studies [9,10], but also the differential effects of fast and slow Ca^{2+} chelators such as BAPTA and EGTA on the activation of Ca^{2+} -dependent cellular responses. Disruption of a Ca^{2+} -sensitive process by millimolar concentrations of BAPTA but not EGTA indicates that the process is located within tens of nanometers from the pore of a nearby Ca^{2+} channel [12,13]. Thus, for many Ca^{2+} -mediated functions, the physiological response is determined by the spatial relationship between Ca^{2+} channels and their Ca^{2+} -sensitive effectors.

For example, at many central synapses, the rapid release of neurotransmitters from presynaptic terminals is due to highly localized Ca^{2+} elevations established by tight molecular coupling of a voltage-gated Ca^{2+} channel to a Ca^{2+} sensor at the transmitter release site [12,14]. This specialized coupling is organized by a protein scaffold that physically tethers Ca^{2+} channel to synaptic vesicle [15]. Since the rate of vesicle exocytosis is steeply dependent on $[\text{Ca}^{2+}]$ [16], the compartmentalization of Ca^{2+} into local signaling domains has important consequences for regulating neurotransmitter release and synaptic strength [17,18]. Another type of specialized coupling arises from the spatial summation of Ca^{2+} entering from a cluster of multiple channels. In vestibular hair cells, adrenal chromaffin cells, and at the neuromuscular junction, voltage-gated Ca^{2+} channel microdomains are

functionally associated with voltage- and Ca^{2+} -activated K^+ (BK) channels [19–21]. The two classes of ion channels are physically assembled into macromolecular channel–channel complexes that underlie their functional relationship [13,21]. As with synaptic vesicles, the close interaction between Ca^{2+} channels and BK channels ensure rapid and robust effector activation.

In an interesting extension of local coupling, Ca^{2+} sensors can detect local signals and relay the signal to targets located outside the microdomain. Of particular importance of this type of coupling to neural development is the control of gene expression by localized Ca^{2+} signals through L-type Ca^{2+} and store-operated CRAC channels [22,23]. In these examples, a Ca^{2+} signal at the mouth of the Ca^{2+} channel activates a Ca^{2+} sensor (e.g., calmodulin), which in turn activates distal signaling pathways (MAP kinase or calcineurin/NFAT) resulting in gene expression in the nucleus [22,23] (as discussed further below). The mechanisms underlying the operation of these diverse localized Ca^{2+} -driven cellular responses are likely to be pervasive throughout nervous system development and in the mature nervous system.

2.2. Store-operated calcium entry

Of the many mechanisms of Ca^{2+} entry found in animal cells, one of the most widespread is store-operated calcium entry (SOCE) [24]. Store-operated channels (SOCs) are so named because they are activated by the depletion of Ca^{2+} from the endoplasmic reticulum (ER) [25]. They deliver Ca^{2+} to refill ER stores as well as evoke sustained Ca^{2+} signals that drive a wide range of effector functions including gene expression, secretion, and motility [24]. Physiologically, SOC activation is triggered through the stimulation of GPCRs or RTKs which act through the phospholipase C (PLC)-inositol 1,4,5-triphosphate (IP_3) signal transduction pathway to stimulate the release of Ca^{2+} from ER stores via IP_3R Ca^{2+} release channels. The Ca^{2+} release-activated Ca^{2+} (CRAC) channel, encoded by the Orai pore proteins (Orai1–3) and activated by the ER Ca^{2+} sensors STIM1 and STIM2, is the most extensively characterized store-operated channel [24,26–28].

The complex choreography of SOCE activation and the calcium current (I_{CRAC}) underlying SOCE is well characterized in many cell types [29]. Upon store-depletion, the luminal EF-hand domain of STIM1 senses the loss of bound Ca^{2+} , triggering a conformation change in STIM1 that leads to the oligomerization and translocation of STIM1 to plasma membrane (PM)-ER junctions where it forms distinct puncta [30–32] (Fig. 1). Orai1 also redistributes within the PM to accumulate at sites opposite to STIM1 [33]. This juxtaposition is mediated by direct interactions between STIM1 and Orai1, leading to Orai1 channel activation [34,35]. The ensuing Ca^{2+} entry into the cytosol is taken up into the ER by the sarco-endoplasmic Ca^{2+} -ATPase (SERCA) pump. Refilling of ER stores is again sensed by STIM1, resulting in dissociation of the Orai1-STIM1 complex and deactivation of the Ca^{2+} channel [36]. Each time an agonist stimulates ER Ca^{2+} release, a fraction of the released Ca^{2+} is transported out of the cell by plasma membrane Ca^{2+} ATPase pumps (PMCA) and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Therefore, SOCE plays a homeostatic role in ensuring adequate repletion of the ER Ca^{2+} stores. CRAC channels also play an active role in inducing both

short-term (i.e., maintenance of cytoplasmic Ca^{2+} oscillations, secretion) as well as long-term (i.e., gene expression) signaling pathways [37].

CRAC channels are endowed with many distinguishing biophysical and regulatory features that make them uniquely suited to generate these Ca^{2+} signals. These features include high Ca^{2+} selectivity and low unitary conductance [24,38]. CRAC channels are not voltage-dependent and can therefore conduct Ca^{2+} at negative membrane potentials, when voltage-sensitive channels such as voltage-gated Ca^{2+} (Ca_v) channels or NMDAR channels are inactive. Their small unitary conductance coupled with their extremely high Ca^{2+} selectivity and tight localization at PM-ER junctions enables these channels to form a highly efficient and selective Ca^{2+} influx mechanism, uniquely poised to create Ca^{2+} microdomains that activate specific Ca^{2+} response pathways. Furthermore, the kinetics of CRAC channel activation and deactivation occurring on slow time scales of tens of seconds to minutes are well suited for mediating oscillations and Ca^{2+} waves that are commonly required to drive key events in development such as gene transcription and cell proliferation. For example, CRAC channels are functionally coupled to the activation of NFAT and c-fos [2,22,39], leukotriene production [40], and PMCA [41].

When expressed heterologously together with STIM1 in HEK293 cells, the three Orai isoforms (Orai1-3) that make up the CRAC channel family produce store-operated currents with only modest differences in their inactivation properties and pharmacology [42,43]. Still, Orai1 remains the best-studied member of the class with numerous mouse models generated to study its role in SOCE in different tissues and a growing list of loss- and gain-of-function mutations linked to human disease [44]. By contrast, there are no genetic models for Orai2 or Orai3 nor have they been conclusively implicated in SOCE in any cell type to date. The three Orai proteins (Orai1-3) are broadly expressed in many tissues and organs including the brain [45–47] and in the murine nervous system, the expression of Orai2 and Orai3 is particularly noteworthy in the cortex, hippocampus, and cerebellum [42,45,48] (Allen Brain Atlas). Further, both in the brain and in other tissues, the expression of Orai1 overlaps with that of Orai2 and/or Orai3, raising the possibility of heteromeric channels composed of multiple Orai isoforms [49,50]. Heteromeric Orai3-Orai1 channels have been reported to show sensitivity to activation by arachidonic acid when expressed in HEK293 cells [51], raising the possibility that such channels may display activation behaviors distinct from canonical store-operated channels. Thus, an interesting possibility is that these differences in gating and regulation give rise to the functional diversity of Ca^{2+} signals noted in different cell types. These and other possibilities require further study using specific mouse models.

Historically, CRAC channels were first described in immune cells and other nonexcitable cells lacking the ability to fire action potentials [reviewed in ref. [24]]. However, they are now known to be present in virtually all cells, and a growing literature on SOCE implicates them in the regulation of numerous Ca^{2+} -mediated functions in the brain [52–56]. Given the neuroectodermal lineage and nonexcitable nature of neural stem/progenitors cells, CRAC channels may have a particularly important role for neurodevelopmental processes including neurogenesis, proliferation, and migration (as discussed further below).

2.3. Spontaneous Ca^{2+} transients and oscillations

Oscillatory Ca^{2+} signals generated by periodic discharges of Ca^{2+} release and Ca^{2+} influx are a widespread presentation of cellular Ca^{2+} signaling through all stages of neural development and are implicated in driving numerous genetic programs [3,8] including proliferation, migration, and differentiation [5,57–60]. For example, in the embryonic mouse neural crest, spontaneous Ca^{2+} transients establish a neuronal preference for undifferentiated neural crest cells [61]. In the embryonic rat neocortical ventricular zone (VZ), patterns of spontaneous $[\text{Ca}^{2+}]_i$ changes in cortical precursor cells influence neurogenesis and proliferation [62]. Likewise, in *Xenopus* and zebrafish spinal neurons, spontaneous Ca^{2+} transients are present at early stages and regulate axonal growth and differentiation [7,63,64] as well as neurotransmitter phenotype of developing neurons [65]. The downstream effects of these Ca^{2+} signals are thought to be mainly achieved through regulation of activity of transcription factors and intermediate signaling proteins.

Experimental and computational models indicate that the periodic discharges of Ca^{2+} arise from coordinated interplay of Ca^{2+} influx and release from intracellular Ca^{2+} stores [66,67]. Oscillations generally dissipate in the absence of Ca^{2+} entry [68–70], indicating a requirement of Ca^{2+} influx for the maintenance of these Ca^{2+} signals. Among the various molecules implicated in the Ca^{2+} influx necessary to sustain oscillations are voltage-gated Ca^{2+} channels and neurotransmitter-gated receptors such as GABA and glutamate. At earliest stages, before synapses have formed in the developing brain, glutamate and GABA signal through NMDA and GABA_A receptors in a paracrine, nonsynaptic mode of intercellular communication [71,72] to elevate $[\text{Ca}^{2+}]_i$. GABA activates depolarizing chloride currents which can open voltage-gated Ca^{2+} channels, while ligand-gated glutamate receptors generate Na^+ and Ca^{2+} influx [73,74]. TRP channels [75], metabotropic transmitter receptors [59,76], and mechanoreceptors [77] also generate spontaneous Ca^{2+} transients that direct neural development. Few studies have as yet examined the potential role of Orai channels for Ca^{2+} oscillations in neural development. However, a study in mouse neural progenitors found that elimination of Orai1 activity through knockin of a loss of function Orai1 mutation (R93W) abrogates Ca^{2+} oscillations seen in heterozygous WT/R93W Orai1 mice [2], suggesting that CRAC channels may be critically important for generating Ca^{2+} oscillations in the developing nervous system.

It is generally believed that oscillations offer an advantage over sustained Ca^{2+} signals in driving many types of effector responses due to the high signal-to-noise discrimination by the effector proteins for digital signals [78–80]. High Ca^{2+} thresholds of the effector responses coupled with slow activation kinetics allow for discrimination of true Ca^{2+} inputs from stray events. In this manner, the signature of the oscillatory signal (frequency, duration, amplitude) also offers a way to discriminate between particular end points that co-exist in the same cell [7,61,78,81]. One of the best-described examples of this type of discrimination is the dependence of the efficacy and specificity of gene expression of the NFAT, NFkB, and Oct1-OAP transcriptional pathways on oscillations of specific frequency and amplitude [78].

3. Physiological roles of Ca^{2+} signaling in neural development

As described in the preceding sections, numerous ion transport pathways are implicated in regulating neurogenesis, from proliferation and migration of neural progenitor cells to differentiation. Below we describe the functional role of specific Ca^{2+} pathways that have been implicated in these processes, with an emphasis on ion channels mediating Ca^{2+} influx. Table 1 provides a list of the Ca^{2+} proteins and ion transports mechanisms that are discussed in the following sections.

3.1. Early neurogenesis

The formation of the vertebrate nervous system is initiated during gastrulation in a process called neural induction, when the cells of the embryonic ectoderm choose neural fate over epidermal fate and give rise to neural progenitors. This decision of cell fate involves inductive signals from a specialized cluster of cells in the dorsal mesoderm called the ‘neural organizing center,’ which triggers neural development in the dorsal ectoderm [82]. A critical signaling mechanism involved in the development of neuroectoderm from the ectoderm is the inhibition of the potent epidermal-inducer, BMP, by factors secreted from the dorsal mesoderm—noggin, chordin, and follistatin [82]. There is increasing evidence that Ca^{2+} signaling has an instructive role in the initiation of the neuroectoderm-specific gene expression programs [83]. In amphibians for example, an increase in $[\text{Ca}^{2+}]_i$ is both necessary and sufficient to commit the ectoderm to a neural fate during embryo development [84]. In *Xenopus laevis* embryos, direct visualization of Ca^{2+} dynamics revealed localized domains of Ca^{2+} transients restricted to the dorsal ectoderm cells, just prior to the onset of neural induction in these cells [85]. In contrast, $[\text{Ca}^{2+}]_i$ elevations are not observed in the ventral ectoderm or mesoderm during this time. Studies in amphibians have found that L-type (or dihydropyridine-sensitive) Ca^{2+} channels in the ectoderm are required for the acquisition of neural fate [85–87]. Moreover, the temporal pattern of channel expression correlates with neural competence; the ability of the ectoderm cells to differentiate toward neural tissue is optimal when the L-type Ca^{2+} channel density is highest [86]. Further, the neural inducer noggin has been shown to activate Ca^{2+} influx through L-type Ca^{2+} channels to induce transcription of the immediate early gene (IEG) *c-fos* [88]. The protein encoded by *c-fos* interacts with the product of another IEG, Jun, to form a heterodimeric transcription factor and has been implicated in the control of cell cycle entry, cell proliferation, and neural differentiation [88–90], suggesting that Ca^{2+} signals promote neural fate by modulating signal transduction pathways that control gene expression. Indeed, Ca^{2+} transients also regulate the expression of early proneural genes such as *Zic3* [85,91]. In amphibian embryos and animal caps, the Ca^{2+} -regulated arginine *N*-methyltransferase *xPRMT1b* has been implicated as a direct link between $[\text{Ca}^{2+}]_i$ increase and the induction of neural fate [92]. The overexpression of *xPRMT1b* induces the expression of *Zic3* and the neural differentiation marker *N-tubulin*. Expression of these genes likely involves Ca^{2+} -mediated gene transcription as discussed further below.

In mammals, an increase in $[\text{Ca}^{2+}]_i$ also appears to be the key signal that controls neural fate determination [93]. However, while the main source of Ca^{2+} increase in amphibian ectoderm is influx through voltage-operated Ca^{2+} channels, mammalian embryonic stem cells do not

express VOCCs and therefore must employ alternate pathways [94,95]. Indeed, Yanagida et al., observed store-operated Ca^{2+} currents in mouse embryonic stem cells [94] which was attributed to TRPC channels based on the detection of TRPC1 and TRPC2 mRNA. However, at the time of that study, the molecular identity of the SOC had not yet been determined and the potential role of the CRAC channel family in mediating these store-operated currents was not evaluated. More recently, a screen to identify neural induction genes in mammals identified neuronatin (Nnat), an ER membrane protein that physically antagonizes SERCA2 activity to regulate the intracellular Ca^{2+} level [93]. Knocking down Nnat inhibits neural induction in ESCs by reducing the level of intracellular Ca^{2+} . Together, these results suggest that a key source of Ca^{2+} for neural induction involves intracellular stores, and potentially, SOCE. Recently, the CRAC channel family was shown to be an important Ca^{2+} entry mechanism for gene expression in embryonic and adult neural stem/progenitor cells [2], but its potential role in regulating the induction of early neural genes remains to be explored.

After neural induction, the neuroectoderm forms the neural plate, which consists of neuroepithelial/neural stem cells that are the precursors to neural tissue. These neuroepithelial cells are successively replaced by radial glial cells, the main progenitor cell type during development of the embryonic and postnatal CNS. The progenitor cell bodies are contained in the ventricular zone (VZ) and through cycles of self-renewal and differentiation will give rise to most of the neurons as well as glial cells in the brain. Recently, Papanayotou et al. described a novel L-type Ca^{2+} channel modulator called calfacilitin that is required for neural plate development in the chick embryo [96]. Calfacilitin binds to L-type $\text{Ca}_v1.2$ channels to slow inactivation thereby facilitating Ca^{2+} influx, which leads to increased expression of neural plate specifier genes *Geminin* and *Sox2*.

3.2. Gene expression

A major mechanism controlling neurogenesis is the regulated expression of specific genes driven by extracellular cues. Ca^{2+} signals mediate a key role in this process by activating signaling pathways that converge on transcription factors which then initiate the expression of a large number of proteins critical for proliferation, migration, and differentiation [97]. Ca^{2+} -dependent activation of transcription factors can occur either directly through Ca^{2+} interaction with transcription factors that are pre-bound to their targets, or indirectly through Ca^{2+} -sensitive proteins that affect transcription factor activity. A well-known example of the former is Ca^{2+} regulation of the transcription factor, Downstream Regulatory Element (DRE) Antagonist Modulator (DREAM) [98]. DREAM is a Ca^{2+} sensor with four EF-hand Ca^{2+} -binding domains and it acts as a transcriptional repressor by binding DNA at DRE sequences. Ca^{2+} stimulation abolishes DREAM's ability to bind DRE and repress transcription. DREAM is highly expressed in the brain and regulates transcription of the early immediate gene *c-fos* [98] as well as expression of voltage-gated Ca^{2+} channels [99].

Among the indirect pathways, a well-characterized example is Ca^{2+} regulation of the NFAT family of transcription factors. NFAT activation is indirectly coupled to Ca^{2+} mobilization: Ca^{2+} elevations activate the phosphatase calcineurin, which dephosphorylates NFAT, causing NFAT to translocate to the nucleus where, working in conjunction with the fos/jun complex, it controls a wide variety of genes in diverse cell types [100]. First shown to be important in

the immune system, NFAT is now known to be widely expressed in the nervous system, although its role in neuronal development is only recently beginning to be understood [101]. At the molecular level, NFAT-directed gene transcription regulates the expression of several key proteins critical for activity responses including IP₃Rs, GluRs, and pro-nociceptive genes in neurons [102–104]. Calcineurin-NFAT signaling is critical for neurotrophin-mediated axonal growth and guidance during vertebrate development [105,106] as well as presynaptic development [107], dendritogenesis [108], and neuronal survival [107–110].

Several sources of Ca²⁺ entry have been shown to regulate NFAT activity. In cortical and hippocampal neurons, for example, neurotrophins binding to Trk receptors activate PLC-IP₃ signaling, causing an increase in intracellular Ca²⁺ levels that activate NFAT transcription via calcineurin [103,105]. Ca²⁺ influx through L-type voltage-gated Ca²⁺ channels has also been shown to activate NFAT, which is especially critical for excitation-dependent gene transcription underlying functions such as synaptic plasticity [111]. Further, influx of Ca²⁺ through NMDA receptor regulates neuronal survival in the cortex through an NFAT-dependent survival pathway [110]. In neural stem/progenitor cells, CRAC channels have a prominent role in activating NFAT-dependent gene expression. Ca²⁺ signaling through CRAC channels stimulates the nuclear translocation of NFAT and NFAT-dependent gene transcription, with murine knockouts of Orai1 and/or STIM1 showing impaired NFAT-dependent gene transcription [2]. Interestingly, the differential effects of BAPTA and EGTA indicate that NFAT activation is stimulated by local Ca²⁺ signals around CRAC channels, suggesting that calcineurin may be physically associated with CRAC channels [2]. This finding is similar to the spatial coupling observed between CRAC channels and NFAT activation in HEK cells and between L-type Ca²⁺ channels and NFAT activation in hippocampal neurons [39,112]. The molecular basis for the tight coupling between calcineurin and the Ca²⁺ channel appears to involve the anchoring protein AKAP79, which targets calcineurin to calmodulin-bound Ca²⁺ channels, so that local Ca²⁺ entering at the plasma membrane can effectively and selectively activate calcineurin/NFAT signaling to the nucleus [112,113].

cAMP response element binding (CREB) protein is another important transcription factor whose transcriptional activity is regulated by calcium-activated signaling pathways and which mediates responses to extracellular cues and local activity in the developing brain [114]. Multiple signaling pathways converge onto CREB including cAMP dependent protein kinase A and Ca²⁺/calmodulin (CaM)-dependent protein kinases (CaMKs), which activate CREB activity by phosphorylation at three critical serine residues (Ser-133, Ser-142 and Ser-143). Ser-133 has been examined extensively and the prevailing evidence shows that this residue becomes phosphorylated by a wide range of stimuli, both Ca²⁺-dependent and independent [115]. However, Ser-142 and Ser-143 are phosphorylated strictly in a Ca²⁺-dependent manner [116]. CREB regulates a wide range of biological processes including cell proliferation, differentiation, and cell survival [115,117]. For example, in the rat embryonic subventricular zone, the action of the second messenger cAMP downstream of GPCR signaling supports the differentiation of neural progenitor cells via up-regulation of L-type voltage-gated Ca²⁺ channels. Moreover, cAMP signaling in conjunction with Ca²⁺ entry through VOCCs promotes the morphological and functional maturation of NSCs, an effect dependent on CREB activation [118]. Activated CREB is largely absent in stem cells,

and transiently upregulated in NSCs and young neurons during the first few weeks of differentiation [119]. Loss of CREB signaling results in cell death and loss of neuronal gene expression [120]. These wide-ranging roles of CREB for circuit development are likely mediated by several cofactors that interact with phosphorylated form of CREB, and which in many cases are themselves regulated by Ca^{2+} (e.g., the CREB binding protein, CBP) [115].

A hallmark of neurogenesis is the expression of the basic Helix-Loop-Helix (bHLH) proneural genes which are critical for cell fate determination and differentiation of progenitor cells. NeuroD is a bHLH transcription factor that plays an important role in the survival and differentiation of many neuronal tissues including the hippocampal dentate gyrus neurons, cerebellar granule neurons, and developing inner ear neurons. Studies have suggested that NeuroD acts as a Ca^{2+} -regulated transcription factor [121,122]. In cerebellar granule neurons, neuronal activity stimulates Ca^{2+} influx through L-type voltage-gated calcium channels to induce CaMKII-mediated phosphorylation of NeuroD and thereby stimulate dendritic growth [121]. In adult neural progenitor cells, excitation is coupled to neurogenesis through L-type Ca^{2+} channel activation, which increases expression of NeuroD and inhibits expression of glial fate genes [122]. Thus, in both embryonic and adult neural progenitor cells, Ca^{2+} signaling regulates specific transcriptional networks to drive specific steps of neurogenesis.

3.3. Proliferation

Calcium signaling regulates many fundamental steps of the cell cycle, including the transitions between the various phases of the cell cycle, the transcription of immediate early genes, and the regulating events that control quiescence and cell division [123–125]. In the brain, the niche of proliferating cells comprise primarily three cell types: type B cells (most stem-like), type C cells (transient amplifying cells), and type A cells (migrating neuroblasts) (Fig. 2). Type B cells are slowly dividing and give rise to the rapidly proliferating type C cells (TAPs) through asymmetric division. Type C cells then go on differentiate into type A cells which acquire a migratory phenotype that is then associated with a decline in proliferation [126]. Proliferation of these progenitors is regulated by a variety of extrinsic factors that include the growth factors EGF and FGF [127–129] and neurotransmitters that evoke Ca^{2+} elevations [59,73,130,131], likely through specific ion transport pathways in each cell type. Some studies have implicated voltage-gated Ca^{2+} channels as an important route of Ca^{2+} entry in late stage neural precursors [132]. However, NSCs are derived from non-excitable epithelial cell lineage and are thus more akin to non-neuronal cell types in terms of Ca^{2+} influx [133], raising the possibility that CRAC channels could function as a major Ca^{2+} regulatory mechanism to regulate NSC proliferation.

Indeed, consistent with this possibility, we have found that CRAC channels encoded by Orai1 and STIM1 comprise the major mechanism for the sustained and oscillatory Ca^{2+} signals activated by EGF and the neurotransmitter, ACh, in mouse subventricular zone NSCs [2]. STIM1 and Orai1-mediated SOCE is seen in NSCs at every age (embryonic, neonatal, and adult NSCs), indicating that this Ca^{2+} entry mechanism is conserved throughout development and is preserved well into adulthood. Knockin of a non-functional Orai1 mutant (R93W Orai1) or conditional knockouts of Orai1 in the brain severely impaired NSC

proliferation both *in vitro* and *in vivo* [2], indicating that CRAC channels have a major influence on the proliferation of subventricular NSCs. Although not formally proven, these effects are likely mediated by calcineurin/NFAT-mediated transcription of cell cycle proteins as inhibiting calcineurin/NFAT signaling also evoked impairment of proliferation comparable to that seen with loss of CRAC channel function [2]. These findings establish CRAC channels as a novel mechanism for regulation of Ca^{2+} signaling, gene expression, and proliferation in NSCs. Because NFAT is upregulated in hypoxia-treated NSCs [134], these results raise the possibility that CRAC channel-mediated regulation of calcineurin/NFAT signaling may also be important for regulating the proliferative response seen following brain injury.

Signaling through cholinergic receptor pathways also plays a major role in neuronal cell proliferation [131]. Acetylcholine (ACh) is present in the brain prior to synaptogenesis and the onset of neurotransmission, suggesting that its action is mediated via non-classical neurotransmitter signaling. Both muscarinic (mAChRs) and nicotinic (nAChRs) acetylcholine receptors are expressed in neuronal progenitor cells [135–139] and their activation is known to increase proliferation and neurogenesis [138,140] likely through stimulation of SOCE [2]. AChRs induce Ca^{2+} influx, which can stimulate mitogen-activated protein kinase (MAPK) and ERK signaling pathways that govern proliferation and DNA synthesis [138,139,141–144]. Intracellular Ca^{2+} can modulate the MAPK cascade through two mechanisms—calcium-dependent tyrosine kinase (PYK2) or calmodulin—that converge upon the Ras pathway leading to MAPK activation [145]. An increase in intracellular Ca^{2+} evoked by thapsigargin is sufficient to induce ERK activation to levels similar to those induced by AChR agonists, underlying the importance of Ca^{2+} in this pathway [146].

In addition to CRAC channels and the aforementioned cholinergic pathways, other influx mechanisms including TRP channels are also implicated in Ca^{2+} influx and regulation of proliferation of neural progenitors [75]. Interestingly, voltage-gated Ca^{2+} channels do not seem to play a role until later stages of NSC development [127,132,147,148] (Fig. 2). These late-stage progenitors become sensitive to GABAergic transmission, which acts as a depolarizing stimulus—opposite to what is seen in mature neurons where GABA is inhibitory. GABAergic signaling appears to be paired with increased expression of voltage-gated Ca^{2+} channels and loss of either excitatory GABAergic transmission or voltage-gated Ca^{2+} channels lead to diminished proliferation [148,149], highlighting the importance of this transition for progenitor function. In addition, glutamatergic signaling involving both metabotropic glutamate receptors [150] as well as NMDA receptors is implicated in the proliferation of DCX positive late stage progenitors [122]. Both NMDA receptor blockade as well as inhibition of L-type Ca^{2+} channels impair proliferation of hippocampal progenitors [122], suggesting a growing importance of activity dependent pathways for nurturing and tuning the proliferative response.

3.4. Migration

A key step in neurodevelopment, one that is also seen following brain injury, is the directed migration of NSCs to widespread and distant areas of the brain. Precise migration of NSCs is regulated by a complex interplay of cell-autonomous programs and environmental factors

in which Ca^{2+} signaling has a critical role. Studies in immune cells have previously shown that Ca^{2+} signaling regulates the migration of hematopoietic cells, in this case functioning as a stop signal [151]. In the thymus, elevations in intracellular Ca^{2+} are sufficient to arrest the motility of migrating thymocytes, a feature that may enable developing T-cells to interact more effectively with antigen presenting cells [152]. The role of Ca^{2+} in NSCs is more complicated as it appears that Ca^{2+} signaling has an important influence both for initiating the transition of highly proliferating NSCs to a migratory phenotype as well as motility itself [4,60]. In the development of the cerebellum, for example, the appearance of N-type Ca^{2+} channels is associated with migration of granule cells [153]. Ca^{2+} influx through N-type Ca^{2+} channels is believed to induce specific Ca^{2+} fluctuations whose amplitude and frequency control the rate of cell migration [154]. However, in the neurogenic region of the subventricular zone, NSCs appear to require low levels of activity, possibly involving gap junctions, to maintain their migratory behavior [155,156]. As NSCs arrive at the sites where they are ultimately incorporated, they exhibit increased Ca^{2+} signaling which arrests their migratory phenotype [156]. Although more studies are needed to understand the basis of the various results, one possibility is that Ca^{2+} both facilitates and inhibits migration based on its amplitude and kinetic signature. Low to modest levels of Ca^{2+} signaling may be required to sustain migratory behavior, but high levels may function to stop motility [156].

Among various extrinsic factors, purinergic agonists [157,158], growth factors [159], and chemokines [160–162] are well-known modulators of NSC migration. For example, ATP regulates the migration of intermediate neuronal progenitors from the ventricular zone to the subventricular zone by acting through the purinergic P2Y1 receptor [157]. Progenitor cells in the ventricular zone are extensively coupled in clusters via gap junctions and can communicate with neighboring cells by releasing ATP through gap junctions/hemichannels [163]. Genetic knockdown of the P2Y1 receptor or blockade of ATP signaling by P2Y1 inhibitors reduces Ca^{2+} transients in intermediate progenitor cells and impairs their migration to the subventricular zone [157]. Likewise, numerous trophic and differentiation factors regulate neuronal migration. For example, one study has shown that neuregulin1 (NRG1), a member of the epidermal growth factor (EGF) family that binds to the tyrosine kinase ErbB4, evokes intracellular signaling that induce migration of neuronal progenitor cells [164]. NRG1/ErbB4 signaling is involved in tangential migration of olfactory bulb interneuron precursors in the rostral migratory stream [165] and in migration of cortical interneuron precursors from the ventral telencephalon [166]. Mechanistically, NRG1 stimulation of ErbB4 results in a sustained $[\text{Ca}^{2+}]$ increase in neural progenitors which involves store-operated calcium entry [159]. NRG1-induced migration is dependent on Ca^{2+} influx and is positively regulated by NMDAR activation [159]. It is known that local polarization of Ca^{2+} elevation can confer proper directionality [167] suggesting that extrinsic chemical gradients might be sensed using finely tuned local Ca^{2+} transients. The Ca^{2+} entry pathways stimulated by the aforementioned extrinsic factors have not yet been identified. However, their ability to stimulate GPCRs leading to release of Ca^{2+} from intracellular stores raises the possibility that CRAC channel mediated Ca^{2+} influx may be a major mechanism of the intracellular response.

3.5. Differentiation

In addition to proliferation and migration, there is extensive evidence for a powerful influence of Ca^{2+} signaling for various aspects of neuronal differentiation, including the appearance of ion channels that confer neuronal excitability, neurotransmitter specification, axonal pathfinding, and dendritic outgrowth [3,63,65,168–170]. Both spontaneous Ca^{2+} oscillations as well as activity driven Ca^{2+} influx through voltage-gated N- and L type channels are implicated in these differentiation events, which is typically accompanied by the appearance of specific neurotransmitters prior to synapse formation [65,168,171]. The initiation of differentiation in the newly emerging cells occurs through induction of transcription factors that promote the expression of ion channels and receptors favoring a neuronal phenotype [3,171]. For example, one study has shown that depolarization of neural progenitors by GABAergic excitation, which would be expected to evoke activation of voltage-gated Ca^{2+} channels, promotes the induction of NeuroD and neuronal differentiation while impairing proliferation [172]. A key hallmark of this process is the dependence on activity, with increases in intracellular Ca^{2+} enhancing the expression of ion channels, receptors, and neurotransmitters that shape the neuronal phenotype [3]. In this manner, the hard-wired stereotypic development of the early brain that is driven by cell autonomous genetic programs can be modified by activity-driven environmental cues to shape overall brain development.

4. Concluding remarks

The central role of Ca^{2+} signaling in directing many aspects of neurogenesis is now well accepted. This has occurred in large part through the development of new tools including genetically encoded Ca^{2+} indicators that have transformed our understanding of how Ca^{2+} signals are generated, compartmentalized, and dissipated. Several key molecular players mediating Ca^{2+} signaling including the molecules of SOCE and nature of the signals linking the opening of these channels to downstream effector responses have been identified. A large body of literature also exists about the properties of numerous Ca^{2+} signals at different stages of neuronal development and their roles for cell autonomous functions such as gene expression or cell proliferation. The largest area of uncertainty perhaps relates to how Ca^{2+} signaling networks regulate the development of specific neuronal circuits, and how aberrant function of particular Ca^{2+} signaling proteins affects the function and development of these circuits. The existence of so many routes of Ca^{2+} influx (STIM/Orai channels, Ca_v channels, TRP channels, and various ligand-gated channels) additionally begets the question of why so many pathways for Ca^{2+} influx are needed in the developing brain. The answer to this teleological question is likely multi-faceted. Multiple pathways are needed not only for conferring functional specificity, but also to bestow a safety net in case one pathway is compromised. Redundancy may explain why brain and T-cell development in mice lacking functional Orai1/STIM1 channels are seemingly normal despite the abrogation of SOCE. In addition, neural stem cells have been lauded for their potential to cure neurodegenerative diseases and ameliorate brain injuries. However, whether functions of particular Ca^{2+} signaling proteins can be controlled in ways to generate specific outcomes of therapeutic value still remains unclear. If the pace of progress in the last several years is any indication, however, it is highly probable that the ability to manipulate Ca^{2+} signals to selectively

influence the behavior and function of neural stem cells for therapeutic applications may not be too far off.

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Fig. 1. Calcium signaling pathways in neural progenitor cells

Extracellular agonists (Ag) bind to cell surface receptors (R), including G protein-coupled receptors (GPCRs) such as mGluR, mAChR, and P2YR, and receptor tyrosine kinases (RTKs) such as Trk receptors and ErbB receptors. GPCRs activate phospholipase C (PLC)- β and RTKs activate PLC- γ through G protein (G) or tyrosine kinase-coupled pathways (TK), respectively. PLC cleaves PIP₂ to produce IP₃, which mobilizes Ca²⁺ from ER stores via IP₃R Ca²⁺ release channels. Upon store depletion, the luminal EF-hand domain of STIM1 senses the loss of ER [Ca²⁺], triggering STIM1 to oligomerize and translocate from the bulk ER to plasma membrane-ER junctions where it activates store-operated Ca²⁺ entry through CRAC channels. Voltage-gated Ca²⁺ channels (VOCCs) mediate Ca²⁺ influx in response to membrane depolarization. NMDA receptors (NMDAR) and nicotinic ACh receptors (nAChRs) are Ca²⁺-permeable channels that are activated by neurotransmitters glutamate and ACh, respectively. Transient receptor potential (TRP) channels are Ca²⁺-permeable channels that are activated by a wide variety of stimuli. These Ca²⁺ influx pathways contribute to a rise in cytosolic [Ca²⁺]_i, which activates various Ca²⁺-dependent downstream gene expression pathways through regulation of transcription factors such as NFAT, CREB, NeuroD, and DREAM. Activation of these pathways has been linked to proliferation, migration, and differentiation of neural progenitor cells.

Fig. 2. Schematic of the different stages of neurogenesis in the developing brain

Neurogenesis proceeds through several overlapping stages: proliferation, differentiation, migration, and maturation. The Ca²⁺ influx pathways expressed during these stages are indicated. Commonly used immunohistological markers for staging neurogenesis are also shown above.

Table 1Ca²⁺ mobilization pathways in neural development.

Channel	Cell types and stages expressed	Physiological role	Reference
<i>Voltage-operated Ca channels (VOCCs)</i>			
L-type Ca ²⁺ (DHP-Ca ²⁺) channel	<i>Xenopus</i> ectoderm cells at blastula stage → end of gastrulation	Neural competence/neural induction	[84–86]
		Gene expression/cell fate	[87,91]
	Zebrafish embryonic spinal cord interneuron progenitors	Neuronal fate/differentiation (secondary to glycinergic depolarization)	[173]
	Mouse postnatal NSCs	Neuronal differentiation	[132]
L-type/N-type Ca ²⁺ channel	Mouse/Rat NSCs	Neuronal differentiation, through increased expression of <i>NeuroD</i>	[122,172,174]
	Mouse cortical neurons	Termination of migration	[156]
	Cerebellar granular neurons	Neuronal migration	[153,154]
<i>Receptor-operated channels (ROCs)</i>			
NMDA glutamate receptor	Mouse embryonic cortical neurons of VZ/SVZ	Neuronal precursor migration	[72,176]
	Rat adult NSCs	Neuronal fate/differentiation	[122]
Nicotinic ACh receptor	Mouse cortical NSCs	Early cortical development	[135]
GABA _A receptor	Mouse postnatal cortical neurons of SVZ	Reducing neuronal precursor migration speed	[177]
	Immature cerebellar granule cells	Proliferation (secondary to Ca ²⁺ influx via VOCCs)	[178]
<i>Store-operated Ca channels (SOCCs)</i>			
Orai1/STIM1	Mouse embryonic and adult NSCs	Proliferation and gene expression	[2]
<i>G-protein-coupled receptors (GPCRs)</i>			
mGluR5	Mouse immature cortical neurons	Spontaneous Ca ²⁺ oscillations/gene expression	[76]
	Mouse adult hippocampal NSCs	Proliferation	[150]
P2YR1	Mouse NSCs	Proliferation	[129,130,158]
	Rat radial glial cells in the cortical VZ	Proliferation (through IP ₃ -mediated Ca ²⁺ release)	[59]
	Mouse intermediate neural progenitors	Migration from the VZ to SVZ	[157]
mAChR	Rat embryonic cortical NSCs	Proliferation	[138,140]
<i>Inositol-1,4,5-triphosphate receptors (IP₃Rs)</i>	Mouse neural crest	Neuronal differentiation	[61]
	Human NSCs	Cell cycle length/neurogenesis	[179]
<i>Gap junctions</i>			
Connexin 43	Rat radial glial cells in the cortical VZ	Proliferation	[59]
	Mouse forebrain precursors	Interkinetic nuclear migration	[154]
<i>Transient receptor potential (TRP) channels</i>			
TRPC3	Mouse embryonic NSCs	Radial glial-mediated neuronal migration	[180]
TRPC1	Rat embryonic neural stem cells	Proliferation	[127]
<i>Stretch channels</i>			
Piezo1	Human embryonic NSCs	Lineage specification (neurogenesis)	[77]
<i>Channel regulators</i>			

Channel	Cell types and stages expressed	Physiological role	Reference
Calfacilitin	Chick embryo neural plate	Neural plate development	[96]
Neuronatin	<i>Xenopus</i> early neuroectodermal cells	Neural induction	[93]

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