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This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/121412 since

Published version:

DOI:10.2174/092986712804143277

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20 April 2024

UNIVERSITÀ DEGLI STUDI DI TORINO

***This is an author version of the contribution published on**:*

Questa è la versione dell'autore dell'opera:

Current Medicinal Chemistry, vol 19, pp.5793-5801 (2002)

10.2174/092986712804143277

The definitive version is available at:

La versione definitiva è disponibile alla URL:

http://www.eurekaselect.com/105166/article

Calcium signalling in neuronal motility: pharmacological tools for investigating specific pathways.

Davide Lovisolo[1,2,3] Paolo Ariano[4], Carla Distasi[3,5]

1. Department of Life Sciences and Systems Biology, University of Torino, Italy
2. NIS Centre of Excellence, University of Torino, Italy
3. Neuroscience Institute of Torino, University of Torino, Italy
4. Center for Space Human Robotics @Polito, Istituto Italiano di Tecnologia, Torino, Italy
5. Department of Pharmaceutical Sciences, University of Eastern Piedmont ‘‘A. Avogadro’’, Novara, Italy

Address correspondence to:

Davide Lovisolo

Department of Life Sciences and Systems Biology,

University of Torino

Via Accademia Albertina 13, 10123 Torino, Italy

davide.lovisolo@unito.it

Key words:

Calcium signalling, neuronal migration, pharmacology

Abstract

Migration of neurons and neuronal precursors from the site of origin to their final location is a key process in the development of the nervous system and in the correct organization of neuronal structures and circuits. Different modes of migration (mainly radial and tangential) have been described in the last 40 years; for these, as for motility processes involving other cellular types, calcium signalling plays a key role, with influx from the extracellular medium representing the main mechanism, and a more delimited but specific role played by release from intracellular stores. Deciphering the involvement of the different calcium influx pathways has been a major task for cellular neurobiologists, and the availability – or lack – of reliable and selective pharmacological tools has represented the main limiting factor. This review addresses the strategies employed to investigate the role of voltage-dependent calcium channels and of neurotransmitter activated channels, either calcium permeable or not, that directly or indirectly can elicit cytosolic calcium increases; in addition, reference to recent findings on the involvement of other families of calcium permeable channels (such as the transient receptor potential superfamily) is presented. Finally, a brief description of the present - and limited - knowledge of the perturbations of calcium signalling involved in neuronal migration pathologies is provided.

1. Introduction: calcium signalling in the development of the nervous system

Calcium is a second messenger unrivalled for ubiquity and pleiotropicity in all eukaryotic cells, and it is not surprising that in neuronal cells, a wide array of mechanisms is involved in the fine tuning of its intracellular concentration as a free ion, relying on an ample set of import and export pathways. Almost all aspects of neuronal function, both during development and in the adult organism, are under the control of calcium signals, tightly regulated in space and time.

In the developing nervous system, the proliferation, survival and migration of neuronal precursors and postmitotic neurons [1-3] and the formation of functional networks thanks to the correct patterning of connections with the right targets [4,5] are all dependent on specific patterns of calcium signals; after the onset of the postnatal circuitry, the transfer and coding of information and its plastic changes in response to environmental inputs depend on intricate calcium signalling mechanisms [6].

In this review we will focus on the former aspect, and will not deal with calcium dependent signalling in mature neurons. Moreover, we will not discuss the huge literature regarding the specificity of calcium mobilizing pathways in the control of neurite growth and orientation; we will restrict our review to the strategies to dissect specific contributions to calcium signalling involved in the control of neuronal motility. For general reviews on calcium-dependent effector proteins, neuronal motility, neurite growth and guidance refer to [7-14].

2. The formation of neural circuitry

During development, neuronal precursors are in general produced at sites different from the final localization, that is reached through a complex process of migration and stepwise differentiation. Two main modalities of neuronal migration have been described: radial migration, in which postimitotic neurons migrate from their place of origin (the surface of the cerebral ventricles) toward the different layers of the cortex moving along radial glia [15,16], and tangential migration, the process by which many interneurons migrate from the ganglionic eminences to the cortex [17-20]. Other neuronal cells and precursors reach their final position by pathways that have been classified as tangential; examples are provided by olfactory and gonadotropin-releasing hormone (GnRH) neurons [20-23]. Some migratory patterns, such as for neural crest cells [24,25] cannot be easily classified into the above categories. All these processes are tightly regulated by extracellular signals and depend on changes in intracellular calcium. After having reached their final localization, neurons emit thin, highly dynamic protrusions, called neurites, that eventually, mainly depending on

the signals present in the extracellular medium, will specialize into axons and dendrites. These structures grow until they reach the correct target; here the growth stops, and the last phase is that of stabilization of the connections and formation of functional synapses. This process is again under the control of attractive and repulsive extracellular cues, and calcium in a key player in this context, too: growth, orientation and stop signals are calcium dependent.(see e.g. [13])

3. Calcium signalling pathways involved in the control of neuronal migration.

The diversity both of developmental events and of neuronal types is reflected in the diversity of calcium mobilizing mechanisms involved in their control. As a preliminary statement, it can be said that calcium influx from the extracellular medium is the main player, with a more restricted, but in some instances crucial, role of release from intracellular stores. As for calcium influx, ample evidence is available for both voltage dependent and independent pathways, deeply interwoven. Discriminating between these mechanisms is of relevance in basic science, but also, in perspective, in the understanding of the bases of migration-related neurological diseases (see Section 7).

The present knowledge on the mechanisms and dynamics of neuronal migration has been strongly dependent on information gained from mouse mutants (mainly the *reeler* mouse; see *e.g.* [20,26]), and from knockout mice [27-30]. (Noteworthily, the ataxic phenotype of another mutant, the *weaver* mouse, that was ascribed to defective migration of granule cells in the developing cerebellum, has been shown to depend on granule cell death [19,31]).
In contrast, the deciphering of calcium mobilizing mechanisms involved in neuronal migratory behaviour has relied mainly on pharmacological tools, with few exceptions [32-35]. This is particularly true for voltage dependent calcium channels and for calcium permeable, neurotransmitter activated channels. The relatively limited number of channel isoforms and the availability of selective blockers has contributed to an early understanding of the role of these channel families in neuronal motility.

4. Voltage dependent and neurotransmitter activated calcium channels

4.1 Radial migration

Interestingly, most of the available data on calcium signalling in radial migration of neurons have been obtained from mouse cerebellar granule neurons. Cerebellar cortex has some advantages, in particular a more defined architecture and a limited number of cell types [10]. The pioneering work

of P. Rackic, H. Komuro and collaborators [7,10, 36-39] has elucidated the major role played in this context by voltage-dependent calcium channels (VDCCs).

In the molecular layer of the cerebellum, granule cells show a saltatory movement along glial fibres, with alternance of forward jumps and stationary phases; this behaviour is paralleled by the generation of spontaneous calcium oscillations, strongly dependent on influx from the extracellular medium, that show a time course and a frequency closely synchronized with cell motility (Fig. 1, from Ref. 38). Dealing with neurons, it was to be expected that voltage dependent calcium channels would be deeply involved; indeed, interfering with calcium influx through N-type calcium channels by means of the specific inhibitor ω-conotoxin-GVIA (3 μM) reduced the migratory rate of granule neurons to about 25% of control values [36]. Remarkably, blocking other types of voltage dependent channels (Na^+, L- and T-type Ca^{2+}, and K^+ channels) with specific inhibitors had no effect on radial migration, pointing to a selective role of calcium influx through N channels in the control of cell motility. In a subsequent paper [38] the same Authors showed that granule neurons migrating from microexplants displayed periodic oscillations in $[Ca^{2+}]_i$, that were synchronous with the saltatory movement of granule cells; ω-conotoxin, in the same concentration range, reduced both the amplitude and frequency of the oscillations by about 50%, with a proportional decrease in motility. Reducing the extracellular calcium concentration reduced both amplitude and frequency of the calcium oscillations, together with the migratory rate; conversely, depolarizing cells with high KCl increased motility, thus providing further evidence for the involvement of calcium influx through voltage dependent calcium channels.

Addition of the *N*-Methyl-D-aspartate (NMDA) glutamatergic receptor antagonist D-AP5 (100 μM) to the extracellular medium induced a comparable reduction in all three parameters (amplitude and frequency of Ca^{2+} oscillations, migratory rate); on the other hand, abolishing release from intracellular stores with 1 μM thapsigargin, an inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA), had a more limited effect on amplitude of oscillations and on movement, with no effect on the frequency of calcium signals.

The possibility that the contribution of Ca^{2+} oscillations to neuronal motility could be explained in terms of an elevation of average $[Ca^{2+}]_i$ was ruled out: increasing basal $[Ca^{2+}]_i$ by means of the Ca^{2+} ionophore ionomycin (50 nM) had no effect on calcium oscillations and on neuronal motility. Adding all the above compounds together reduced oscillations and motility even more dramatically, notwithstanding the elevated basal $[Ca^{2+}]_i$.

These observations were obtained thanks to reliable pharmacological agents, were confirmed by subsequent observations (see e.g. [10]), were extended to cortical neurons [40], and set the basis of our understanding of the calcium dependence of radial neuronal migration for the last two decades.

Fig 2 (from Ref. 10) presents a detailed description of the calcium transients observed in the different phases of granule cell migration, form tangential to radial, and shows that the loss of the calcium transients is the signal for completion of migration.

What emerges from these studies is a complex and not yet fully resolved picture, in which influx from the extracellular medium plays a key role, but a contribution of release form internal stores is also present. Moreover, blocking NMDA receptors may have a negative effect on the activation of N-type channels: the two pathways are deeply interwoven and are not easily evaluated separately. Other voltage-dependent calcium channels may be involved: in *weaver* mice, blocking L-type channels by means of 1-5 μM verapamil, a L–type calcium channel blocker, rescued the neurite outgrowth of granule cells in culture, a process considered to be preliminary to neuronal migration along radial glia [41], pointing to a specific role for this channel subtype in a particular and still controversial neurological disorder [31]. Data about the involvement of N-type, but also of L-type channel homologues in neuronal migration have been obtained in the nematode *Caenorhabditis elegans* by means of mutant analysis [33].

Calcium mobilizing mechanisms in radially migrating neurons may be modulated by other extracellular cues, in addition to classical neurotransmitters, and these effects may be dependent on a specific developmental stage. Somatostatin [42], Slit-2 [43] and pituitary adenylate cyclase-activating enzyme (PACAP) [44] have been shown to modulate both calcium oscillations and motility in cerebellar neurons in *in vitro* experiments.

4.2 Tangential migration

In the case of tangential migration of cortical neurons, the scenario is markedly different and seems to be dominated by neurotransmitter activated channels.

Soria and Valdeolmillos [45] investigated the relationship between calcium signalling and tangential migration in rat cortical slices. The specific agonists NMDA, kainate (for AMPA glutamatergic receptors) and muscimol (for $GABA_A$ channels), all superfused at a concentration of 50 μM, induced slow and long lasting increases in $[Ca^{2+}]_i$, providing evidence that glutamate, via both AMPA and NMDA receptors, and GABA, via $GABA_A$ receptors, modulate calcium signalling in these cells. The responses were abolished by specific inhibitors, respectively APV (200 μM), CNQX (50 μM), bicuculline (50 μM). It must be recalled that at early neuronal developmental stages, $GABA_A$ activation has a depolarizing and excitatory role, due to the low expression levels of the K^+/Cl^- cotransporter KCC2 and the consequent high $[Cl^-]_i$ and relatively depolarizing Cl^- reversal potential [46]. Interestingly, in this paper, calcium transients were not observed in migrating neurons and no frequency coding was reported.

Blocking the generation of action potentials by 1 µM tetrodotoxin, a selective blocker of voltage activated Na^+ channels, had no significant effect on the increases in $[Ca^{2+}]_i$ induced by the glutamate analogues, while it nearly abolished responses to muscimol, thus showing that glutamate can induce calcium signals independently from the electrical activity, while the GABA-induced increases in $[Ca^{2+}]_i$ require the generation of action potentials.

A different, and more complex picture emerges from the data provided by Bortone and Polleaux [47]. These Authors combined observation from acute medial ganglionic eminence (MGE) slices and 2-D cocultures of MGE explants on a layer of dissociated pyramidal neurons, and utilized a more sophisticated set of analysis tools to quantify motion parameters and calcium signals. During migration, a subpopulation of interneurons showed spontaneous Ca^{2+} transients, in the absence of any pharmacological intervention; this activity, as well migration, was enhanced by knocking down the KCC2 cotransporter and was suppressed by overexpressing the transporter or by blocking $GABA_A$ receptors with 10 µM bicuculline. Chelating intracellular calcium by means of 25 µM BAPTA-AM significantly reduced the percentage of migrating interneurons in acute telelencephalic slices. Blocking of L-type Ca^{2+} channels by means of 10 µM nifedipine reduced the migratory behaviour, while block of N-type channels resulted in a minor effect. A cooperative effect of $GABA_A$ and glutamate receptor activation on interneuron migration was also evidenced.

These observations indicate that spontaneous $[Ca^{2+}]_i$ oscillations are present also in tangentially migrating interneurons and that their modulation by the combined action of glutamate and GABA, both exerting a depolarizing effect and eliciting voltage-dependent Ca^{2+} influx, influences migration; at later stages, the increased expression of the KCC2 transporter and the ensuing establishment of a more negative Cl^- reversal potential shifts the action of GABA from excitatory to inhibitory and serves a stop signal.

Studies on hippocampal slices [48,49] have provided a partially different picture: tangential migration of interneurons is positively modulated by AMPA, but not by NMDA receptors, in contrast to radial migration that is influenced by both NMDA and $GABA_A$ receptors.

As an added complexity, in the cerebral cortex activation of AMPA receptors has been reported to have an inhibitory effect on tangential migration [50]. These discrepancies may be explained by the different subunit composition (and consequent calcium permeability) of AMPA receptors in different regions of the CNS and at different developmental stages.

Finally, other neurotrasmitters, such as serotonin, have been shown to affect tangential migration of cortical interneurons [51], but no data on their effect on calcium signalling is at present available.

Other neurons migrate along pathways that have been classified as tangential: the best example are interneurons of the olfactory bulb and GnRH neurons. In mouse E11.5-E15.5 intact olfactory bulbs, the average velocity of migration of neuronal precursors was markedly reduced by chelating extracellular calcium with BAPTA and by application of 10 μM nifedipne, pointing to a role of calcium influx through voltage dependent L-type channels; on the other hand, 50 μM APV, blocker of NMDA channels, had no effect [52]. Interestingly, in olfactory bulb slices form postnatal mice, the L-type calcium channel blocker nimodipine (30 μM) strongly reduced spontaneous $[Ca^{2+}]_i$ oscillations in neural precursors cells, without affecting migration [53]. Thus, the coupling of calcium signalling to the control of neuronal migrations appears to be stage- and age-dependent, at least in this system, characterized by postnatal neurogenesis.
Another group [54] reported that the velocity of migration of neuronal precursors in the SVZ is reduced by ambient GABA via $GABA_A$ receptors, by showing that the specific blocker bicuculline (100 μM) increases this parameter, while Cd^{2+} and Ni^{2+}, blockers of voltage dependent calcium channels, had no effect. The authors attributed this effect to a $GABA_A$-mediated release from intracellular stores, although no description of the mechanism was provided.
GnRH neurons migrate from the nasal placode in a partially parallel pathway, using olfactory neuron axons as a guide. Their migration has been extensively studied in nasal explants, and it has been shown to be reduced, at initial migratory stages, by removing extracellular calcium and by blocking N-type channels with ω-conotoxin-GVIA (100 nM), while nifedipine was not effective [55]. Since the effects of calcium removal were more marked than those induced by the N-type channel blocker, other, voltage-independent, influx pathways are probably involved. Neurotransmitters (GABA) have a role in this process, too [56,57]: but no data on calcium signalling are at present available.

5. Other calcium mobilizing pathways

5.1 Calcium release from intracellular stores

From the data discussed so far, the role of calcium release from intracellular stores has emerged as marginal and at most secondary to calcium influx. However, in a few cases, this pathway has been shown to be the primary mechanism responsible for the calcium signals involved in the control of neuronal motility and migration, either directly or through activation of store operated calcium influx (SOCE), *i.e.* the mechanism of calcium entry from the extracellular medium gated by the emptying of the intracellular calcium stores, in particular in the endoplasmic reticulum [58].
Kumada et al. [59] reported that the autonomous turning of cerebellar granule cells, that can be observed in vitro, is dependent on both calcium influx (through voltage dependent and NMDA

channels) and release from intracellular stores: stimulating the release through ryanodine receptors with caffeine (1 mM) or the IP3 receptors with the agonist thimerosal (5 μM) strongly increased the frequency of turning events.

In ST14A cells, an immortalized line obtained from rat striatal primordia, Pregno et al. [60] investigated the migratory activity induced by Neuregulin1 and its calcium dependence. Chelating intracellular calcium with BAPTA-AM strongly reduced cell motility; Neuregulin1 induced long lasting increases in $[Ca^{2+}]_i$ that were dependent on release from intracellular stores and ensuing activation of SOCE, as assessed in experiments with 100 μM thapsigargin, an inhibitor of calcium pumps of the endoplasmic reticulum plasmamembrane that causes the emptying of these stores.

A final, and quite different, example comes from the analysis of the *in vitro* migration of a neural crest derivative, embryonic chick ciliary ganglion neurons.

In dissociated cultures, it has been shown that non-random, directional movement of these neurons (that in these experimental conditions tend to form aggregates connected by highly fasciculated neurite bundles, [61]) is dependent on their association with glial cells [62]. Subsequently, the relationship between calcium signalling and migration was investigated [63]. Neurons showed spontaneous oscillations in $[Ca^{2+}]_i$, with fast spikes, abolished by the voltage dependent calcium channel blockers nifedipine and ω-conotoxin, superimposed onto slow waves; both components were independent from release from intracellular stores. Glial cells showed slow oscillations which were dependent on both release and influx and were unaffected by voltage dependent channel blockers; on the other hand, blocking release completely abolished oscillations. Interestingly, blockers of voltage dependent Ca^{2+} channels had no effect on the velocity of the neuronal-glial complexes, while stimulating release in glial cells with the IP3 receptor agonist thimerosal (0.2 μM) increased it. This is another case for the involvement of glial cells in neuronal migration: but, differently from what happens in the cortex, in this *in vitro* model it is the migrating glial cell that physically carries the neuron to its final location in the cell aggregate.

5.2 A new entry in calcium dependent neuronal migration: the TRP channels

A cell line derived from GnRH neurons has recently attracted interest as a model to study the role of calcium signalling by a different class of receptor activated channels, the TRP superfamily [11], in neuronal migration. The GN11 immortalized cell line is derived from a tumour induced in the olfactory bulb [54]; these cells show many properties of immature GnRH neurons, including a strong proliferative and migratory activity in the presence of foetal calf serum (FCS). Moreover, while primary GnRH neurons express voltage dependent calcium channels at least starting from stage E 12.5, GN11 cells do not express functional voltage dependent calcium channels [65,66];

therefore, they are a good model to investigate voltage-independent calcium signalling. Zaninetti et al. [34] combined pharmacological and overexpression approaches to show that activation of the TRPV4 channel induces increases in $[Ca^{2+}]_i$ that are related to a reduction in cell migration and chemotaxis. TRPV4 channels display constitutive activity at 37 °C and a number of diverse stimuli can modulate their behavior: the activation of the TRPV4 channels in migrating cells could occur via both mechanical and osmotic stimuli [67,68].

In a consistent percentage of these cells, 1 µM of the selective agonist 4a-phorbol 12,13 didecanoate (4α-PDD) [69] induced $[Ca^{2+}]_i$ increases dependent on influx from the extracellular medium, while the nonspecific TRPV inhibitor Ruthenium Red (RR; 1 µM) abolished it. The agonist, and its analogue 4α-PDH [70] strongly reduced the percentage of cells migrating in response to a foetal calf serum (FCS) gradient, and this reduction was partially reverted by RR. TRPV4 overexpression provided converging results, reducing the migratory behaviour of GN11 cells. Interestingly, migration was also negatively modulated by two proposed TRPV4 activators [71], arachidonic acid (5 µM) and 5,6-epoxyeicosatrienoic acid (3 µM). Finally, another, not structurally related TRPV4 agonist, GSK1016790A [72], reduced migration in a wound healing assay.

The involvement of TRPC channels has been investigated in two recent papers, too, providing a more controversial picture. Ariano et al. [73] analyzed the spontaneous calcium oscillations in GN11 cells and their enhancement in the presence of FCS. The nonspecific TRPC inhibitors, SKF96365 (5 µM) and La^{2+} (1 mM), strongly reduced amplitude and frequency of the calcium oscillations and the migratory activity. A blocking function anti-TRPC1 antibody caused comparable reduction of calcium oscillations and migration, Partially contrasting results have been provided by Storch et al. [35]: they showed that in heterologous systems, TRPC1 assembly in heterotetramers reduced calcium permeation through the channels; in GN11 neurons, TRPC1 downregulation increased calcium permeability and migratory velocity and directionality. These discrepancies can be explained by the fact that TRPC1 channels, as stated above, form heterotetramers with the other subunits (these cells express also TRPC2, 5 and, according to [35], TRPC6): the anti-TRPC1 Ab, by interfering with this subunit, may modify the permeability of the whole assembly, thus blocking the heterotetrameric channel.

These findings are a strong reminder that due to the lack of specificity of the available pharmacological and molecular tools, only a combination of experimental approaches can give unequivocal results, and are indicative of the problems encountered when studying this channel subfamily. While evidence of their role in neuronal migration is just beginning to be provided, they are considered among the main players in the game of another aspect of neuronal motility, neurite growth and orientation [11]; therefore, a digression on these problems may be useful. The

limitations are both in the specificity of the available pharmacological tools and in the understanding of the activation mechanisms. When these channels began to be actively studied, it was widely accepted that they represented the molecular identities responsible for the so called store-operated calcium entry (SOCE). Further studies showed that they can be generally considered as receptor-activated channels downstream of the phospholipase C (PLC) pathway, with several lipidic second messengers, such as diacylglycerol (DAG) and free fatty acids as the main activators [74-76], even if there is evidence that some of them are involved in SOCE [77].
Their controversial nature is reflected in the poor specificity of the pharmacological inhibitors available. The case of the imidazole derivative SKF96365 is the best exemplification of this issue. Initially described as a generic blocker of receptor activated calcium influx and of SOCE [78,79], it has been widely used as a nonspecific blocker of TRPC channels, also in neurons (see e.g. [80,81]). In contrast, several reports [78, 82-84] have evidenced its poor selectivity, since it can block other types of calcium permeable channels, mainly voltage dependent ones, and interfere with other ionic mechanisms. However, most of these effects have been observed at concentrations in the 10-100 μM range, while at concentrations of 5 μM or lower it can be considered a good preliminary tool to block TRPC-mediated influx. The lack of better options can explain its continued use in many studies, in particular in the field of neurite growth and orientation [80,85,86]. Inorganic ions, such as La^{3+} (and Gd^{3+}), can block most of these channels [87], but with complex dose-response relationships; therefore, these tools have to be employed with caution.
In summary, the lack of pharmacological tools has led some authors [88] to state that the study of TRPC channels must rely on knockdown and silencing techniques; however, even if a large amount of relevant data has been obtained thanks to these approaches, they still present several limitations, since, because of the heteromeric nature of TRPC channels, and of compensatory mechanisms, data obtained form knockout and knockdown protocols are not always univocally interpreted; in some case double or multiple knockouts are necessary to get a full understanding of the role of single members (see e.g. [89]). The pharmacological approach is therefore still a reasonable option, in most cases as a complement to other tools.

6. Calcium signalling and neuronal migration disorders

Mutations in mice that affect the correct patterning of the cerebral cortex have provided relevant information about the role of neuronal migration in cortical development. The *reeler* mutant mice [20,26] are characterized by an "upside down" organization of the cortical layers, due to perturbations in pyramidal neuron migration; similar disturbances have been observed in other mutants, such as *weaver* mice [31,90]. The product of the *reeler* gene, Reelin, is an extracellular

matrix protein that controls the exact positioning of radially migrating neurons and the correct organization of the six cortical layers [91]. In humans, mutations in several genes [92], including the gene encoding Reelin have been associated with some forms of lissencephaly [93], a developmental disorder dependent on impairment of neuronal migration that results in abnormal layering and reduction of cortical folds [94].

Despite the ample literature on the genetic bases of these cortical malformations, the knowledge of the molecular biology of the pathogenetic mechanisms is still far from being understood, and very little is known about the involvement of anomalies in calcium signalling. It has been shown that Reelin interacts, via membrane receptors, with motor and cytoskeletal proteins [94,95], likely targets of calcium dependent pathways. Actually, it has been shown that the product of the *LIS1* gene, whose mutation leads to Type I lissencephaly, regulates in a calcium influx-dependent way some RhoGTPases involved in cytoskeletal dynamics [96].

Calcium signalling has been involved in other aspects of reelin-and *LIS1* function, not related to neuronal migration and reported in postnantal mice, such as NMDAR activation and LTP [97], and seizures and increased excitability [98].

However, a hint of the relevance of alterations in calcium signalling in cortical pathologies linked to impaired neuronal migration emerges not from genetic mutations, but from environmental perturbations: Kumada et al. [99] have shown that foetal alcohol syndrome (FAS), a serious disorder of brain development caused by maternal alcohol abuse during pregnancy, and other related disturbances, are linked to reduced calcium transients frequency and migratory activity of cerebellar granule cells; caffeine (1mM), by stimulating release form intracellular stores, and NMDA (30 μM), by inducing Ca^{2+} influx, significantly reduced the effects of ethanol (25-100 mM) on the Ca^{2+} transients and on migration. Similar effects were observed following inhibition of the cAMP pathway. (Notably, migration is antagonistically regulated by Ca^{2+} and cAMP pathways not only in cerebellar granule cells [44] but also in ciliary ganglion glial cells [100]).

Thus, alcohol appears to affect neuronal migration by interfering with multiple signal transduction pathways, among them multiple calcium mobilizing mechanisms.

7. Conclusions and future developments

The findings described in the preceding chapters point to a crucial role of calcium signalling not only in the physiology, but also in the pathology of neuronal migration, and prelude to further achievements in our understanding of the processes that lead to normal and abnormal development of the nervous system.

From the neuropathological perspective, the picture of the genetic and epigenetic mechanisms that lead to migration-dependent neurological disorders is far from being complete, and this is particularly true for alterations in the calcium signalling mechanisms. It can be envisioned that future focus will be, more than on calcium signalling *per se*, on the field of calcium-dependent proteins involved in cytoskeletal organization and in organelle trafficking, as pointed by the Reelin-lissencephaly case [94-96]. However, it cannot be excluded that the TRP channel superfamily, just a newcomer in this context, may provide some surprise – and some unexpected answers. The role of these channels in the control of neurite growth and orientation is now established [12,13,85,86], and they play crucial roles in many other aspects of neuronal function, from synaptic transmission [101,102] to sensory transduction [103]; on the other hand, many members are putatively involved in a wide spectrum of pathologies of the nervous system [104,105]. If this will be the case, the development of more specific and selective agonists and antagonists will become the critical issue.

Aknowledgments

Support from the Compagnia di S. Paolo to the NIS Centre of Excellence is gratefully acknowledged.

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Figure legends

Fig. 1. Correlation between spontaneous calcium fluctuations and motility in migrating granule cells from microexplants cultures of P2-P5 mouse cerebella.

Upper: time course of changes in Fluo-3/Fura-Red ratio signal. Upward deflections in Fluo-3/Fura-Red ratio signals represent elevations of [Ca2+]i and downward deflections indicate decreases of [Ca2+]i.

Lower: The direction and distance traversed by the same cell during each 30 s of the testing period (From Komuro and Rakic, 1996)

Fig. 2. Dynamic changes in the frequency of Ca2+ transients (blue lines) in migrating cerebellar granule cells are related to cell movement (red lines). The numbers at top of each graph represent the position of granule cells along the migratory pathway and the stage of the differentiation. in order: (1-5) granule cell precursor migrating from top to bottom of the external granular layer (EGL); (6-8) radial migration along the Bergmann glial process in the molecular layer (ML); (9–10) stationary period in the Purkinje cell layer (PCL); (11–13) radial migration at the top and middle of the internal granular layer (IGL); (14) completion of migration at the bottom of the IGL; (15) post-migratory granule cell at the bottom of the IGL. (From Komuro and Kumada, 2005).

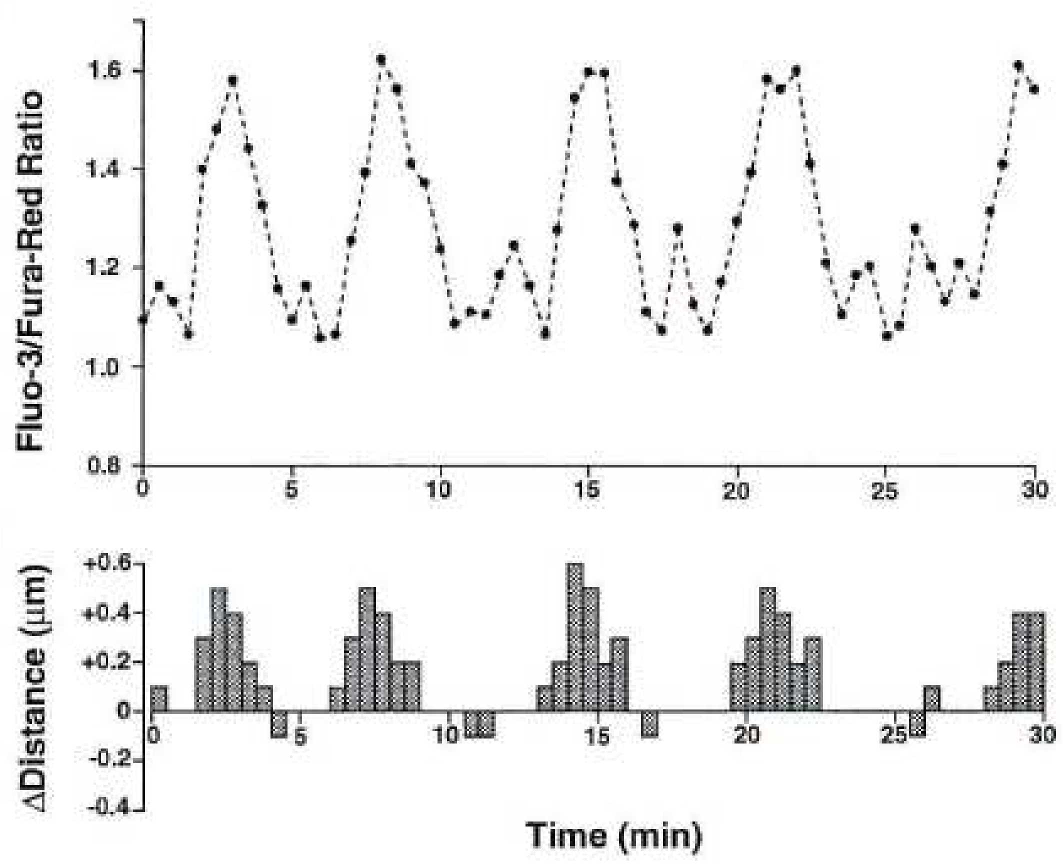

Fig. 1

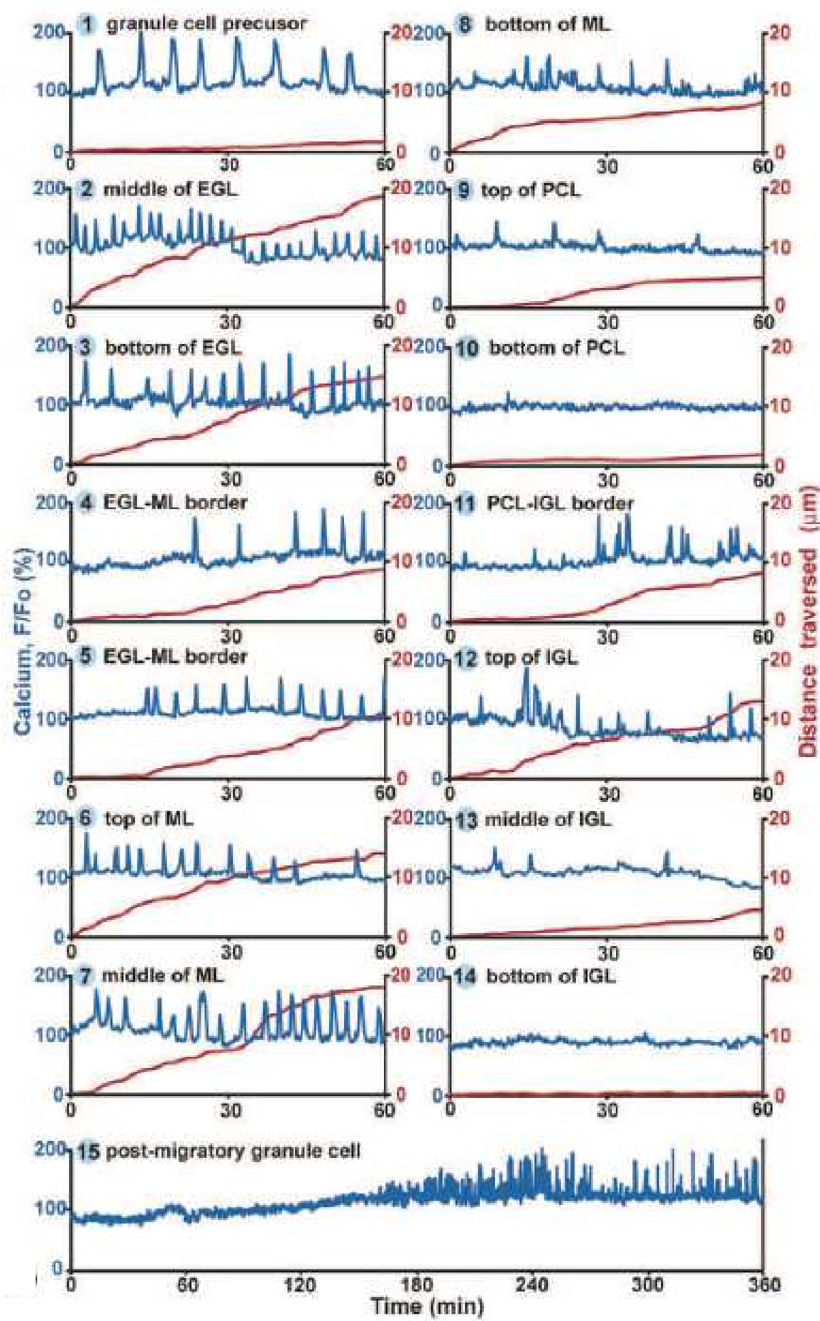

Fig. 2