Emerging Functions of the "Ca2+ Buffers" Parvalbumin, Calbindin D-28k and Calretinin in the Brain

CHAPTER · JANUARY 1970

DOI: 10.1007/978-0-387-30379-6_5

1 AUTHOR:

Beat Schwaller
Université de Fribourg
126 PUBLICATIONS **4,552** CITATIONS

SEE PROFILE

11 Calcium-binding Proteins

B. Schwaller

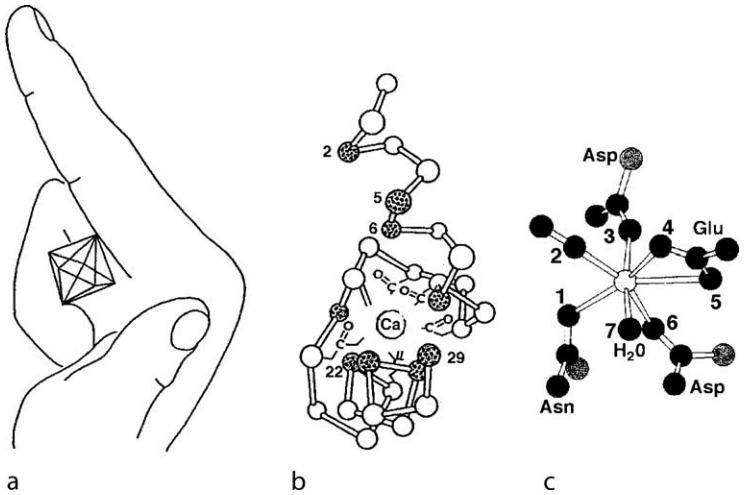
Abstract: Together with the ubiquitous calmodulin (CaM), the EF-hand containing calcium-binding proteins (CaBPs), parvalbumin (PV), calbindin D-28k (CB), and calretinin (CR), are the most abundantly expressed members of this family in the brain. Formerly, they were classified as simple buffers serving to "clamp" the intracellular calcium concentration $[Ca^{2+}]_i$. But recent studies often using transgenic mice have revealed these molecules to play pivotal roles in Ca^{2+} homeostasis and signaling. And research conducted during the last 5 years indicates that they are important for synaptic plasticity and related rhythmic activities within neuronal networks. For CB, an additional modulator role in inositol-1,4,5-trisphosphate (IP_3)-signaling pathways was reported, indicating additional sensor functions. In this chapter, I summarize the current knowledge on the three CaBPs in the brain revealing their important roles in the CNS.

1 Introduction

Calcium ions (Ca^{2+}) serve as an indispensable second messenger in many vital cellular functions. Processes as diverse as fertilization, muscle contraction, neuronal signaling, cell cycle regulation, and regulated cell death depend on spatially and temporally precise Ca^{2+} signals (Berridge, 1998; Berridge et al., 2003). In neurons, Ca^{2+} signals play key roles in intrinsic membrane excitability, gene transcription, neurotransmitter release and associated synaptic plasticity (long-term potentiation (LTP) and long-term depression (LTD)), oscillatory activity, and neuronal motility and morphology, including the growth and branching of dendrites and the formation of dendritic spines. To achieve precision in Ca^{2+} signaling, cells express specific components, which permit the influx of this cation across the plasma membrane or its release from intracellular stores such as the endoplasmic reticulum or mitochondria. A Ca^{2+} transient is terminated by the activity of specific Ca^{2+} pumps, which extrude the cation either into the extracellular space or back into the intracellular stores. Plasmalemmal Ca^{2+}-ATPases (PMCAs) and Na^+/Ca^{2+} exchangers (NCXs) are involved in the former process, while the sarcoplasmic reticulum Ca^{2+}-ATPases (SERCAs) and the yet uncharacterized mitochondrial Ca^{2+} uniporter are responsible for the latter. Cytosolic proteins capable of binding Ca^{2+} with precisely tuned affinities are important modulators of intracellular Ca^{2+} signals. Numerous possibilities exist by which Ca^{2+}-binding sites in proteins may be constructed; several distinct protein families contain various evolutionarily well-conserved Ca^{2+}-binding domains. These include the EF-hand proteins, the annexins, and the C2 domain proteins (for review see Celio et al., (1996); Schwaller, (2004)). Among the calcium-binding proteins (CaBPs), those possessing the EF-hand domain (Celio et al., 1996; Kawasaki et al., 1998) are the most common. Analysis of the human genome has revealed 242 such proteins (Lander et al., 2001). This feature also renders them one of the largest groups of proteins sharing a common motif. The canonical EF-hand domain (helix–loop–helix motif) consists of a conserved stretch of usually 29 amino acids (two more in the S100-specific N-terminal Ca^{2+}-binding loop), which are formed sequentially into an α helix, a Ca^{2+}-binding loop, and a second α helix orientated perpendicular to the first (❯ *Figure 11-1*). The structure of this motif was elucidated by X-ray analysis of carp parvalbumin (Kretsinger et al., 1973). The three helical pairs were named AB, CD, and EF, and the domain containing the C-terminal α helices 5 and 6 (EF) conferred the entire family its name. The EF-hand domain can be visualized by the thumb and the index of the right hand, which represent the two helices, and by the bent major finger, which represents the loop providing the usually six oxygen atoms that coordinate Ca^{2+}. The seventh ligand is typically an oxygen atom from a water molecule. Hence, the seven oxygen ligands form a pentagonal bipyramid (❯ *Figure 11-1*). An EF-hand protein contains multiple, and usually an even number (two, four, six, or eight) of EF-hand domains. The exceptions are parvalbumin and oncomodulin with three domains, and the family of penta-EF-hand proteins with five domains (Maki et al., 2002). Typically, not all EF-hand domains are functional. For example, in parvalbumin only the CD and EF domains, not the AB site, bind Ca^{2+}. In this chapter, emphasis is placed on the CaBPs parvalbumin (PV), calbindin D-28k (CB), and calretinin (CR), which represent the major species in the central nervous system (CNS).

Figure 11-1

The EF-hand motif. (a) The three-dimensional organization of the EF-hand motif can be simulated using the right hand: the index finger represents the E-helix (residues 1–10), the bent middle finger symbolizes the 12 amino acids of the Ca^{2+}-binding loop (10–21), and the thumb depicts the F-helix (19–29). The seven oxygen ligands coordinating the Ca^{2+} ion are located in the seven corners of a pentagonal bipyramid (modified from Celio et al. (1996)). (b) X-ray crystal structure from the EF-domain of parvalbumin (modified from Kretsinger et al. (1973)). (c) Coordination of the Ca^{2+} ion in calmodulin (CaM) with the seven oxygen ligands: five from side chains, one from a carbonyl group of the backbone, and the seventh from a water molecule



2 Buffering vs. Sensor Function of CaBPs

The EF-hand CaBPs are structurally and functionally grouped into two categories: Ca^{2+} sensors/modulators and Ca^{2+} buffers (Skelton et al., 1994; Ikura, 1996; Nelson et al., 1998) (see the chapter **XY** by Heizmann et al. (Leclerc et al., 2006)). Ca^{2+} sensors are characterized by their ability to undergo significant Ca^{2+}-dependent conformational changes, which permit their interaction with specific targets in a Ca^{2+}-regulated manner. The archetypical, best-described, and most representative example of the sensor class of EF-hand proteins is calmodulin (CaM) (Cohen et al., 1988). It is a small protein (M_r 17 kDa; 148 amino acid residues) ubiquitously expressed and highly conserved among different species. In the presence of Ca^{2+}, the protein undergoes a conformational change from a dumbbell shape, with pairs of EF-hand domains representing the two peripheral globes and a central long helix, to a more compact globular structure. CaM is implicated in many CNS functions, including synaptic transmission, neuronal plasticity associated with short-term and long-term potentiation, and learning and memory processes (see chapter **XY** by Heizmann et al. (Leclerc et al., 2006)) . Other families of Ca^{2+} of sensors include the S100 proteins (Marenholz et al., 2004) and the neuronal calcium sensors (NCS) (Braunewell et al., 1999; Burgoyne et al., 2001).

The two "classical" examples of Ca^{2+}-buffering EF-hand proteins are PV (official human gene symbol: PVALB) and calbindin D-9k (also known as calbindin 3, CaBP-9k, and CABP1; official gene symbol: S100G) (Marenholz et al., 2004). The latter is a member of the S100 family. Both proteins undergo conformational changes upon Ca^{2+}-binding. But globally, the structure is essentially the same in the metal-bound and free (apo) states (Skelton et al., 1995). To date, no binding partners for the two proteins have been reported, indicating their principally Ca^{2+}-buffering roles. Among the species so far investigated, CB D-9k has not been detected in the CNS. With respect to CR (also known as calbindin 2, protein 10 (Winsky et al., 1989) and calbindin 30 kDa (Hubbard et al., 1995); official gene symbol: CALB2) and CB D-28k (also

known as calbindin 1, CaBP-28k, and spot 35 (Yamakuni et al., 1987); official gene symbol: CALB1) the situation is more complicated. In many reports, they are still considered as Ca^{2+}-buffering proteins, but recent progress indicates that they have additional "sensor" functions.

3 Important Parameters Describing the Properties of Ca^{2+} Buffers

In many respects, the term "Ca^{2+} buffer" is rather a feeble one. But for mainly historical reasons and in the absence of a better alternative, it will still be used in this chapter. In chemical terms, a pH-buffering system consists of an acid and base, which is optimized to "clamp" the pH to a predetermined value in such a way that the addition of an acid or a base elicits only a negligible change in the pH of the solution. Hence, the "buffering" capacity is highest when the pH is identical, or close to, the p*K* value of the corresponding acid–base pair. But this is not the case for Ca^{2+}-buffering proteins. Under resting conditions, the intracellular cytosolic concentration of Ca^{2+}, $[Ca^{2+}]_i$, is in almost all cells, including those of the CNS (neurons and glia), in the order of 50–100 nM. However the dissociation constants (K_D) of most CaBPs are in the low micromolar range. Thus, under resting conditions, most of the Ca^{2+}-buffering protein molecules are in the Ca^{2+}-free form. Nevertheless, if $[Ca^{2+}]_i$ increases due to Ca^{2+} influx through Ca^{2+}-permeable channels or receptor channel complexes in the plasma membrane or by a release from internal stores, Ca^{2+}-buffering proteins will modulate the spatiotemporal aspects of Ca^{2+} signals. The most important parameters relating to this buffering function include: the cytosolic concentration of the buffers (❯ *Sect. 2.1*), their affinity for Ca^{2+} and other metal ions (❯ *Sect. 2.2*), the kinetics of Ca^{2+} binding and release (❯ *Sect. 2.3*), and their mobility (❯ *Sect. 2.4*). These parameters have not been determined for PV, CB, and CR with precision in vivo. But recent progress in the field is summarized.

AU1

3.1 Intracellular Concentration

An accurate determination of the cytosolic concentrations of CaBPs in neurons is not easy. Reported results were obtained using biochemical, immunocytochemical, radioimmunological (RIA), and electrophysiological methods. Additionally, the recording of $[Ca^{2+}]_i$ transients using fluorescent Ca^{2+} indicator dyes in combination with modeling the effects of buffers on $[Ca^{2+}]_i$ transients allows to estimate the binding properties and intracellular concentration. Evidently, in the latter case, the quality of the results is critically dependent on the quality of the metal-binding parameters determined by various other methods (for methodological details, see Cox, 1996; Lee et al., 2000; Nagerl et al., 2000b). Each of the three proteins PV, CB, and CR is characterized by a very restricted pattern of expression within neurons of the CNS (see ❯ *Sect. 3*), which, together with the complexity of neuronal morphologies, renders the determination of CaBP concentrations extremely difficult. In single, tall saccular hair cells of the frog, levels of CR—determined by Western blot—are extremely high (1.2 mM (Edmonds et al., 2000)), whereas in the inner and outer hair cells of the rat cochlea they are significantly lower. Quantitative immunohistochemistry and ultrastructural immunostaining have revealed these to be 19 ± 2 μM and 35 ± 3 μM, respectively (Hackney et al., 2005). A similar CR concentration range (30–40 μM) has been proposed for cerebellar granule cells; CR−/− neurons display an altered neuronal excitability that can be compensated by injecting 150 μM BAPTA (Gall et al., 2003). Taking into account that CR has five Ca^{2+}-binding sites (Cheung et al., 1993; Schwaller et al., 1997), 30 μM CR is roughly equivalent to 150 μM BAPTA, although this needs to be considered with precaution (see ❯ *Sect. 2.5*). The CB-concentration range in different neuronal populations is generally higher: 150–360 μM in cerebellar Purkinje cells (for review see Schwaller et al., (2002)), and 40–50 μM in mature hippocampal dentate gyrus granule cells, CA3 stratum radiatum interneurons, and in CA1 pyramidal cells as determined by quantitative immunohistochemistry (Muller et al., 2005). Estimates of the PV concentration in Purkinje cells range from 80 μM in the mouse (Schmidt et al., 2003b) to 116 ± 30 μM in the rat (Hackney et al., 2005). A value of 150 μM for molecular-layer interneurons of the murine cerebellum fits best with the experimental data (Collin et al., 2005). Using ELISA and ultrastructural immunostaining methods, values ranging from 50 μM (Plogmann et al., 1993) up to 1 mM (Kosaka et al.,

1993) have been reported. The estimated concentrations of PV and CB are in line with experimental data revealing the very high Ca^{2+}-buffering capacities of Purkinje cells (Fierro et al., 1996; Maeda et al., 1999). The contribution of other CaBPs to the total Ca^{2+}-buffering capacity of Purkinje cells is likely to be minor (Schmidt et al., 2003b). There remains one point of concern: all quantitative data (obtained using immunohistochemistry, ELISA, RIA) are based on the use of antibodies that specifically recognize the three proteins of interest. Several reports indicate that CaBP antibody recognition depends on the Ca^{2+}-binding status of the investigated proteins (Winsky et al., 1996; Zimmermann et al., 2002). Hence, if the relative amounts of the Ca^{2+}-bound and the Ca^{2+}-free forms are different in nitrocellulose membranes (from Western blots) or in other in vitro preparations used for calibrative comparison with those in the cytoplasm of chemically-fixed tissue sections (Kosaka et al., 1993), the "apparent" differences in CaBP concentrations that have been reported using different methods and by different laboratories could reflect these technical obstacles. However, in general, the agreement between reported values is fairly good.

3.2 Metal-Binding Affinities

In EF-hand proteins, two types of Ca^{2+}-binding sites are distinguished on the basis of their different selectivities and affinities for Ca^{2+} and Mg^{2+} (Celio et al., 1996). CB and CR contain only the so-called Ca^{2+}-specific sites (four functional sites in CB and five in CR; reviewed in Celio et al. (1996)). In general, the affinities of these Ca^{2+}-specific sites for Ca^{2+} (K_{Ca}) are in the order of 10^{-3}–10^{-7} M and are significantly lower for Mg^{2+} ($K_{Mg} = 10^{-1}$–10^{-2} M). The most accurate values for CB have been obtained in vitro by using UV-flash photolysis of DM-nitrophen in combination with a model-based analysis of fluorescence transients using the Ca^{2+} indicator Oregon Green 488 BAPTA-5N (Nagerl et al., 2000b). UV laser flashes of variable intensity elicit rapid, step-like increases in $[Ca^{2+}]_i$. If synthetic buffers such as EGTA or recombinant CB are added, the decay in flash-induced fluorescence can be attributed to the presence of the Ca^{2+} buffer (EGTA or CB). Subsequent modeling of the transients yields the kinetic information. Using this tool, CB has been shown to possess two different types of binding sites with different K_{Ca} and kinetic Ca^{2+}-binding rates (k_{on}): two sites are high-affinity sites, which bind Ca^{2+} with a k_{on} comparable to that of EGTA ($\approx 1 \times 10^7$ M^{-1} s^{-1}); the others are of lower affinity, with an approximately eight-fold faster k_{on}. However, the mathematical model did not incorporate cooperativity, a mechanism known to operate within most CaBPs, including CB (Berggard et al., 2002a). Therefore, a more refined analysis is awaited. A similar evaluation for the alternatively spliced form of CR, CR-22k (Schwaller et al., 1995), has been undertaken, but only preliminary data are as yet available in the form of a meeting abstract (Faas et al., 2003). In a "resting" neuron with a $[Ca^{2+}]_i$ of 40–100 nM, the Ca^{2+}-binding sites of CB and CR are assumed to be essentially Ca^{2+}-free (less than 9% of the Ca^{2+}-bound form of CB has been reported to exist in a resting cell) (Berggard et al., 2002a), and thus ready for Ca^{2+} binding with fast kinetics when $[Ca^{2+}]_i$ is raised.

The second, mixed Ca^{2+}/Mg^{2+} site (two of which exist in PV) binds Ca^{2+} with high affinity and Mg^{2+} with moderate affinity in a competitive manner (dissociation constants: $K_{Ca} = 10^{-7}$–10^{-9} M; $K_{Mg} = 10^{-3}$–10^{-5} M) (see ❯ *Table 11-1*). Thus, in the cytoplasm of a "resting" neuron, the two mixed Ca^{2+}/Mg^{2+} sites of PV are occupied principally by Mg^{2+}, which must dissociate before Ca^{2+} binding can occur. EF-hand domains usually exist in pairs, which form a tandem domain consisting of two helix–loop–helix regions linked by a short stretch of 5–10 amino acid residues. Hence, the majority of these proteins have an even number of EF-hand domains (six in CB and CR), the uneven number (three) for PV being rather an exception. The tandem domains are not only important for the structural stability of EF-hand domains but also for the binding of Ca^{2+} to one site that allosterically affects the affinity and the binding kinetics of the second (Nelson et al., 2002).

3.3 Metal-Binding Kinetics

Within a cell, Ca^{2+}-binding kinetics (k_{on}) can vary by more than two orders of magnitude. For proteins with Ca^{2+}-specific sites implicated in very rapid biological processes, such as muscle contraction (e.g., troponin C), k_{on} can exceed 10^8 M^{-1} s^{-1}, whereas for the slow-onset buffer PV, values in the order

Table 11-1

Properties of the CaBPs, PV, CR, and CB, and of the synthetic chelators EGTA and BAPTA (modified from Dargan et al. (2004); Schwaller et al. (2002)). Published values are marked by superscript alphabets ([a]–[j]). Other values are assumptions based on experimental data and are explained below

	EGTA	BAPTA	PV	CB slow [#]	CB fast [#]	CR
Ca^{2+}-binding sites (functional)	1 (1)	1 (1)	3 (2)	2	2	6 (5)
Apparent K_d (nM) (at pH 7.2)	150[a]	160[a]	150*[b,c]	200[d]	500[d]	1500**[e]
k_{on} (μM^{-1} s^{-1})	3–10[d,f]	100–1000[d,f]	6*[b,c]	10[d]	80[d]	100–1000***
k_{off} (s^{-1}) = K_d * k_{on}	0.5–1.5	16–160	0.9[b]	2	40	150–1500
Dwell time τ_{dwell} (ms) $1/K_{off}$	700–2000	6–60	1050	500	25	0.7–7
$D_{Cabuffer}$ (μm^2 s^{-1})	200[g]	200[g]	43[h]	25[i]	25[i]	≈25
Shuttle distance $d_{shuttle}$ (μm) $\sqrt{(6D_{Cabuffer} * \tau_{dwell})}$	28–50	3–9	16	8.5	1.9	0.3–1.1
Capture time $\tau_{capture}$ (ms)	4–10	0.04–0.4	3	5.4	0.4	0.007–0.07
$1/(K_{on} * [B])$	$[B] = 270$ μM	$[B] = 270$ μM	$[B] = 540$ μM	$[B] = 540$ μM	$[B] = 540$ μM	$[B] = 1.35$ mM
Capture distance $d_{capture}$ (μm)	0.7–1	0.07–0.2	0.4	0.8	0.2	0.03–0.09
$\sqrt{(6D_{Ca} * \tau_{capture})}$	$[B] = 270$ μM	$[B] = 270$ μM	$[B] = 540$ μM	$[B] = 540$ μM	$[B] = 540$ μM	$[B] = 1.35$ mM

[#]CB has six EF-hand domains, four of which are functional: two with fast binding kinetics and two with slower binding kinetics (Nagerl et al., 2000b). In the table, the two sets of binding sites are treated as separate entities, but evidently in the intact CB molecule binding sites are in close proximity. Cooperativity of Ca^{2+}-binding sites was not included in the model to calculate the different parameters. On- and off-rates were derived assuming $k_{off} = K_d k_{on}$

*K_d and k_{on} for PV are highly dependent on $[Mg^{2+}]$—the values stated (Schwaller et al., 2002) are estimates at physiological cytosolic $[Mg^{2+}]$ (0.6–0.9 mM)

**CR has multiple sites with different K_ds—the half-saturation value is shown ($[Ca^{2+}]_{50} = 1.5$ μM) (Schwaller et al., 1997), but higher affinities (0.3–0.4 μM for chick CR) have been reported (Stevens et al., 1997)

***The stated k_{on} for the estimated "fast" binding site(s) of CR (Edmonds et al., 2000) was used to derive the other kinetic parameters. More recent data (G. Faas, unpublished) suggests that the multiple sites of CR have differing kinetics similar to CB. The diffusion coefficients $D_{Ca\ buffer}$ for PV and CB were obtained by FRAP experiments

[a]Morris et al. (1999)

[b]Lee et al. (2000)

[c]Schwaller et al. (2002)

[d]Nagerl et al. (2000b)

[e]Schwaller et al. (1997)

[f]Naraghi (1997)

[g]Allbritton et al. (1992). Dwell times (τ_{dwell}), reflecting how long Ca^{2+} will remain bound to each buffer is defined as $1/k_{off}$. The corresponding mean distances over which the Ca^{2+} buffer complexes will diffuse before releasing bound Ca^{2+} ($d_{shuttle}$) were estimated as $\sqrt{(6D_{Ca\ buffer}\ \tau_{dwell})}$. The parameter $d_{shuttle}$ for CR was calculated assuming $D_{Ca\ buffer} = 25$. Mean capture times before a Ca^{2+} ion binds to PV, CB, or CR were calculated $\tau_{capture} = 1/(k_{on}B)$ (Stern, 1992; Roberts, 1994), where B is the concentration of Ca^{2+}-free binding sites on the buffer, assuming that Ca^{2+} ions in the cytosol are bound to immobile endogenous buffers for 90% of the time. Corresponding mean capture distances were estimated using the relation $d_{capture} = \sqrt{6D_{Ca}\tau_{capture}}$, assuming an apparent diffusion coefficient (D_{Ca}) of 20 $\mu m^2\ s^{-1}$ for Ca^{2+} in the presence of immobile endogenous buffers

[h]Schmidt et al. (2003a)

[i]Schmidt et al. (2005) and the similar value assumed for CR is based on the similar molecular mass of CB and CR. Values for EGTA and BAPTA are based on the reported mobility (283 $\mu m^2\ s^{-1}$) of another small mobile molecule, IP_3

of 3×10^6 M^{-1} s^{-1} have been reported and are directly linked to the particular Mg^{2+}/Ca^{2+} binding sites of PV (*Table 11-1* and references cited therein). In the absence of Mg^{2+}, the on-rate of Ca^{2+} binding to PV is very rapid (1.08×10^8 M^{-1} s^{-1}) (Lee et al., 2000). But at the resting cytosolic [Mg^{2+}] levels (0.3–0.9 mM) in neurons and muscle (Li-Smerin et al., 2001; Watanabe et al., 2001), the rate for Ca^{2+} binding, which is determined by the rather slow Mg^{2+} off-rate (Lee et al., 2000) (*Table 11-1*), is much slower. As aforementioned, k_{on} values for CR and CB are only now emerging. But it is generally agreed that for the fastest site(s) of CR, k_{on} probably exceed(s) 10^8 M^{-1} s^{-1} (Edmonds et al., 2000); the Ca^{2+}-binding kinetics for CB are likely to be slower (Nagerl et al., 2000b) and those for PV the slowest of the trio. The importance of the kinetic properties of PV and CB will be discussed in *Sect. 4*, where the role played by CaBPs in short-term modulation of synaptic plasticity is addressed.

3.4 Mobility and Interaction with Ligands

As a general rule, the cytosolic mobility of a molecule is proportional to its size (molecular mass). However, there are notable exceptions. In *Xenopus laevis* oocytes, the cytosolic diffusion rate of the intracellular second messenger inositol-1,4,5-trisphophate (IP_3) (283 μm^2 s^{-1}) (Allbritton et al., 1992) is much higher than that of Ca^{2+}, whose movement is impeded by the presence of slowly mobile or almost completely immobile Ca^{2+} buffers. The diffusion rate of Ca^{2+} under resting conditions ($D^* = 13$ μm^2 s^{-1}) increases to 65 μm^2 s^{-1} by raising $[Ca^{2+}]_i$ to 1 μM, at which level the immobile buffer sites are presumably saturated. The apparent mobility of Ca^{2+} can also be increased by the presence of mobile Ca^{2+} buffers, which "shuttle" this cation through the sea of immobile buffers. The mobility of PV and CB in the cytoplasm of Purkinje cells has been determined by two-photon fluorescence recovery after photobleaching (FRAP) experiments. From the recovery time constant (τ) and the mean geometry of spine heads and necks of Purkinje cells, the calculated apparent diffusion coefficients (D^*) are 43 μm^2 s^{-1} (Schmidt et al., 2003a) and 26 μm^2 s^{-1} (Schmidt et al., 2005) for PV and CB, respectively. As yet, no measurements have been reported for CR, but judging from the similarity of their molecular masses, values comparable to those for CB are expected (apparent M_r 28 kDa for CB and 30 kDa for CR). The manner in which a Ca^{2+} transient is affected by the presence of a Ca^{2+} buffer is also linked to the intracellular localization of the buffer, i.e., whether it is freely diffusible or bound to organelles such as the plasma membrane or the cytoskeleton. In Purkinje cells, PV behaves like a freely diffusible molecule, indicating that its main role therein is to act as a slow-onset Ca^{2+} buffer modulating the spatiotemporal aspects of Ca^{2+} signals (Schmidt et al., 2003b). CB on the other hand, is partially immobilized within the spines and dendrites, but not within the axons of cerebellar Purkinje cells (Schmidt et al., 2005). Immobilization occurs over several seconds and is enhanced by suprathreshold synaptic activity, and can be relieved by a synthetic peptide that resembles the putative CB-binding site of *myo*-inositol monophosphatase (Berggard et al., 2002b), thereby indicating that CB binds to immobilized *myo*-inositol monophosphatase. Consequently, CB has been postulated to act as an activity-dependent sensor targeting membrane/cytoskeleton-bound *myo*-inositol monophosphatase within neurons.

3.5 Models Used to Investigate the Role of Synthetic Ca^{2+} Buffers and CaBPs

The *Xenopus* oocyte model is widely used to study the function of channels, and more recently, Ca^{2+} signaling. The Ca^{2+} liberation through inositol-1,4,5-trisphosphate receptors (IP_3R), also occurring by a mechanism known as Ca^{2+}-induced Ca^{2+} release (CICR), is modulated by the mobile synthetic cytosolic Ca^{2+} buffers EGTA and BAPTA (Dargan et al., 2003): EGTA speeds Ca^{2+} signals and "disperses" Ca^{2+} waves into local "puffs," whereas BAPTA slows Ca^{2+} responses and promotes the "globalization" of spatially uniform Ca^{2+} signals. The distinct kinetics of individual Ca^{2+} buffers thus influences the time course and spatial distribution of IP_3-evoked Ca^{2+} signals. Like EGTA, an injection or the overexpression of PV evokes Ca^{2+} puffs, which are inhibited by the IP_3R blocker heparin. An injection of CB does not induce Ca^{2+} puffs, thereby indicating that CaBP-specific kinetics is of utmost importance (John et al., 2001). In the *Xenopus* oocyte model, the response to CR differs in several respects from that evoked by the fast buffer BAPTA

(Dargan et al., 2004). At low doses, CR induces Ca^{2+} puffs, which are never observed with BAPTA. This finding may reflect the different kinetic characteristics of the five Ca^{2+}-binding sites of CR (Faas et al., in preparation). CaBPs not only influence the release of Ca^{2+} from internal stores, but also modulate the activity of Ca^{2+} channels, including the N- and P/Q-type Ca^{2+} channels as they affect the processes of inactivation and facilitation, and which are intimately involved in Ca^{2+} influx. Modulation of the N-type Ca^{2+} channels by CaM was reported first, then that of the P/Q-type. In the former case, CaM binds to the isoleucine–glutamine motif in the carboxy terminus of the α1C subunit, and in the latter, on the α1A subunit of the channel (Lee et al., 1999; Zühlke et al., 1999). In the presence of low concentrations of intracellular Ca^{2+} chelators, Ca^{2+} influx through P/Q-type channels enhances inactivation, increases recovery from inactivation, and elicits a long-lasting facilitation of the Ca^{2+} current (Lee et al., 1999). A role for PV and CB in modulating this Ca^{2+}-dependent inactivation has been demonstrated in 293T cells transfected with a Cav2.1 (P/Q-type) Ca^{2+} channel cDNA (Kreiner et al., 2005). Interestingly, the effects are not the same as those induced by the synthetic buffers EGTA and BAPTA. The Ca^{2+}-dependent inactivation of Cav2.1 has been postulated to depend on Ca^{2+} microdomains located immediately beneath the plasma membrane. These are believed to be affected by the amplitude of the Ca^{2+} current and to be differentially modulated by PV and CB (Kreiner et al., 2005).

AU2

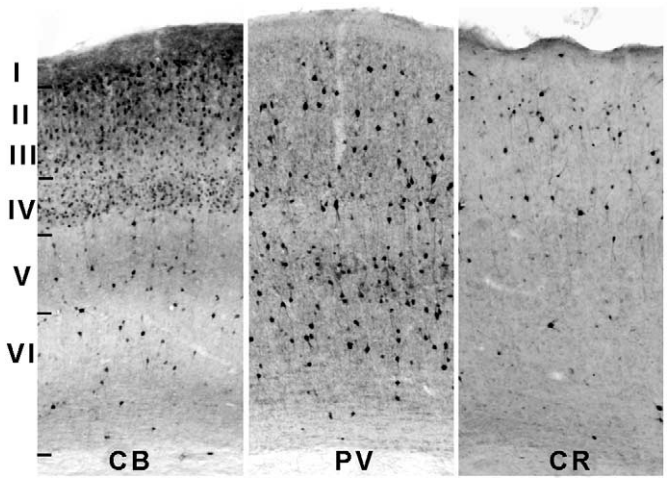
4 Distribution of PV, CB, and CR in the Brain

Each of the three CaBPs has a very distinct expression pattern in the brain exemplified in the mouse temporal cortex (◉ *Figure 11-2*) (for details see figure legend). In the next section, the principal expression patterns for PV, CB, and CR are summarized.

4.1 PV

The expression of PV in the CNS is restricted to the neurons (Celio et al., 1981) and by large, to a GABAergic subpopulation (Celio, 1986). The pattern of PV expression in the rat brain was first described

◘ Figure 11-2
Immunohistochemistry of mouse brain sections (temporal cortex) for the three CaBPs CB (left), PV (middle), and CR (right). The number of immunoreactive interneurons in the mouse cortex increases in the order with those of PV>CB>CR. Besides the dark-stained population of CB-immunoreactive interneurons, there is also a lighter-stained population composed of many pyramidal cells distributed throughout layers 2 and 3. Thus, the total number of cortical CB-immunoreactive neurons is the highest of all three CaBPs

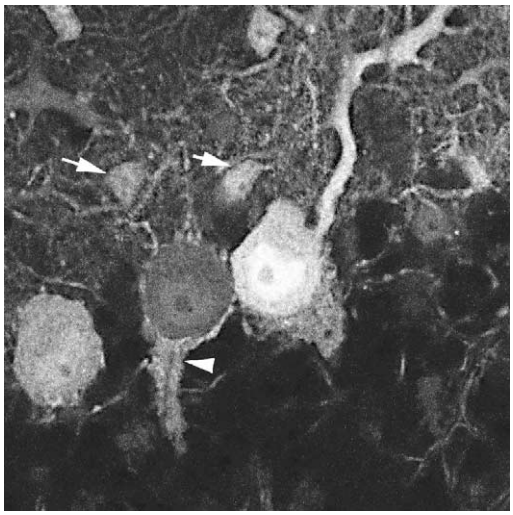


by Celio (1990). Subsequently, many reports have appeared, dealing with its developmental patterns of expression (Hendrickson et al., 1991; Solbach et al., 1991), its expression in different species (Hof et al., 1999), and changes in its expression profile under pathological conditions (Heizmann et al., 1992) (for review see Andressen et al. (1993)). In almost all brain regions, networks of GABAergic interneurons, and in particular PV-immunoreactive neurons, seem to be important in generating and promoting synchronous activity and are involved in producing coherent oscillations. PV-immunoreactive neurons exert strong inhibition: In the neocortex, the chandelier and basket cells (Kawaguchi et al., 1998; Gupta et al., 2000), in the hippocampus, the axo-axonic and basket cells (Freund et al., 1996), and in the cerebellum, the molecular layer interneurons, the stellate and basket cells (Celio, 1990; Kosaka et al., 1993), are responsible for axonic, perisomatic, and dendritic inhibition. In all these three regions consisting of many repetitive modular structures termed "microcircuits" (Grillner et al., 2005), the PV-immunoreactive neurons are essential components of the networks. In thalamic structures, specific neurons express PV (Jones et al., 1989), and in particular, in the thalamic reticular nucleus (RTN) almost the entire neuronal population is PV-immunoreactive (Seto-Ohshima et al., 1989). In the adult rat thalamus the neurons of the reticular nucleus display PV-immunostaining and PV-positive fibers densely innervate most of the dorsal thalamic domains (Frassoni et al., 1991). Furthermore, previously unobserved PV-immunoreactive GABAergic calyciform terminals, originating from local PV-immunoreactive interneuronal perikarya, have been shown to synapse with large PV-negative dendrites (Csillik et al., 2005). It has been postulated that these synapses could be instrumental in intrinsic cell-to-cell communications, possibly those involved in synchronizing thalamocortical oscillations (Csillik et al., 2005). Reciprocal connections between PV-immunoreactive interneurons are also typically encountered within microcircuits of the neocortex, the hippocampus, and the cerebellum (Grillner et al., 2005). Interestingly, these neurons are connected not only via chemical synapses, but also via electrical ones (gap junctions) involving connexin 36 (Gibson et al., 1999; Fukuda et al., 2000; Galarreta et al., 2001). In connexin-36 knockout mice, the amplitude, but not the dominant frequency of γ oscillations is reduced (Hormuzdi et al., 2001). One of the exceptions concerning the preferred relationship between GABA and PV expression is the large excitatory synapse, the calyx of Held in the medial nucleus of the trapezoid body (MNTB) of rodents. From postnatal day 6 P6 to P31, the expression of PV (and CR) increases in the presynaptic calyces, and PV is also expressed in the somata of the MNTB principal neurons (Felmy et al., 2004). The advantage of this giant excitatory synapse (the calyx of Held) in the brainstem is its accessibility to experimental manipulations: a presynaptic terminal can be patch-clamped and the Ca^{2+} signals simultaneously measured (Schneggenburger et al., 2000).

4.2 CB

The variety of neurons expressing CB is much greater than for PV (Celio, 1990) (for review see Andressen et al. (1993)) and there are significant differences between species (Hof et al., 1999). In rats, CB-immunoreactivity appears on embryonic day E 14 in the CNS and the sensory organs and on E15 in the peripheral nervous system. The adult pattern of CB expression is attained before birth in most regions of the brain. In general, the pattern manifested during brain development corresponds to that in the adult organism (for further details, see Enderlin et al. (1987); Hof et al. (1999)). This situation contrasts to that for CR, which is often only transiently expressed during development (❯ *Sect. 3.3*). CB-immunoreactive neurons are mainly local-circuit neurons (interneuron type). They are most abundant in the upper cortical layers II and III. In rodents, they include the small multipolar neurons with ascending dendrites ramifying in the molecular layer, small bitufted cells, pyramid-like cells in layer II, horizontal neurons in the molecular layer, multipolar neurons with long descending dendrites, and large double-bouquet cells (Ferrer et al., 1992). A subpopulation of CB-immunoreactive double-bouquet cells has been identified in the monkey, which is also immunoreactive for CR (Zaitsev et al., 2005). CB-immunoreactive, somatostatin-expressing Martinotti cells have also been observed (Kawaguchi et al., 1997). For details on the coexpression of CaBPs and neuropeptides and the electrophysiological classes of interneurons, see ❯ *Figure 11-3* in Markram et al. (2004) and Toledo-Rodriguez et al. (2005). Also, a subpopulation of pyramidal cells is weakly CB-positive.

Figure 11-3
Enhanced green fluorescent protein (EGFP) expression in cerebellar molecular-layer interneurons (*arrows*) and Purkinje cells in PV-EGFP mice (Meyer et al., 2002). PV-immunoreactive basket cell terminals form an axonal plexus around the Purkinje cell main dendrite, the soma, and also make a unique complex synapse—the "pinceau" (*arrowhead*)—to the initial segment of the Purkinje cell axon (Palay et al., 1974; Korn et al., 1980). Similar to native PV expression in Purkinje cells, EGFP expression levels in transgenic mice may vary from cell to cell as seen in the three depicted Purkinje cells (PV-EGFP mice were a kind gift from H. Monyer, Dept. of Clinical Neurobiology, Heidelberg, Germany; image courtesy of M. Chat, INSERM, UMR 8118, Paris, France)



In the hypothalamus, CB-immunoreactive cells are widely distributed. In the hippocampus, CB-immunoreactive interneurons are observed within all subdivisions. In the human dentate gyrus, CB is strongly AU3 expressed in granule, but not in CA1 pyramidal cells, unlike the situation in rodents, where CA1 cells are also CB-immunoreactive (Sloviter et al., 1991). In the cerebellum, Purkinje cells are the only cell type expressing CB (for a review of CB, CR, and PV expression in the cerebellum, see Bastianelli (2003)).

4.3 CR

In the human cortex, CR, like PV and CB, is principally expressed within a subclass of interneurons that occurs in all cortical layers, but most abundantly in layers II and III, and less frequently in layer VI. In layer I, the large horizontal (Cajal–Retzius) cells are CR-immunoreactive (Vogt Weisenhorn et al., 1994; Belichenko et al., 1995) (for a review of CR distribution in the rat fore- and hindbrain, see Arai et al. (1991); Jacobowitz et al. (1991); Résibois et al. (1992)). The morphology of CR-immunoreactive neurons ranges from "bipolar, bitufted, fusiform to double bouquet cells, whose long axes lie parallel to the radial axis of the cortex" (Fonseca et al., 1995). Despite the virtually identical morphological features of CR-immunoreactive and certain CB-immunoreactive neurons (double bouquet cells), colocalization of CB and CR in the same neurons is rare (Schwaller et al., 1999a). Consequently, double bouquet cells are considered to be a chemically heterogeneous neuronal population (for marker studies, see Toledo-Rodriguez et al. (2005)). In the main and the accessory olfactory bulbs, the granule, periglomerular, and mitral cells are CR-immunoreactive (Jacobowitz et al., 1991). CR-positive cells are also found in the substantia nigra compacta, in the ventral tegmental area, and in the nigrostriatal and mesolimbic projections. In the hippocampus, specific CR-immunoreactive interneurons occur within all subfields of the CA1–CA3 regions and within the dentate gyrus (Gulyas et al., 1992; Miettinen et al., 1992; Freund et al., 1996) and more than 80% of these are GABA-positive. GABA-negative CR-immunoreactive neurons are located in the hilus of the

dentate gyrus and in the stratum lucidum of the CA3 subfield; they are characterized by a spiny morphology. Spine-free CR-immunoreactive cells are usually GABA-positive. Hence, two morphologically and neurochemically distinct subpopulations of CR-immunoreactive neurons exist. CR-immunoreactive neurons of the hippocampus never coexpress PV, and rarely express CB (<5%). Thus, there is only a minimal overlap of the CR-, CB-, and PV-expressing populations. In the thalamus, CR-immunoreactive cells occur within the periventricular, the reticular, the lateral habenula, and the reunions nuclei. In the cerebellum, CR is expressed principally in the granule cells and their parallel fibers (Arai et al., 1993). The unipolar brush cells (Floris et al., 1994), the Golgi- and Lugaro cells, and a recently described neuronal type, the monodendritic cell (Braak et al., 1993), also express CR. Some mossy fibers terminating on the granule cells also show CR expression.

5 Transgenic Models Revealing the Functions of CaBPs in the Brain

In this section, current knowledge relating to the physiological functions of CB, CR, and PV within their respective neurons is summarized. This information has been acquired from experiments with different strains of knockout and transgenic mice. The first knockout mouse strain (generated by homologous recombination) was reported for CB (Airaksinen et al., 1997a), shortly followed by one for CR (Schurmans et al., 1997) and 2 years later, for PV (Schwaller et al., 1999b). The first genetically modified mouse with decreased CB expression levels using an antisense strategy was reported in 1996 (Molinari et al., 1996). Since then, several strains of mice with ectopic expression or tissue/cell-specific ablation, as well as "rescue" mice have been produced. The characteristics of these mice and their potential applications are discussed below. In the presynaptic terminals or postsynaptic regions (soma, dendrites, and spines) of neurons, repetitive Ca^{2+} transients occurring at short time intervals are a typical physiological signaling event. The manner in which a particular Ca^{2+} buffer influences the spatiotemporal characteristics of these transients (concentration, Ca^{2+} affinity, binding kinetics, and diffusion rate) has been addressed above, and the relevant information is summarized below.

5.1 PV Knockout Mice

The initial phenotype described in PV knockout (PV−/−) mice (systematic name $Pvalb^{tm1Swal}$) is related to PV's function in fast-twitch muscles (Schwaller et al., 1999b) and is well suited to appreciate the function of PV not only in muscle cells, but also in the nervous system. As a slow-onset Ca^{2+} buffer, PV does not affect the rapid rise in $[Ca^{2+}]_i$ occurring during the contraction phase, thus not competing for Ca^{2+} binding with troponin C. However, it does increase the initial rate of $[Ca^{2+}]_i$ decay, which accelerates the relaxation of an electrically induced single twitch in fast fibers (Schwaller et al., 1999b). The reverse holds true for a wild-type muscle that has undergone prolonged tetanic stimulation (for 0.2–3.2 s). Under these conditions, PV becomes saturated with Ca^{2+} and, in acting as a transient Ca^{2+} source, prolongs the decay in $[Ca^{2+}]_I$, and thus also the relaxation phase (Raymackers et al., 2000). The role of PV in pre- and postsynaptic Ca^{2+} signaling has been investigated in cerebellar neurons. In wild-type mice, and using a paired-pulse protocol, short-term depression is observed at the synapses between stellate/basket cells and Purkinje cells. In PV−/− mice, the amplitude of the second inhibitory postsynaptic potential (IPSP) is increased (facilitated), most dramatically after short interspike intervals (30 ms) (Caillard et al., 2000). At that time point, the presumably higher residual $[Ca^{2+}]_i$ in the presynaptic terminal of PV−/− cells has been postulated to cause facilitation. If the pulses are delivered at longer intervals (300 ms), between which residual $[Ca^{2+}]_i$ decays to basal levels, irrespective of the presence of PV, paired-pulse depression is observed in both wild-type and PV−/− mice. Direct measurements of $[Ca^{2+}]_i$ within varicosities of the stellate- and basket cell axons of WT and PV−/− mice have revealed that the presence of PV changes the decay in $[Ca^{2+}]_i$ from a monoexponential to a biexponential mode. This finding confirms that PV plays an essential role in modulating short-term synaptic transmission (Collin et al., 2005). In addition, the slow decay component is responsible for a robust, PV-dependent, delayed transmitter release at interneuron–interneuron synapses

subsequent to presynaptic bursts of action potentials. Thus, PV-immunoreactive molecular-layer interneurons give a random, almost steady synaptic output between bursts, with an average intensity that is proportional to the number of action potentials in the previous burst, and this can be considered as an integrating signaling mode (Collin et al., 2005). Yet in the dentate gyrus of the hippocampal formation, asynchronous release at synapses between interneurons and principal cells is mainly observed in cholecystokinin (CCK)-immunoreactive interneurons, much less in PV-interneurons, but the role of PV in this process was not directly assessed (Hefft et al., 2005).

In the hippocampus, the role of PV in axo-axonic and basket cells is very similar to the situation in cerebellar interneurons, albeit initially finding no differences between wild type and PV−/− neurons when applying a paired-pulse protocol (Vreugdenhil et al., 2003). Evidently, the steady-state level of $[Ca^{2+}]_i$ during burst-like action potentials depends upon $\Delta t/\tau$, where Δt is the time interval between action potentials, and τ the Ca^{2+}-relaxation time constant of individual Ca^{2+} transients. Hence, only when a series of 10 pulses at 33, 50, and 100 Hz are applied to hippocampal slices do differences between wild type and PV−/− neurons emerge. At frequencies greater than 20 Hz, facilitation is higher in PV−/− than in wild-type tissue slices, but the difference attains significance only after 4–5 pulses. This finding indicates that PV-immunoreactive hippocampal neurons probably possess faster Ca^{2+}-extrusion systems than Purkinje cells, such that the effects of residual $[Ca^{2+}]_i$ emerge only after repetitive stimulation. In cerebellar Purkinje cells, the mechanisms governing the kinetics of climbing fiber-mediated Ca^{2+} transients in the spiny dendrites, and in particular the role played by PV and CB, have been quantified using high-resolution confocal Ca^{2+} imaging (Schmidt et al., 2003b). As in other neurons, the spineous peak amplitude is not affected by PV deficiency. However, the biphasic nature of the $[Ca^{2+}]_i$ decay is less pronounced than in the presence of this CaBP—the typical signature of the slow binding kinetics of PV. The effects of eliminating CB from the spines and dendrites of Purkinje cells are more dramatic and are summarized in ❯ *Sect. 4.2*. As aforementioned, the subpopulation of PV-immunoreactive GABAergic interneurons is, in almost all brain regions, critically involved in strong perisomatic inhibition, and thus in controlling the output of the principal cells. A change in the inhibitory activity of these neurons in the neocortex has been proposed as a major mechanism underlying epileptic seizures (Mihaly et al., 1997). The role of PV in maintaining the stability of neuronal networks in vivo has been assessed in PV−/− mice (Schwaller et al., 2004). Pentylenetetrazole (PTZ)-induced seizures are more severe in PV−/− than in wild-type animals, although their onset is delayed. The latter findings accord with the observation in PV−/− mice that the inhibitory effect exerted by PV interneurons of the cerebellum and hippocampus is heightened in the absence of PV. In the hippocampus, PV-deficiency facilitates the $GABA_A$ergic current reversal induced by high-frequency stimulation, and thus the proconvulsive GABA-mediated depolarizing postsynaptic potential. This finding indicates that PV plays a key role in regulating the local inhibitory effects exerted by GABAergic interneurons on pyramidal neurons.

AU4

5.2 CB Knockout- and CB Antisense Mice

Similar to PV−/− mice, CB-null mutant mice (systematic name: $Calb1^{tm1Mpin}$) manifest no overt phenotype related to development, histology of the nervous system, or behavior (Airaksinen et al., 1997a). Subtle deficits in motor coordination implicate functional disturbances in the cerebellar pathways involving Purkinje neurons, since this is the only efferent of the cerebellar cortex, and the Purkinje cell is the only neuronal type that expresses CB in the cerebellum. To demonstrate the Purkinje-cell specificity of the motor phenotype, Purkinje cell-specific CB−/− mice have been generated (Barski et al., 2002) by crossing a transgenic strain expressing Cre recombinase controlled by the L7/pcp-2 gene promoter (Barski et al., 2000) with another expressing a "floxed" CB gene (Barski et al., 2002). Like the "global" CB−/− mice, these Purkinje cell-specific CB−/− mice manifest an impaired motor coordination phenotype (Barski et al., 2003). In both strains, similar changes in synaptically evoked (either after parallel or climbing fiber stimulation) postsynaptic Ca^{2+} transients are observed: the amplitude is increased and their fast, but not their slow $[Ca^{2+}]_i$ decay component has larger amplitudes in CB−/− mice than in wild-type mice (Airaksinen et al., 1997a). However, the delayed metabotropic glutamate receptor-mediated Ca^{2+} transients

were similar to those in wild-type animals (Barski et al., 2003). A detailed quantitative analysis of the role played by CB in the dendritic Ca^{2+} transients of Purkinje cells has been undertaken (Schmidt et al., 2003b). By comparing the signals from PV−/− cells with those from PV−/−CB−/− cells, the contribution of CB could be deduced and the roles of both Ca^{2+} buffers, as well as their concentration in Purkinje cells, could be modeled. In the absence of PV and CB, peak $[Ca^{2+}]_i$ amplitudes are about two-fold higher than in wild-type cells, and the decay in $[Ca^{2+}]_i$ becomes almost monophasic. Hence, the biphasic $[Ca^{2+}]_i$ decay kinetics are attributable not only to the slow binding sites of PV, but also to the high-affinity sites of CB, which are characterized by rather slow kinetics (Nagerl et al., 2000b). Interestingly, ablation of the CB gene in Purkinje cells has no effect on the LTD of Purkinje cell parallel fiber synaptic transmission, which is considered as a critical determinant of normal cerebellar function (Barski et al., 2003). This is unlike in mice deficient for components of the metabotropic glutamate receptor/IP_3/Ca^{2+} signaling pathway, where in mice lacking type 1 IP_3R the motor control impairment (severe ataxia) is associated with impairment of LTD (Matsumoto et al., 1996). In summary, motor coordination deficits in CB−/− mice are probably caused by subtle disturbances in the Ca^{2+} signaling of Purkinje cells, which, at the network level, result in the emergence of 160 Hz oscillations (Servais et al., 2005).

The role of CB in short-term plasticity has been addressed in a newly described interneuron type, the multipolar bursting cell (Blatow et al., 2003b). On the basis of existing data, saturation of a mobile cytosolic Ca^{2+} buffer has been proposed as a mechanism leading to paired-pulse facilitation (Rozov et al., 2001). More recently, saturation of CB has been shown to play a major role in paired-pulse facilitation at CB-containing synapses (Blatow et al., 2003a). Washout of cytosolic constituents via the patch pipette increases the amplitude of the first response and decreases paired-pulse facilitation, which could be restored by adding either the fast synthetic buffer BAPTA or recombinant CB. The effect is termed pseudofacilitation, since the main effect of CB (a decrease in the amplitude of the inhibitory postsynaptic potential) is on the first response in the paired-pulse protocol. The same mechanism is also present at the CB-immunoreactive facilitating excitatory mossy fiber CA3 pyramidal cell synapse, and experiments with CB−/− mice have confirmed Ca^{2+}-buffer saturation to be responsible for this presynaptic mechanism of synaptic plasticity. In many studies, a neuroprotective role for CaBPs, including CB, has been reported, details of which are summarized in ❯ *Sect. 5.* In the postmortem brains of patients with mesial temporal lobe epilepsy (mTLE), which is the predominant form of the disease in adults as well as in animal models, a loss of CB from granule cells of the dentate gyrus is observed. CB's function was addressed in both CB−/− mice (Klapstein et al., 1998) and tissue obtained from patients with Ammon's horn sclerosis (AHS) (Nagerl et al., 2000a). CB significantly increases the Ca^{2+}-dependent inactivation of voltage-dependent Ca^{2+} currents (I_{Ca}), thereby diminishing Ca^{2+} influx during repetitive neuronal firing. Addition of recombinant CB in the pipette restores I_{Ca} inactivation to levels recorded in cells with normal CB content harvested from mTLE patients without AHS. In CB−/− mice, functional properties such as the altered adaptation of action potential firing and altered paired-pulse and frequency potentiation at affected synapses are consistent with the absence of an intracellular Ca^{2+}-buffer (Klapstein et al., 1998). Thus, this is a clear example, where the absence of CB contributes to increasing the resistance against excitotoxicity, and the loss of CB in dentate gyrus granule cells may be viewed as a homeostatic mechanism to protect the surviving neurons.

Putative roles for CB in the visual and auditory systems have also been investigated. CB is abundant within hair cells of the inner ear and within distinct neurons of the auditory pathway, and it has been postulated to act as a fast buffer to speed the return of potentially toxic $[Ca^{2+}]_i$ levels to basal values. However, experiments with wild-type and CB−/− mice reveal no differences in the auditory brainstem-evoked response, in the distortion-product otoacoustic emissions, or in noise-induced trauma that results in hair cell loss in both strains (Airaksinen et al., 2000). These results argue against an essential role for CB either in hearing or in protecting against moderate noise-induced inner ear traumata, at least in mice. Furthermore, the absence of CB from horizontal cells in the retina of CB−/− mice has no apparent effect on the histology of this layer, which indicates that CB is not required for the structural maintenance of the differentiated retina (Wassle et al., 1998).

Another approach to study the role of CB was the generation of a transgenic mouse strain producing CB antisense mRNA (Molinari et al., 1996). In these mice, impairment of plasticity (LTP in hippocampal CA1 neurons by modulation of NMDA receptors (Jouvenceau et al., 1999)) and spatial learning have

been reported and linked to limiting elevations in $[Ca^{2+}]_i$ (Jouvenceau et al., 2002). Indeed, elevations in $[Ca^{2+}]_i$ effected by NMDA- or K^+-induced depolarization are prolonged in these CB-antisense mice (Pasti et al., 1999).

5.3 CR Knockout Mice

As for the other gene ablations discussed above, CR null-mutant mice (CR−/−) (systematic name: $Calb2^{tm1Map}$) are phenotypically only subtly compromised. The first reported alteration was impaired LTP in the hippocampus (Schurmans et al., 1997). In CR−/− mice, basal synaptic transmission between the perforant pathway and granule cells and between the Schaffer commissural input and CA1 pyramidal neurons is not changed. But the induction of LTP is impaired in the dentate gyrus. The impairment is of a similar magnitude to that in CR+/− mice indicating that a CR expression level of approximately 50% does not suffice to sustain normal LTP (Gurden et al., 1998). In the cerebellum, CR is prominently expressed in the granule cells that provide the major excitatory input to Purkinje cells via the parallel fibers, and long-term synaptic plasticity at the parallel fiber–Purkinje cell synapse is thought to underlie forms of motor learning also in part due to variations in $[Ca^{2+}]_i$ in presynaptic terminals of parallel fibers. Impairment in motor coordination tests is observed in CR−/− mice and is linked to altered Ca^{2+} homeostasis in Purkinje cells, which is indirectly supported by the increased Ca^{2+} saturation of CB in these cells (Schiffmann et al., 1999). As in CB−/− and PV−/− mice, the firing properties of Purkinje cells are affected in alert CR−/− mice. The most notable changes include an increase in the simple spike firing rate, a shortening of the complex spike duration, and a shortening of the spike pause (Schiffmann et al., 1999). These changes are not detected in brain slices, which indicates that CR is involved at the network level in cerebellar physiology. Direct patch-clamp recordings of mature CR−/− granule cells reveal these to have faster action potentials and to generate repetitive spike discharge showing an enhanced frequency increase with injected currents. Coinjection of the exogenous fast Ca^{2+} buffer BAPTA (150 μM) restores the wild-type situation indicating that the absence of CR-mediated Ca^{2+} buffering in CR−/− cells is responsible for the observed effects (Gall et al., 2003). In alert CR−/− mice, multielectrode recordings reveal the presence of 160 Hz local field potential oscillations in the cerebellar cortex (Cheron et al., 2004), similar to those observed in PV−/− and CB−/− mice (Servais et al., 2005). Such oscillations have never been detected in wild-type mice. After the injection of gap-junction-, $GABA_A$-, or NMDA blockers, these 160 Hz oscillations are reversibly attenuated, which suggests that they emerge via a mechanism that synchronizes Purkinje cell assemblies (mediated by excitation of the parallel fibers) and the network of coupled molecular-layer interneurons (stellate/basket cells). As CR-immunoreactive granule cells are not the only CR-expressing neurons involved in motor coordination and motor learning, the neuronal specificity of these 160 Hz oscillations has been addressed using "rescue" mice, where CR is selectively expressed in the granule cells (Bearzatto et al., 2006). In transgenic mice that express CR under the control of the $GABA_A$ receptor α6 promoter (Aller et al., 2003), granule cell-specific CR expression is achieved. When these mice are crossed with CR−/− mice, cerebellar granule cells remain the only neuronal subpopulation expressing CR. In alert "rescue" mice of this strain, granule cell excitability and Purkinje cell firing do not differ from the responses in wild-type animals, and neither 160 Hz oscillations nor an impairment in motor coordination is observed. These results demonstrate that CR expression in granule cells of the cerebellar cortex is a requirement for correct computation. Therefore, fine-tuning of granule cell excitability via Ca^{2+} homeostasis regulation is crucial for the coding and storage of information in the cerebellum. The same holds true for the subtle Ca^{2+} regulation in molecular-layer interneurons and in CB-expressing Purkinje cells (Servais et al., 2005).

5.4 Multitransgenic and Reporter Strains: CaMII-PV, Thy-1-PV, PV-EGFP, and Cre-PV

AU5 Ectopic CaBP expression has been reported only in PV transgenic mice. In the first mouse strain, the transgene consists of the rat CaMII promoter followed by the rat PV cDNA. PV expression is seen in the

spinal cord, and additionally, the rat PV transcript is detected in liver, kidney, and muscle (Beers et al., 2001). In the second transgenic line, under the control of the Thy-1 promoter (Chang et al., 1985), ectopic PV expression is detected within neurons of the CNS, and also in kidney, thymus, and spleen (Van Den Bosch et al., 2002). Using this model, the putative neuroprotective role of PV in striatal neurons and motoneurons has been addressed (see ❯ *Sect. 5*). In one interesting reporter strain, enhanced green fluorescent protein (EGFP) is expressed in virtually all PV-expressing neurons in various regions of the brain (Meyer et al., 2002). The protein is visible not only in the somata, but also in the dendrites and even in the axons (❯ *Figure 11-3*).

The high fidelity of expression is achieved by using an artificial bacterial chromosome (BAC) clone, which contains most (if not all) regulatory elements of the PV promoter. These mice have been used to investigate the electrical coupling between PV-immunoreactive cells in brain slices, i.e., between dentate gyrus basket cells in the hippocampus and multipolar cells in layer II/III of the neocortex (Meyer et al., 2002). In another study, paired recordings from PV-EGFP hippocampal basket cells were carried out to unravel the underlying mechanisms that generate γ frequency oscillations (Bartos et al., 2002). The decay in unitary IPSCs at basket cell–basket cell synapses is significantly faster than that at basket cell–principal cell synapses, indicating target cell-specific differences in IPSC kinetics. Finally, these mice have also been used for performing two-photon targeted patching that uses two-photon imaging to guide in vivo whole-cell recordings of EGFP-labeled PV-immunoreactive interneurons in the somatosensory cortex (Margrie et al., 2003). A so-called "knockin" mouse strain has also been produced, wherein an IRES-Cre-pA targeting cassette is integrated into the 3′-UTR of exon 5 of the PV gene (Hippenmeyer et al., 2005). When crossed with the appropriate floxed strain, these mice may serve to selectively express a protein in the population of PV-immunoreactive neurons. Mouse strains with a Purkinje cell-specific ablation of CB (Barski et al., 2003) and a selective rescue of CR in granule cells (Bearzatto et al., 2006) have been described. For the three CaBPs, CB, CR, and PV, all conceivable deletion combinations have been produced and described: CB−/− CR−/− (Cheron et al., 2004), CB−/−PV−/− (Vecellio et al., 2000), and CR−/−PV−/− (Bouilleret et al., 2000). The results obtained with these mice are described in ❯ *Sect. 5*. Knockout mice for all three CaBPs have also been produced and are currently undergoing investigation (B. Schwaller et al., unpublished data). Such triple-knockout mice are viable, fertile, and have no striking phenotype when maintained under normal housing conditions. And the histology of the brain also appears to be unaltered.

6 Alterations in CaBP Expression and Relation to Brain Pathologies

Numerous studies have reported alterations in the expression of CaBPs within diverse regions of the brain of deceased patients with specific pathologies, as well as in animal models of these diseases. These pathologies include Alzheimer's, Huntington's, and Parkinson's diseases, various types of ataxia, and disorders such as epilepsy, schizophrenia, bipolar disorder, and depression (for a detailed review, see Baimbridge et al. (1992); Heizmann et al. (1992); Andressen et al. (1993); Schwaller et al. (2002)). Since the early 1990s, many more reports on this topic have been published with often contradictory results. The reason for this discrepancy is discussed next.

6.1 Neuronal Loss vs. Loss of CaBP Immunoreactivity

The overt finding may be a relatively simple one: The staining of brain sections with an antibody against CB, CR, or PV may reveal region-specific differences between control samples and specimens derived from patients with a particular neurological disorder. The following questions arise from these observations: Does the immunostaining pattern reflect a downregulation in protein expression? Is it attributable to a loss of the neuronal population expressing this particular CaBP? If so, is there a correlation or a causal relationship between the disappearance of this neuronal population and CaBP expression? Unfortunately, many studies lack the appropriate controls to answer any of these questions with certainty. If a neuronal population expressing a particular CaBP in a given paradigm has a higher survival rate than a neighboring

one, this fact is incorrectly attributed to a CaBP-mediated neuroprotective effect. But how can one avoid making the most obvious mistakes? In general, most of the neurons expressing CB, CR, or PV selectively coexpress other neuronal markers. For example, PV-immunoreactive neurons are, in most regions, characteristically surrounded by a special kind of extracellular matrix termed the perineuronal net (for review see Celio et al. (1994); (1998)). In PV−/− mice, the number of neurons surrounded by a perineuronal net does not change, either in the cortex (Schwaller et al., 2004) or in the hippocampus (Vreugdenhil et al., 2003). Hence, the population of neurons that would express PV in wild-type mice does not disappear in the absence of this CaBP. Other useful markers for the PV-immunoreactive neurons are the voltage-dependent K^+ channels Kv3.1 (Du et al., 1996) and connexin 36 (Belluardo et al., 2000). Other regional markers exist also for CB- and CR-immunoreactive neurons, and those pertaining to the neocortex have been particularly well characterized (Toledo-Rodriguez et al., 2005). The question as to whether a correlation or a causal relationship exists between the disappearance of a neuronal population and CaBP expression is experimentally demanding to answer. This issue is addressed in the next two sections.

6.2 Correlation vs. Cause vs. Secondary Adaptive Changes

The putative neuroprotective roles of CB and CR in excitotoxicity have been recently reviewed (Schwaller et al., 2002), and the main conclusions are recapitulated here. In CB−/− mice, the increased resistance to ischemia manifested by hippocampal neurons in vitro and in vivo (Klapstein et al., 1998) is partially attributable to an enhanced Ca^{2+}-dependent inactivation of Ca^{2+} channels, which in turn, decreases the total Ca^{2+} load (Nagerl et al., 1998). This mechanism may also underlie the survival of dentate granule cells in mTLE patients with AHS (Nagerl et al., 2000a). This is in contrast to an earlier view that the absence of CB from the dentate granule cells of human epileptic patients "results in hyperexcitability of the dentate gyrus, which may then function as a motor for seizures" (Magloczky et al., 1997). Hence, a causal relationship between CB expression and enhanced (not decreased) vulnerability to cell death is a novel insight derived from experiments with CB−/− mice. A neuroprotective role of CB was also not supported in another model based on the presence of CB-immunoreactive neurons in the substantia nigra and the ventral tegmental, which were found to be more resistant to MPTP toxicity (Iacopino et al., 1992), an experimental model of Parkinson's; the extent of neurodegeneration is identical in CB−/− mice (Airaksinen et al., 1997b). The same holds true in weaver mice, a genetic model of degeneration of both midbrain dopaminergic neurons and cerebellar granule cells (Airaksinen et al., 1997b). Just as not to leave one with the impression that all studies using transgenic (knockout) mice yield consistent results, the extent of kainate-induced excitotoxicity (a model of mTLE) was independent of the presence of the CaBPs, CB, CR, and PV (Bouilleret et al., 2000). These findings contradict others relating both to CB−/− mice (Nagerl et al., 1998) and mTLE patients described above (Nagerl et al., 2000a). However, it is necessary to take into account the various changes that occur within the hippocampal GABAergic interneuron circuits, which play a central role in epileptogenesis (Magloczky et al., 2005) and which may be differentially affected in human mTLE and in murine models of the disease. Furthermore, cell survival in humans might be correlated with, but not causally related to, the absence of CB. A protective role for CB in other paradigms have also been reported (e.g., overexpression of CB in the striatum of rats in vivo (Yenari et al., 2001) and in vitro (D'Orlando et al., 2001)). In conclusion, the role of a particular CaBP in neuroprotection cannot be generalized; it must be carefully evaluated for each model and for each neuronal population.

AU6

The data for CR are also discrepant. In vivo, CR-containing neurons have shown to be less vulnerable than other neuronal populations to excitatory insults (Möckel et al., 1994). In several in vitro models, a neuroprotective effect of CR has been demonstrated (Pike et al., 1995; Diop et al., 1996; D'Orlando et al., 2002), although not in all (Kuznicki et al., 1996; Isaacs et al., 2000). In correlative in vivo studies, a selective sparing of CR-containing neurons in specific regions of the brain has been demonstrated for several neurodegenerative disorders such as the striatum in Huntington's disease (Cicchetti et al., 1996), in the substantia nigra pars compacta in Parkinson's disease (Mouatt-Prigent et al., 1994), and in the neocortex in Alzheimer's disease (Sampson et al., 1997). Nevertheless, in none of these studies was a causal relationship between CR expression and increased resistance (survival) corroborated.

AU7

Recent reports on the role of PV in neuroprotection using transgenic mouse models are summarized here. In CaMII-PV mice, PV overexpression protects vulnerable motoneurons from amyotrophic lateral sclerosis (ALS) patient's IgG-mediated increases in $[Ca^{2+}]_i$ (Beers et al., 2001). Furthermore, PV expression rescues motoneurons in an animal model of familial ALS: when mice expressing mutant human Cu^{2+}/Zn^{2+} superoxide dismutase (mSOD1), an enzyme involved in free oxygen radical metabolism, are crossed with CaMII-PV mice, there is a significant delay in the onset of the disease as compared with the mSOD1 mice (Beers et al., 2001). Thy-PV mice ectopically expressing PV also in motoneurons were used to address whether the increase in the Ca^{2+}-buffering capacity of motoneurons is protective. Previously, the selective vulnerability of motoneurons in ALS was attributed to the extremely low Ca^{2+}-buffering capacity of these cells (Palecek et al., 1999) in contrast to oculomotor neurons, which are extremely resistant in ALS models (Vanselow et al., 2000) and express high levels of PV. Cultured motoneurons in vitro from Thy-PV mice are significantly more resistant to kainate-induced excitotoxicity than those from control mice (Van Den Bosch et al., 2002). Furthermore, kainate-induced Ca^{2+} transients mediated via Ca^{2+}-permeable AMPA receptors—but not those induced by depolarization—were attenuated. The role of ectopic PV expression in motoneurons has also been investigated in vivo. Following nerve injury in neonatal rats, a large percentage of motoneurons die, probably owing to glutamate excitotoxicity, and as proposed also for motoneuron degenerative diseases such as ALS. In this study, one of the hindlimb sciatic nerves of newborn Thy-PV- and wild-type mice was crushed, and the effects on motoneuron survival were assessed 8 weeks later by retrograde labeling of the motoneurons innervating the tibialis anterior muscle (Dekkers et al., 2004). Motoneuron survival was more than two-fold higher in the Thy-PV mice than in the controls. However, this dramatic increase was not reflected in a significant improvement in muscle function. The putative neuroprotective role of PV in the brain has also been assessed in adult Thy-PV mice after injecting the glutamate agonist ibotenic acid into the striatum (Maetzler et al., 2004). The result was a local loss of nerve cells and reactive astrogliosis. Contrary to the expectations of a neuroprotective role for PV, an enlarged and accelerated neurodegenerative process was observed. This finding indicates that an increase in the cytosolic Ca^{2+}-buffering capacity impairs other systems involved in Ca^{2+}homeostasis and sequestration. The reduced mitochondrial volume in striatal neurons of Thy-PV mice resulting from a homeostatic mechanism induced by ectopic PV expression may render these mice more vulnerable to excitotoxic stress (for details see *Sect. 5.3*).

6.3 The "Ca^{2+} Homeostasome"

Constitutive genetic ablation of a particular gene might induce compensatory or homeostatic mechanisms. Interestingly, in knockout mice for CB, CR, and PV, the two remaining proteins are expressed at normal levels, and in none other than the wild-type neuronal populations. Hence, if compensatory mechanisms are at play, they are not operative at the level of the two remaining CaBPs. Indeed, there is no evidence that other CaBPs are significantly upregulated in these knockout strains. It has been postulated that either the promoter regions of other CaBPs are permanently inactivated in the neuronal population lacking a particular CaBP or that the relevant parameters for the two remaining CaBPs (affinities, kinetics, and diffusion) are not suited to compensate for the missing one (Schwaller et al., 2002). Nevertheless, putative adaptive changes have been reported. The spine morphology of Purkinje cells is selectively affected by the absence of CB (increased length and volume), but not by that of PV (Vecellio et al., 2000). On the other hand, the volume of mitochondria, organelles involved in Ca^{2+} sequestration and acting as a transient Ca^{2+} store, is almost twice as large in fast-twitch muscles of PV−/− mice (Chen et al., 2001). The reverse effect is observed in neurons that express PV ectopically (Maetzler et al., 2004). Within striatal neurons, which do not normally express PV, the mitochondrial volume is reduced by almost 50%, which accounts for the heightened excitotoxic injury provoked by a local injection of ibotenic acid into Thy-PV mice. In several neurological diseases, including schizophrenia, a decrease in the population of prefrontal GABAergic PV-immunoreactive neurons has been reported (Lewis et al., 2005). In the same subpopulation, mRNAs for the 67-kDa isoform of glutamate decarboxylase (GAD67), an enzyme for GABA synthesis and for the GABA membrane transporter (GAT1), were decreased, which resulted in impaired neurotransmission. Deficient

neurotrophin signaling via the tyrosine kinase receptor B was postulated as the underlying pathogenetic mechanism. The downregulation of PV observed under these conditions is viewed as a compensatory mechanism to increase GABAergic transmission (Lewis et al., 2005). This postulate is moreover based on the finding that repetitive inhibitory postsynaptic currents are facilitated, and the power of γ oscillations is increased in the hippocampus of PV−/− mice (Vreugdenhil et al., 2003), indicating increased inhibition. Hence, the "Ca^{2+} buffers," CR, CB, and PV, constitute an integral part of the precisely tuned system involved in Ca^{2+} homeostasis and Ca^{2+} signaling. Elimination of a CaBP does not induce the obvious compensatory mechanism (i.e., the upregulation of another Ca^{2+} buffer), but leads to more subtle changes at the level of cell morphology or within specific Ca^{2+}-uptake or -release systems. Apparently, CaBP-deficient cells make use of the components of the "Ca^{2+}-signaling toolkit" (Berridge et al., 2003), which they adapt to conform to the situation prevailing in "normal" wild-type cells.

AU8

7 Conclusion

The three EF-hand Ca^{2+}-binding proteins, CB, CR, and PV, which, in classical terminology, are referred to as "Ca^{2+} buffers," are integral components of Ca^{2+}-signaling and Ca^{2+} homeostatic mechanisms in specific CNS neurons. The properties of PV, particularly its great mobility and inability to interact with other cellular components, render this molecule the prototype of a slow-onset Ca^{2+} buffer. While most of the functions of CB and CR can be explained in terms of the fast Ca^{2+}-buffering kinetics of their Ca^{2+}-specific binding sites, their additional roles as Ca^{2+} modulators are now starting to emerge. The best-characterized interaction is the binding of CB to the enzyme *myo*-inositol monophosphatase, which links IP_3- and Ca^{2+}-signaling pathways, at least in Purkinje cells. Novel insights into the important functions of CB, CR, and PV in the CNS have been obtained from genetically modified mice using both in vitro and in vivo approaches. Further progress in the field is expected using even more subtle genetic tools, such as subpopulation-specific inducible CaBP "knockout" and "knockin" strains, as well as multitransgenic mice with alterations to several components of the neuronal Ca^{2+}-signaling system. These models should help us tackle the tremendous complex mechanisms underlying intraneuronal Ca^{2+} signaling and Ca^{2+} homeostasis.

Acknowledgments

The help of M. Celio, Fribourg, Switzerland and I. Llano, Paris, France, for the critical reading of the manuscript is highly appreciated. ❯ *Figure 11-3* was provided by M. Chat, INSERM, UMR 8118, Paris, France. This work was partially supported by a grant from the Swiss National Science Foundation (no. 3100A0-100400/1 to B. S.).

References

Airaksinen MS, Eilers J, Garaschuk O, Thoenen H, Konnerth A, et al. 1997a. Ataxia and altered dendritic calcium signaling in mice carrying a targeted null mutation of the calbindin D-28k gene. Proc Natl Acad Sci USA 94: 1488-1493.

Airaksinen MS, Thoenen H, Meyer M. 1997b. Vulnerability of midbrain dopaminergic neurons in calbindin D-28k-deficient mice: Lack of evidence for a neuroprotective role of endogenous calbindin in MPTP-treated and weaver mice. Eur J Neurosci 9: 120-127.

Airaksinen L, Virkkala J, Aarnisalo A, Meyer M, Ylikoski J, et al. 2000. Lack of calbindin D-28k does not affect hearing level or survival of hair cells in acoustic trauma. ORL J Otorhinolaryngol Relat Spec 62: 9-12.

Allbritton NL, Meyer T, Stryer L. 1992. Range of messenger action of calcium ion and inositol-1,4,5,-trisphophate. Science 258: 1812-1815.

Aller MI, Jones A, Merlo D, Paterlini M, Meyer AH, et al. 2003. Cerebellar granule cell Cre recombinase expression. Genesis 36: 97-103.

Andressen C, Blümcke I, Celio MR. 1993. Calcium-binding proteins: Selective markers of nerve cells. Cell Tissue Res 271: 181-208.

Arai R, Jacobowitz DM, Deura S. 1993. Ultrastructural localization of calretinin immunoreactivity in lobule V of the rat cerebellum. Brain Res 613: 300-304.

Arai R, Winsky L, Arai M, Jacobowitz DM. 1991. Immunohistochemical localization of calretinin in the rat hindbrain. J Comp Neurol 310: 21-44.

Baimbridge KG, Celio MR, Rogers JH. 1992. Calcium-binding proteins in the nervous system. Trends Neurosci 15: 303-308.

Barski JJ, Dethleffsen K, Meyer M. 2000. Cre recombinase expression in cerebellar Purkinje cells. Genesis 28: 93-98.

Barski JJ, Hartmann J, Rose CR, Hoebeek F, Morl K, et al. 2003. Calbindin in cerebellar Purkinje cells is a critical determinant of the precision of motor coordination. J Neurosci 23: 3469-3477.

Barski JJ, Morl K, Meyer M. 2002. Conditional inactivation of the calbindin D-28k (Calb1) gene by Cre/loxP-mediated recombination. Genesis 32: 165-168.

Bartos M, Vida I, Frotscher M, Meyer A, Monyer H, et al. 2002. Fast synaptic inhibition promotes synchronized γ oscillations in hippocampal interneuron networks. Proc Natl Acad Sci USA 99: 13222-13227.

Bastianelli E. 2003. Distribution of calcium-binding proteins in the cerebellum. Cerebellum 2: 242-262.

Bearzatto B, Servais L, Roussel C, Gall D, Baba-Aissa F, et al. 2006. Targeted calretinin expression in granule cells of calretinin-null mice restores normal cerebellar functions. FASEB J 20: 380-382.

Beers DR, Ho BK, Siklos L, Alexianu ME, Mosier DR, et al. 2001. Parvalbumin overexpression alters immune-mediated increases in intracellular calcium, and delays disease onset in a transgenic model of familial amyotrophic lateral sclerosis. J Neurochem 79: 499-509.

Belichenko PV, Vogt WD, Myklossy J, Celio MR. 1995. Calretinin-positive Cajal–Retzius cells persist in the adult human neocortex. Neuroreport 6: 1869-1874.

Belluardo N, Marko G, Trovato-Salinaro A, Le Gurun S, Charollais A, et al. 2000. Expression of connexin36 in the adult and developing rat brain. Brain Res 865: 121-138.

Berggard T, Miron S, Onnerfjord P, Thulin E, Akerfeldt KS, et al. 2002a. Calbindin D-28k exhibits properties characteristic of a Ca^{2+} sensor. J Biol Chem 28: 28.

Berggard T, Szczepankiewicz O, Thulin E, Linse S. 2002b. *Myo*-inositol monophosphatase is an activated target of calbindin D-28k. J Biol Chem 277: 41954-41959.

Berridge MJ. 1998. Neuronal calcium signaling. Neuron 21: 13-26.

Berridge MJ, Bootman MD, Roderick HL. 2003. Calcium signaling: Dynamics, homeostasis, and remodeling. Nat Rev Mol Cell Biol 4: 517-529.

Blatow M, Caputi A, Burnashev N, Monyer H, Rozov A. 2003a. Ca^{2+} buffer saturation underlies paired-pulse facilitation in calbindin D-28k-containing terminals. Neuron 38: 79-88.

Blatow M, Rozov A, Katona I, Hormuzdi SG, Meyer AH, et al. 2003b. A novel network of multipolar bursting interneurons generates θ frequency oscillations in neocortex. Neuron 38: 805-817.

Bouilleret V, Schwaller B, Schurmans S, Celio MR, Fritschy JM. 2000. Neurodegenerative and morphogenic changes in a mouse model of temporal lobe epilepsy do not depend on the expression of the calcium-binding proteins parvalbumin, calbindin, or calretinin. Neuroscience 97: 47-58.

Braak E, Braak H. 1993. The new monodendritic neuronal type within the adult human cerebellar granule cell layer shows calretinin-immunoreactivity. Neurosci Lett 154: 199-202.

Braunewell KH, Gundelfinger ED. 1999. Intracellular neuronal calcium sensor proteins: a family of EF-hand calcium-binding proteins in search of a function. Cell Tissue Res 295: 1-12.

Burgoyne RD, Weiss JL. 2001. The neuronal calcium sensor family of Ca^{2+}-binding proteins. Biochem J 353: 1-12.

Caillard O, Moreno H, Schwaller B, Llano I, Celio MR, et al. 2000. Role of the calcium-binding protein parvalbumin in short-term synaptic plasticity. Proc Natl Acad Sci USA 97: 13372-13377.

Celio MR. 1986. Parvalbumin in most γ-aminobutyric acid-containing neurons of the rat cerebral cortex. Science 231: 995-997.

Celio MR. 1990. Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience 35: 375-475.

Celio MR, Blümcke I. 1994. Perineuronal nets—a specialized form of extracellular matrix in the adult nervous system. Brain Res Rev 19: 128-145.

Celio MR, Heizmann CW. 1981. Calcium-binding protein parvalbumin as a neuronal marker. Nature 293: 300-302.

Celio M, Pauls T, Schwaller B, editors. 1996. Guidebook to the Calcium-Binding Proteins. Oxford: Oxford University Press.

Celio MR, Spreafico R, De Biasi S, Vitellaro-Zuccarello L. 1998. Perineuronal nets: Past and present. Trends Neurosci 21: 510-515.

Chang HC, Seki T, Moriuchi T, Silver J. 1985. Isolation and characterization of mouse Thy-1 genomic clones. Proc Natl Acad Sci USA 82: 3819-3823.

Chen G, Carroll S, Racay P, Dick J, Pette D, et al. 2001. Deficiency in parvalbumin increases fatigue resistance in fast-twitch muscle and upregulates mitochondria. Am J Physiol (Cell Physiol) 281: C114-C122.

Cheron G, Gall D, Servais L, Dan B, Maex R, et al. 2004. Inactivation of calcium-binding protein genes induces 160 Hz oscillations in the cerebellar cortex of alert mice. J Neurosci 24: 434-441.

Cheung WT, Richards DE, Rogers JH. 1993. Calcium binding by chick calretinin and rat calbindin D-28k synthesized in bacteria. Eur J Biochem 215: 401-410.

Cicchetti F, Parent A. 1996. Striatal interneurons in Huntington's disease: Selective increase in the density of calretinin-immunoreactive medium-sized neurons. Mov Disord 11: 619-626.

Cohen P, Klee CB. 1988. Calmodulin. Amsterdam-New York-Oxford: Elsevier

Collin T, Chat M, Lucas MG, Moreno H, Racay P, et al. 2005. Developmental changes in parvalbumin regulate presynaptic Ca^{2+} signaling. J Neurosci 25: 96-107.

Cox JA. 1996. Techniques for measuring the binding of Ca^{2+} and Mg^{2+} to calcium-binding proteins. Guidebook to the Calcium-Binding Proteins. Celio M, Pauls T, Schwaller B, editors. Oxford: Oxford University Press, pp. 1-12.

Csillik B, Mihaly A, Krisztin-Peva B, Chadaide Z, Samsam M, et al. 2005. GABAergic parvalbumin-immunoreactive large calyciform presynaptic complexes in the reticular nucleus of the rat thalamus. J Chem Neuroanat 30: 17-26.

D'Orlando C, Celio MR, Schwaller B. 2002. Calretinin and calbindin D-28k, but not parvalbumin, protect against glutamate-induced excitotoxicity in transfected N18-RE 105 neuroblastoma–retina hybrid cells. Brain Res 945: 181-190.

D'Orlando C, Fellay B, Schwaller B, Salicio V, Bloc A, et al. 2001. Calretinin and calbindin D-28k delay the onset of cell death after excitotoxic stimulation in transfected P19 cells. Brain Res 909: 145-158.

Dargan SL, Parker I. 2003. Buffer kinetics shape the spatiotemporal patterns of IP3-evoked Ca^{2+} signals. J Physiol 553: 775-788.

Dargan SL, Schwaller B, Parker I. 2004. Spatiotemporal patterning of IP3-mediated Ca^{2+} signals in *Xenopus* oocytes by Ca^{2+}-binding proteins. J Physiol 556: 447-461.

Dekkers J, Bayley P, Dick JR, Schwaller B, Berchtold MW, et al. 2004. Overexpression of parvalbumin in transgenic mice rescues motoneurons from injury-induced cell death. Neuroscience 123: 459-466.

Diop AG, Dussartre C, Barthe D, Hugon J. 1996. Neuroprotective properties of calretinin against the HIV-1 gp120 toxicity. Neurosci Res Commun 18: 107-114.

Du J, Zhang L, Weiser M, Rudy B, McBain CJ. 1996. Developmental expression and functional characterization of the potassium channel subunit Kv3.1b in parvalbumin-containing interneurons of the rat hippocampus. J Neurosci 16: 506-518.

Edmonds B, Reyes R, Schwaller B, Roberts WM. 2000. Calretinin modifies presynaptic calcium signaling in frog saccular hair cells. Nat Neurosci 3: 786-790.

Enderlin S, Norman AW, Celio MR. 1987. Ontogeny of the calcium-binding protein calbindin D-28k in the rat nervous system. Anat Embryol 177: 15-28.

Faas GC, Schwaller B, Vergara JL, Mody I. 2003. Binding kinetics of calretinin-22k determined by flash photolysis of caged calcium. Soc Neurosci Abstr p. 791.4

Felmy F, Schneggenburger R. 2004. Developmental expression of the Ca^{2+}-binding proteins, calretinin and parvalbumin, at the calyx of Held of rats and mice. Eur J Neurosci 20: 1473-1482.

Ferrer I, Tunon T, Soriano E, del Rio A, Iraizoz I, et al. 1992. Calbindin immunoreactivity in normal human temporal neocortex. Brain Res 572: 33-41.

Fierro L, Llano I. 1996. High endogenous calcium buffering in Purkinje cells from rat cerebellar slices. J Physiol 496: 617-625.

Floris A, Dino M, Jacobowitz DM, Mugnaini E. 1994. The unipolar brush cells of the rat cerebellar cortex and cochlear nucleus are calretinin-positive: a study by light and electron microscopic immunocytochemistry. Anat Embryol (Berl) 189: 495-520.

Fonseca M, Soriano E. 1995. Calretinin-immunoreactivity neurons in the normal temporal cortex and in Alzheimer's disease. Brain Res 691: 83-91.

Frassoni C, Bentivoglio M, Spreafico R, Sanchez MP, Puelles L, et al. 1991. Postnatal development of calbindin and parvalbumin immunoreactivity in the thalamus of the rat. Brain Res Dev Brain Res 58: 243-249.

Freund TF, Buzsaki G. 1996. Interneurons of the hippocampus. Hippocampus 6: 347-470.

Fukuda T, Kosaka T. 2000. Gap junctions linking the dendritic network of GABAergic interneurons in the hippocampus. J Neurosci 20: 1519-1528.

Galarreta M, Hestrin S. 2001. Electrical synapses between GABA-releasing interneurons. Nat Rev Neurosci 2: 425-433.

Gall D, Roussel C, Susa I, D&Angelo E, Rossi P, et al. 2003. Altered neuronal excitability in cerebellar granule cells of mice lacking calretinin. J Neurosci 23: 9320-9327.

Gibson JR, Beierlein M, Connors BW. 1999. Two networks of electrically coupled inhibitory neurons in neocortex. Nature 402: 75-79.

Grillner S, Markram H, De Schutter E, Silberberg G, Le Beau FE. 2005. Microcircuits in action—from CPGs to neocortex. Trends Neurosci 28: 525-533.

Gulyas AI, Miettinen R, Jacobowitz DM, Freund TF. 1992. Calretinin is present in nonpyramidal cells of the rat hippocampus—I. A new type of neuron specifically associated with the mossy fibre system. Neuroscience 48: 1-27.

Gupta A, Wang Y, Markram H. 2000. Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. Science 287: 273-278.

Gurden H, Schiffmann SN, Lemaire M, Bohme GA, Parmentier M, et al. 1998. Calretinin expression as a critical component in the control of dentate gyrus long-term potentiation induction in mice. Eur J Neurosci 10: 3029-3033.

Hackney CM, Mahendrasingam S, Penn A, Fettiplace R. 2005. The concentrations of calcium-buffering proteins in mammalian cochlear hair cells. J Neurosci 25: 7867-7875.

Hefft S, Jonas P. 2005. Asynchronous GABA release generates long-lasting inhibition at a hippocampal interneuron–principal neuron synapse. Nat Neurosci 8: 1319-1328.

Heizmann CW, Braun K. 1992. Changes in Ca^{2+}-binding proteins in human neurodegenerative disorders. Trends Neurosci 15: 259-264.

Hendrickson AE, Van Brederode JF, Mulligan KA, Celio MR. 1991. Development of the calcium-binding protein parvalbumin and calbindin in monkey striate cortex. J Comp Neurol 307: 626-646.

Hippenmeyer S, Vrieseling E, Sigrist M, Portmann T, Laengle C, et al. 2005. A developmental switch in the response of DRG neurons to ETS transcription factor signaling. PLoS Biol 3: e159.

Hof PR, Glezer II, Conde F, Flagg RA, Rubin MB, et al. 1999. Cellular distribution of the calcium-binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: Phylogenetic and developmental patterns. J Chem Neuroanat 16: 77-116.

Hormuzdi SG, Pais I, Le Beau FE, Towers SK, Rozov A, et al. 2001. Impaired electrical signaling disrupts γ frequency oscillations in connexin 36-deficient mice. Neuron 31: 487-495.

Hubbard MJ, McHugh NJ. 1995. Calbindin 28 kDa and calbindin 30 kDa (calretinin) are substantially localized in the particulate fraction of rat brain. FEBS Lett 374: 333-337.

Iacopino A, Christakos S, German D, Sonsalla PK, Altar CA. 1992. Calbindin D-28K-containing neurons in animal models of neurodegeneration: Possible protection from excitotoxicity. Brain Res Mol Brain Res 13: 251-261.

Ikura M. 1996. Calcium binding and conformational response in EF-hand proteins. Trends Biochem Sci 1: 14-17.

Isaacs KR, Wolpoe ME, Jacobowitz DM. 2000. Vulnerability to calcium-induced neurotoxicity in cultured neurons expressing calretinin. Exp Neurol 163: 311-323.

Jacobowitz DM, Winsky L. 1991. Immunocytochemical localization of calretinin in the forebrain of the rat. J Comp Neurol 304: 198-218.

John LM, Mosquera-Caro M, Camacho P, Lechleiter JD. 2001. Control of IP_3-mediated Ca^{2+} puffs in *Xenopus laevis* oocytes by the Ca^{2+}-binding protein parvalbumin. J Physiol 535: 3-16.

Jones EG, Hendry SH. 1989. Differential calcium-binding protein immunoreactivity distinguishes classes of relay neurons in monkey thalamic nuclei. Eur J Neurosci 1: 222-246.

Jouvenceau A, Potier B, Battini R, Ferrari S, Dutar P, et al. 1999. Glutamatergic synaptic responses and long-term potentiation are impaired in the CA1 hippocampal area of calbindin D-28k-deficient mice. Synapse 33: 172-180.

Jouvenceau A, Potier B, Poindessous-Jazat F, Dutar P, Slama A, et al. 2002. Decrease in calbindin content significantly alters LTP but not NMDA receptor and calcium channel properties. Neuropharmacology 42: 444-458.

Kawaguchi Y, Kubota Y. 1997. GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb Cortex 7: 476-486.

Kawaguchi Y, Kubota Y. 1998. Neurochemical features and synaptic connections of large physiologically identified GABAergic cells in the rat frontal cortex. Neuroscience 85: 677-701.

Kawasaki H, Nakayama S, Kretsinger RH. 1998. Classification and evolution of EF-hand proteins. Biometals 11: 277-295.

Klapstein GJ, Vietla S, Lieberman DN, Gray PA, Airaksinen MS, et al. 1998. Calbindin D-28k fails to protect hippocampal neurons against ischemia in spite of its cytoplasmic calcium-buffering properties: Evidence from calbindin D-28k knockout mice. Neuroscience 85: 361-373.

Korn H, and Axelrad H. 1980. Electrical inhibition of Purkinje cells in the cerebellum of the rat. Proc Natl Acad Sci USA 77: 6244-6247.

Kosaka T, Kosaka K, Nakayama T, Hunziker W, Heizmann CW. 1993. Axons and axon terminals of cerebellar Purkinje cells and basket cells have higher levels of parvalbumin immunoreactivity than somata and dendrites: Quantitative analysis by immunogold labeling. Exp Brain Res 93: 483-491.

Kreiner L, Lee A. 2005. Endogenous and exogenous Ca^{2+} buffers differentially modulate Ca^{2+}-dependent inactivation of CAV2.1 Ca^{2+} channels. J Biol Chem 281: 4691-4698.

Kretsinger RH, Nockolds CE. 1973. Carp muscle calcium-binding protein. II. Structure determination and general description. J Biol Chem 248: 3313-3326.

Kuznicki J, Isaacs KR, Jacobowitz DM. 1996. The expression of calretinin in transfected PC12 cells provides no protection against Ca^{2+}-overload or trophic factor deprivation. Biochim Biophys Acta 1313: 194-200.

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, et al. 2001. Initial sequencing and analysis of the human genome. Nature 409: 860-921.

Leclerc I, Sturchler E, Heizmann CW. 2006. Calcium regulation by EF-hand proteins in the brain. Handbook of Neurochemistry and Molecular Neurobiology 3rd ed. Lajtha A, editor. New York: Springer.

Lee A, Wong ST, Gallagher D, Li B, Storm DR, et al. 1999. Ca^{2+}/calmodulin binds to and modulates P/Q-type calcium channels. Nature 399: 155-159.

Lee SH, Schwaller B, Neher E. 2000. Kinetics of Ca^{2+} binding to parvalbumin in bovine chromaffin cells: Implications for $[Ca^{2+}]$ transients of neuronal dendrites. J Physiol 525 Pt 2: 419-432.

Lewis DA, Hashimoto T, Volk DW. 2005. Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6: 312-324.

Li-Smerin Y, Levitan ES, Johnson JW. 2001. Free intracellular Mg^{2+} concentration and inhibition of NMDA responses in cultured rat neurons. J Physiol 533: 729-743.

Maeda H, Ellis-Davies GC, Ito K, Miyashita Y, Kasai H. 1999. Supralinear Ca^{2+} signaling by cooperative and mobile Ca^{2+} buffering in Purkinje neurons. Neuron 24: 989-1002.

Maetzler W, Nitsch C, Bendfeldt K, Racay P, Vollenweider F, et al. 2004. Ectopic parvalbumin expression in mouse forebrain neurons increases excitotoxic injury provoked by ibotenic acid injection into the striatum. Exp Neurol 186: 78-88.

Magloczky Z, Freund TF. 2005. Impaired and repaired inhibitory circuits in the epileptic human hippocampus. Trends Neurosci 28: 334-340.

Magloczky Z, Halasz P, Vajda J, Czirjak S, Freund TF. 1997. Loss of calbindin D-28K immunoreactivity from dentate granule cells in human temporal lobe epilepsy. Neuroscience 76: 377-385.

Maki M, Kitaura Y, Satoh H, Ohkouchi S, Shibata H. 2002. Structures, functions, and molecular evolution of the penta-EF-hand Ca^{2+}-binding proteins. Biochim Biophys Acta 1600: 51-60.

Marenholz I, Heizmann CW, Fritz G. 2004. S100 proteins in mouse and man: From evolution to function and pathology (including an update of the nomenclature). Biochem Biophys Res Commun 322: 1111-1122.

Margrie TW, Meyer AH, Caputi A, Monyer H, Hasan MT, et al. 2003. Targeted whole-cell recordings in the mammalian brain in vivo. Neuron 39: 911-918.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, et al. 2004. Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 5: 793-807.

Matsumoto M, Nakagawa T, Inoue T, Nagata E, Tanaka K, et al. 1996. Ataxia and epileptic seizures in mice lacking type 1 inositol-1,4,5-trisphosphate receptor. Nature 379: 168-171.

Meyer AH, Katona I, Blatow M, Rozov A, Monyer H. 2002. In vivo labeling of parvalbumin-positive interneurons and analysis of electrical coupling in identified neurons. J Neurosci 22: 7055-7064.

Miettinen R, Gulyas AI, Baimbridge KG, Jacobowitz DM, Freund TF. 1992. Calretinin is present in nonpyramidal cells of the rat hippocampus—II. Coexistence with other calcium-binding proteins and GABA. Neuroscience 48: 29-43.

Mihaly A, Szente M, Dubravcsik Z, Boda B, Kiraly E, et al. 1997. Parvalbumin- and calbindin-containing neurons express c-fos protein in primary and secondary (mirror) epileptic foci of the rat neocortex. Brain Res 761: 135-145.

Möckel V, Fischer G. 1994. Vulnerability to excitotoxic stimuli of cultured rat hippocampal neurons containing the calcium-binding proteins calretinin and calbindin D-28k. Brain Res 648: 109-120.

Molinari S, Battini R, Ferrari S, Pozzi L, Killcross AS, et al. 1996. Deficits in memory and hippocampal long-term potentiation in mice with reduced calbindin D-28k expression. Proc Natl Acad Sci USA 93: 8028-8033.

Morris SA, Correa V, Cardy TJ, O'Beirne G, Taylor CW. 1999. Interactions between inositol trisphosphate receptors and fluorescent Ca^{2+} indicators. Cell Calcium 25: 137-142.

Mouatt-Prigent A, Agid Y, Hirsch EC. 1994. Does the calcium-binding protein calretinin protect dopaminergic neurons against degeneration in Parkinson's disease? Brain Res 668: 62-70.

Muller A, Kukley M, Stausberg P, Beck H, Muller W, et al. 2005. Endogenous Ca^{2+} buffer concentration and Ca^{2+} microdomains in hippocampal neurons. J Neurosci 25: 558-565.

Nagerl UV, Mody I. 1998. Calcium-dependent inactivation of high-threshold calcium currents in human dentate gyrus granule cells. J Physiol 509 (Pt 1): 39-45.

Nagerl UV, Mody I, Jeub M, Lie AA, Elger CE, et al. 2000a. Surviving granule cells of the sclerotic human hippocampus have reduced Ca^{2+} influx because of a loss of calbindin D-28k in temporal lobe epilepsy. J Neurosci 20: 1831-1836.

Nagerl UV, Novo D, Mody I, Vergara JL. 2000b. Binding kinetics of calbindin D-28k determined by flash photolysis of caged Ca^{2+}. Biophys J 79: 3009-3018.

Naraghi M. 1997. T-jump study of calcium-binding kinetics of calcium chelators. Cell Calcium 22: 255-268.

Nelson MR, Chazin WJ. 1998. Structures of EF-hand Ca^{2+}-binding proteins: Diversity in the organization, packing, and response to Ca^{2+} binding. Biometals 11: 297-318.

Nelson MR, Thulin E, Fagan PA, Forsen S, Chazin WJ. 2002. The EF-hand domain: a globally cooperative structural unit. Protein Sci 11: 198-205.

Palay SL, Chan-Palay V. 1974. The Cerebellum. Berlin-New York: Springer Verlag.

Palecek J, Lips MB, Keller BU. 1999. Calcium dynamics and buffering in motoneurons of the mouse spinal cord. J Physiol (Lond) 520 Pt 2: 485-502.

Pasti L, Carmignoto G, Pozzan T, Battini R, Ferrari S, et al. 1999. Cellular calcium handling in brain slices from calbindin D-28k-deficient mice. Neuroreport 10: 2367-2372.

Pike CJ, Cotman CW. 1995. Calretinin-immunoreactivity neurons are resistant to β-amyloid toxicity in vitro. Brain Res 671: 293-298.

Plogmann D, Celio MR. 1993. Intracellular concentration of parvalbumin in nerve cells. Brain Res 600: 273-279.

Raymackers JM, Gailly P, Schoor MC, Pette D, Schwaller B, et al. 2000. Tetanus relaxation of fast skeletal muscles of the mouse made parvalbumin deficient by gene inactivation. J Physiol 527: 355-364.

Résibois A, Rogers JH. 1992. Calretinin in rat brain: An immunohistochemical study. Neurosci 46: 101-134.

Roberts WM. 1994. Localization of calcium signals by a mobile calcium buffer in frog saccular hair cells. J Neurosci 14: 3246-3262.

Rozov A, Burnashev N, Sakmann B, Neher E. 2001. Transmitter release modulation by intracellular Ca^{2+} buffers in facilitating and depressing nerve terminals of pyramidal cells in layer 2/3 of the rat neocortex indicates a target cell-specific difference in presynaptic calcium dynamics. J Physiol 531: 807-826.

Sampson VL, Morrison JH, Vickers JC. 1997. The cellular basis for the relative resistance of parvalbumin- and calretinin-immunoreactive neocortical neurons to the pathology of Alzheimer's disease. Exp Neurol 145: 295-302.

Schiffmann SN, Cheron G, Lohof A, d&Alcantara P, Meyer M, et al. 1999. Impaired motor coordination and Purkinje cell excitability in mice lacking calretinin. Proc Natl Acad Sci USA 96: 5257-5262.

Schmidt H, Brown EB, Schwaller B, Eilers J. 2003a. Diffusional mobility of parvalbumin in spiny dendrites of cerebellar Purkinje neurons quantified by fluorescence recovery after photobleaching. Biophys J 84: 2599-2608.

Schmidt H, Schwaller B, Eilers J. 2005. Calbindin D-28k targets *myo*-inositol monophosphatase in spines and dendrites of cerebellar Purkinje neurons. Proc Natl Acad Sci USA 102: 5850-5855.

Schmidt H, Stiefel KM, Racay P, Schwaller B, Eilers J. 2003b. Mutational analysis of dendritic Ca^{2+} kinetics in rodent Purkinje cells: Role of parvalbumin and calbindin D-28k. J Physiol 551: 13-32.

Schneggenburger R, Neher E. 2000. Intracellular calcium dependence of transmitter release rates at a fast central synapse. Nature 406: 889-893.

Schurmans S, Schiffmann SN, Gurden H, Lemaire M, Lipp H-P, et al. 1997. Impaired LTP induction in the dentate gyrus of calretinin-deficient mice. Proc Natl Acad Sci USA 94: 10415-10420.

Schwaller B. 2004. Calcium-binding Proteins. In Nature Encyclopedia of Life Sciences, p. E0390502 (doi:0390510.0391038/npg.els.0003905) http://www.els.net/. London: Nature Publishing Group.

Schwaller B, Bruckner G, Celio MR, Hartig W. 1999a. A polyclonal goat antiserum against the calcium-binding protein calretinin is a versatile tool for various immunochemical techniques. J Neurosci Methods 92: 137-144.

Schwaller B, Celio MR, Hunziker W. 1995. Alternative splicing of calretinin mRNA leads to different forms of calretinin. Eur J Biochem 230: 424-430.

Schwaller B, Dick J, Dhoot G, Carroll S, Vrbova G, et al. 1999b. Prolonged contraction–relaxation cycle of fast-twitch muscles in parvalbumin knockout mice. Am J Physiol (Cell Physiol) 276: C395-403.

Schwaller B, Durussel I, Jermann D, Herrmann B, Cox JA. 1997. Comparison of the Ca^{2+}-binding properties of human recombinant calretinin-22k and calretinin. J Biol Chem 272: 29663-29671.

Schwaller B, Meyer M, Schiffmann SN. 2002. "New" functions for "old" proteins: The role of the calcium-binding proteins calbindin D-28k, calretinin, and parvalbumin, in cerebellar physiology. Studies with knockout mice. Cerebellum 1: 241-258.

Schwaller B, Tetko IV, Tandon P, Silveira DC, Vreugdenhil M, et al. 2004. Parvalbumin deficiency affects network properties resulting in increased susceptibility to epileptic seizures. Mol Cell Neurosci 25: 650-663.

Servais L, Bearzatto B, Schwaller B, Dumont M, De Saedeleer C, et al. 2005. Mono- and dual-frequency fast cerebellar oscillation in mice lacking parvalbumin and/or calbindin D-28k. Eur J Neurosci 22: 861-870.

Seto-Ohshima A, Emson PC, Berchtold MW, Heizmann CW. 1989. Localization of parvalbumin mRNA in rat brain by in situ hybridization histochemistry. Exp Brain Res 75: 653-658.

Skelton NJ, Kördel J, Akke M, Forsén S, Chazin WJ. 1994. Signal transduction versus buffering activity in Ca^{++}-binding proteins. Nat Struct Biol 1: 239-245.

Skelton NJ, Kordel J, Chazin WJ. 1995. Determination of the solution structure of Apo calbindin D-9k by NMR spectroscopy. J Mol Biol 249: 441-462.

Sloviter RS, Sollas AL, Barbaro NM, Laxer KD. 1991. Calcium-binding protein (calbindin D-28K) and parvalbumin immunocytochemistry in the normal and epileptic human hippocampus. J Comp Neurol 308: 381-396.

Solbach S, Celio MR. 1991. Ontogeny of the calcium-binding protein parvalbumin in the rat nervous system. Anat Embryol 184: 103-124.

Stern MD. 1992. Buffering of calcium in the vicinity of a channel pore. Cell Calcium 13: 183-192.

Stevens J, Rogers JH. 1997. Chick calretinin: Purification, composition, and metal-binding activity of native and recombinant forms. Protein Expr Purif 9: 171-181.

Toledo-Rodriguez M, Goodman P, Illic M, Wu C, Markram H. 2005. Neuropeptide and calcium-binding protein gene expression profiles predict neuronal anatomical type in the juvenile rat. J Physiol 567: 401-413.

Van Den Bosch L, Schwaller B, Vleminckx V, Meijers B, Stork S, et al. 2002. Protective effect of parvalbumin on excitotoxic motor neuron death. Exp Neurol 174: 150-161.

Vanselow BK, Keller BU. 2000. Calcium dynamics and buffering in oculomotor neurons from mouse that are particularly resistant during amyotrophic lateral sclerosis (ALS)-related motoneuron disease. J Physiol 525 Pt 2: 433-445.

Vecellio M, Schwaller B, Meyer M, Hunziker W, Celio MR. 2000. Alterations in Purkinje cell spines of calbindin D-28k and parvalbumin knockout mice. Eur J Neurosci 12: 945-954.

Vogt Weisenhorn DM, Prieto EW, Celio MR. 1994. Localization of calretinin in cells of layer I (Cajal–Retzius cells) of the developing cortex of the rat. Dev Brain Res 82: 293-297.

Vreugdenhil M, Jefferys JGR, Celio MR, Schwaller B. 2003. Parvalbumin-deficiency facilitates repetitive IPSCs and γ oscillations in the hippocampus. J Neurophysiol 89: 1414-1422.

Wassle H, Peichl L, Airaksinen MS, Meyer M. 1998. Calcium-binding proteins in the retina of a calbindin-null mutant mouse. Cell Tissue Res 292: 211-218.

Watanabe M, Konishi M. 2001. Intracellular calibration of the fluorescent Mg^{2+} indicator furaptra in rat ventricular myocytes. Pflugers Arch 442: 35-40.

Winsky L, Kuznicki J. 1996. Antibody recognition of calcium-binding proteins depends on their calcium-binding status. J Neurochem 66: 1-8.

Winsky L, Nakata H, Martin BM, Jacobowitz DM. 1989. Isolation, partial amino acid sequence, and immunohistochemical localization of a brain-specific calcium-binding protein. Proc Natl Acad Sci USA 86: 10139-10143.

Yamakuni T, Kuwano R, Odani S, Miki N, Yamaguchi K, et al. 1987. Molecular cloning of cDNA to mRNA for a cerebellar spot 35 protein. J Neurochem 48: 1590-1596.

Yenari MA, Minami M, Sun GH, Meier TJ, Kunis DM, et al. 2001. Calbindin D-28k overexpression protects striatal neurons from transient focal cerebral ischemia. Stroke 32: 1028-1035.

Zaitsev AV, Gonzalez-Burgos G, Povysheva NV, Kroner S, Lewis DA, et al. 2005. Localization of calcium-binding proteins in physiologically and morphologically characterized interneurons of monkey dorsolateral prefrontal cortex. Cereb Cortex 15: 1178-1186.

Zimmermann L, Schwaller B. 2002. Monoclonal antibodies recognizing epitopes of calretinins: Dependence on Ca^{2+}-binding status and differences in antigen accessibility in colon cancer cells. Cell Calcium 31: 13-25.

Zühlke RD, Pitt GS, Deisseroth K, Tsien RW, Reuter H. 1999. Calmodulin supports both inactivation and facilitation of L-type calcium channels. Nature 399: 159-162.