CALRETININ EXPRESSION IN THE MAMMALIAN NEOCORTEX : A REVIEW

Filip Barinka [1], Rastislav Druga [1, 2, 3*]

[1] Department of Anatomy, Charles University in Prague, 2nd Faculty of Medicine, Prague, Czech Republic

[2] Department of Anatomy, Charles University in Prague, 1st Faculty of Medicine, Prague, Czech Republic

[3] Department of Developmental Epileptology, Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

[*] Corresponding author: Prof. MUDr. Rastislav Druga, DrSc., Department of Anatomy, Charles University in Prague, 2nd Faculty of Medicine, U Nemocnice 3, 128 00 Prague, Czech Republic, Tel.: +420 224 965 823, Fax: +420 224 965 770, E-Mail: rastislav.druga@lf1.cuni.cz; anatomie@lfmotol.cuni.cz.

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Summary: In the mammalian neocortex, the calcium-binding protein calretinin is expressed in a subset of cortical interneurons. In the recent years, research on interneurons is one of the most rapidly growing fields in neuroscience. This review summarizes the actual knowledge of the functions of calretinin in neuronal homeostasis and particularly of the distribution, connectivity and physiological properties of calretinin expressing interneurons in the neocortex of rodents and primates, including human. The possible neuroprotective role of calretinin and the presumed "resistance" of calretinin expressing interneurons to various pathological processes are also discussed.

INTRODUCTION

Neuronal population in mammalian cerebral cortex consists of two distinct groups. Pyramidal neurons, which constitute approximately 70-80 % of total neuronal population, are uniformly excitatory, using glutamate as a neurotransmitter. The other group is formed by interneurons, also known as local circuit neurons. Most of them are inhibitory, using GABA as a principal neurotransmitter. It is now increasingly appreciated that the GABAergic interneurons play many important roles in cortical neuronal networks both in normal and pathological states. Various strategies to sort GABAergic interneurons into distinct subgroups have been adopted and this process is far from being completed yet. Based on the expression of three different calcium-binding proteins (CaBP), namely Parvalbumin (PV), Calbindin D-28k (CB) and Calretinin (CR), it is possible to divide the cortical GABAergic interneurons into three largely non-overlapping populations (with some limitations, see below). Here we focus on CR and especially on CR expressing cortical neurons in the neocortex.

EF-hand family of calcium-binding proteins

Calretinin belongs to the so-called EF-hand family of CaBP. The EF-hand is an evolutionary well preserved amino acid domain with a characteristic three-dimensional structure and with a high affinity for calcium ions (Moews and Kretsinger 1975). More then 600 proteins containing EF-hand motive are already known across different species (Carafoli *et al.* 2001). The best known one is probably calmodulin, which mediates numerous intracellular processes after binding of Ca^{2+}, hence acting like a Ca^{2+} sensor. The Ca^{2+} sensors are intracellular proteins, which after binding of free calcium ions undergo conformational change and subsequently activate or deactivate various target molecules (enzymes, transport proteins,

etc.), thereby affecting various regulatory processes in the cell. On the contrary, Ca^{2+} buffers are another group of cytosolic Ca^{2+} binding proteins, which do not show any significant conformational change upon Ca^{2+} binding. Ca^{2+} buffers are involved in shaping both the amplitude and the duration of Ca^{2+} signals and in limiting the spatial spreading of local Ca^{2+} signals (Berridge *et al.* 2000, Schwaller 2009).

CALRETININ

Calretinin, first described in 1987, acquired its name based on the structural similarity to calbindin D28k and the site of first detection (retina) (Rogers 1987). Calretinin in general shares many features with calbindin D28k and belongs to calbindin D28k subfamily of CaBP. The gene for CR is located on chromosome 16 (Parmentier *et al.* 1991). CR is composed of 269-271 amino acid residues and contains six EF-hand domains. Four of them bind Ca^{2+} with high affinity in a cooperative manner, one with low affinity and the last one is non-functional, without Ca^{2+} binding ability (Schwaller *et al.* 1997, Stevens and Rogers 1997). Besides Ca^{2+} binding properties, CR also shows affinity for copper ion Cu^{2+} (Groves and Palczewska 2001), which upon binding to CR antagonizes Ca^{2+} binding to CR. The mammalian neuronal cytoplasmic concentration of CR was estimated to be in order of tens of micromoles (Hackney *et al.* 2005). Although usually considered to be freely diffusible and uniformly distributed in the cytoplasm, at a certain time in development it was shown to be highly concentrated beneath the cell membrane (Hack *et al.* 2000).

While PV acts as a so called "slow" buffer (exerts slow Ca^{2+} binding kinetics) and CB like a "fast" buffer (exerts fast Ca^{2+} binding kinetics), CR was first shown to behave as a fast buffer in modifying presynaptic signalling in frog saccular hair cells (Edmonds *et al.* 2000), but recently it was found that CR shares some kinetic properties of both slow and fast buffers in

modifying dendritic Ca^{2+} transients (Faas *et al.* 2007). Such dual kinetic properties are mainly consequence of the abovementioned cooperative Ca^{2+} binding by CR. These studies also show that CR affects intracellular calcium signals both pre- and postsynaptically. Further elucidation of CR function came from the studies on CR-/- (Schurmans *et al.* 1997, Schiffmann *et al.* 1999, Gall *et al.* 2003, Cheron *et al.* 2004, 2005), CR+/- (Gurden *et al.* 1998) and CR "rescue" (CR-/- with selective reexpression in certain cellular population) (Aller *et al.* 2003, Bearzatto *et al.* 2006) mice. In these studies, impairment of long-term potentiation in the dentate gyrus and especially abnormal excitability in the cerebellar neuronal network with mild impairment of motor coordination were shown to be a result of CR deficiency in mossy cells of dentate gyrus and granule cells of cerebellar cortex, respectively. Together, these studies indicate that modulation of calcium signalling by CR (and CB and PV) is important for precise timing and plasticity of synaptic events in neuronal networks. To make things more complicated, it seems that CR, similarly to CB, might also act as a calcium sensor protein (Billing-Marczak and Kuznicki 1999).

Another still controversial topic is the possible neuroprotective role of CR. While some studies performed mainly on isolated cells or tissue cultures found neuroprotective effect against calcium-induced cytotoxicity (Lukas and Jones 1994, Isaacs *et al.* 1996, Marini *et al.* 1997, D'Orlando *et al.* 2001, 2002), other works using similar techniques gave opposite results (Mockel and Fischer 1994, Kuznicki *et al.* 1996, Bouilleret *et al.* 2000, Isaacs *et al.* 2000). Finally, neuroprotective effect of CR against cellular damage mediated by very low Ca^{2+} concentration has been described recently (Lema Tome *et al.* 2006, Turner et al. 2007).

CALRETININ-EXPRESSING NEURONS IN THE NEOCORTEX

After the discovery of calretinin (Rogers 1987), a basic description of CR distribution in rodent brain emerged in the following years from work of several authors (Pochet *et al.* 1989,

Winsky *et al.* 1989, Rogers *et al.* 1990, Jacobowitz and Winsky 1991, Resibois and Rogers 1992), reviewed in (Baimbridge *et al.* 1992, Andressen *et al.* 1993).

In this paper, the neurons expressing CR will uniformly be described as CR+ neurons, regardless of the method used for CR detection in the particular cited articles (immunohistochemistry, in-situ hybridization etc.).

In this review, we will focus mainly on CR+ neurons in rodents and primates, including human. For information about CR+ neocortical neurons in other species and about their relationship to mammalian brain phylogenesis, see (Hof *et al.* 1999).

Ontogenesis of neocortical calretinin-expressing interneurons

At least in rodents, the CR+ interneurons differ from the other interneuronal subtypes in their site of origin. Unlike the pyramidal neurons, which originate in the ventricular zone of the dorsal telencephalon, the precursors of neocortical interneurons proliferate in the ganglionic eminences in the ventral telencephalon and migrate tangentially to neocortex during the embryonic period. While the PV+ and CB+ cells derive predominantly from the medial ganglionic eminence, the CR+ neurons develop in the caudal ganglionic eminence (Xu *et al.* 2003, Wonders and Anderson 2006). While the molecular mechanisms of CR+ interneuronal development and migration are not completely understood yet, the attention was recently drawn to the importance of estrogen receptor *β* expression for the development of CR+ GABAergic interneurons in rodent brain (Fan *et al.* 2006).

Co-expression of other markers in calretinin-expressing neurons

As already mentioned in the introduction, the three calcium-binding proteins CR, PV and CB tend to be expressed in non-overlapping populations of cortical interneurons (Conde *et al.* 1994, Kubota *et al.* 1994, Gabbott and Bacon 1996a, del Rio and DeFelipe 1996, Kawaguchi

and Kubota 1997, Zaitsev *et al.* 2005, 2009). However, a low degree of co-localization between CR and CB was described by some authors (e.g. del Rio and DeFelipe 1996, 1997a, Cauli *et al.* 1997, Park *et al.* 2002), see also (DeFelipe 1997) for review. Nevertheless, the usefulness of utilization of PV, CB and CR for categorizing of interneuronal subpopulations was recently validated by gene cluster analysis (Toledo-Rodriguez *et al.* 2004).

In rodents, near to 100 % of CR+ neurons in the neocortex seem to use GABA as a neurotransmitter (Kubota *et al.* 1994, Gonchar and Burkhalter 1997, Gonchar *et al.* 2007). On the other hand, in monkeys' (Melchitzky *et al.* 2005) and human (del Rio and DeFelipe 1996) neocortex, app. 25 % of CR+ neurons were found not to express GABA. These non-GABAergic CR+ neurons are discussed below.

Substantial co-localization of CR with vasoactive intestinal peptide (VIP) and choline acetyltransferase (ChAT) in neocortical neurons was described (Rogers 1992, Kubota *et al.* 1994, Cauli *et al.* 1997, Kawaguchi and Kubota 1997, Gabbott and Bacon 1997, Porter *et al.* 1998, von Engelhardt *et al.* 2007). As long as both VIP and acetylcholine are involved in the regulation of energy metabolism and blood flow dynamics in the cortex (Magistretti 1990, Cauli *et al.* 2004), an important role of CR/VIP/ChAT positive neurons in "the local regulation of tissue physiology within a radial column of cortex" can be presumed (Gabbott *et al.* 1997b, Cauli *et al.* 2004).

For additional information on co-expression of various other neuronal substances (other neuropeptides, receptor subunits etc.) in CR+ neurons, consult following papers (Rogers 1992, DeFelipe 1993, Dun *et al.* 1994, Cauli *et al.* 2000, Gonzalez-Albo *et al.* 2001, Toledo-Rodriguez *et al.* 2004, 2005, Gonchar *et al.* 2007).

Distribution and somatodendritic morphology of calretinin-expressing neurons in the neocortex

CR+ neurons in neocortex are concentrated predominantly in cortical layers II and III, both in rodents and primates. The density of CR+ neurons decreases with increasing depth in neocortex and therefore they are quite rare in infragranular layers, when compared to the supragranular ones. The CR+ neurons most commonly possess bipolar or bitufted (two tufts of dendrites originating from the opposite cellular poles) vertically oriented somatodendritic morphology, with fusiform or oval perikaryon (Jacobowitz and Winsky 1991, Resibois and Rogers 1992, Glezer *et al.* 1992, Kubota *et al.* 1994, Conde *et al.* 1994, Fonseca and Soriano 1995, Leuba and Saini 1996, Gabbott and Bacon 1996a, Gabbott *et al.* 1997a, 1997b, Meskenaite 1997, Park *et al.* 2002, Gonchar and Burkhalter 2003, Zaitsev *et al.* 2005, 2009, Desgent *et al.* 2005). The dendrites often extend to layer I superficially and to infragranular layers deeply. On the contrary, the dendritic tree is quite narrow in horizontal direction. Besides these bipolar neurons, other morphological types of CR+ neurons, most typically multipolar and horizontally oriented neurons were consistently found in the neocortex in the majority of the above cited studies.

Interareal and interspecies differencies

The distribution of CR+ neurons is similar but not absolutely homogenous among various neocortical areas. In owl monkey, CR+ neuronal density varies between individual areas with higher values in the visual cortices, lower counts in the prefrontal association cortex and the motor and premotor cortices and lowest counts in the primary somatosensory cortex (Elston and Gonzalez-Albo 2003). In human, although the general pattern of CR immunoreactivity was found to be similar in all inspected association areas, the density of CR+ neurons was significantly lower in the frontal than in the temporal, occipital and parietal association cortices (Hof *et al.* 1993, Barinka *et al.* 2009).

Gabbott *et al.* systematically described morphology and distribution of CR+ neurons in the medial prefrontal cortex (mPFC; Brodmann areas 24, 25 and 32) of the rat (Gabbott *et al.*

1997a) , monkey (Gabbott and Bacon 1996a, 1996b) and human (Gabbott *et al.* 1997b). Their work confirmed the overall resemblance of neocortical CR+ population among examined species. However, significant difference in overall CR+ neuronal counts between the rat neocortex on one hand and primate (monkey and human) on the other was documented. In mPFC, the CR+ neurons constituted 4 % of the total neuronal population in the rat, but 11 % in the monkey and 8 % in the human. Further, the ratio between CR+, PV+ and CB+ cells in mPFC was found to be app. 1.2 : 1.7 : 1 in the rat, but 2.2 : 1.2 : 1 in the monkey. These results correspond well with the findings of other authors (Conde *et al.* 1994, Kubota *et al.* 1994, del Rio and DeFelipe 1996, Gonchar and Burkhalter 1997, Tamamaki *et al.* 2003). Altogether, the CR+ neurons are significantly more numerous in primate frontal cortex when compared to rodents, both absolutely and relatively to the other GABA+ interneuronal types. This seems to be true also in other neocortical fields, as for example in parietal and temporal associational areas (our unpublished observation in human and rat neocortex).

Connectivity and physiological properties of calretinin-expressing neurons

For determination of individual interneuronal types, the axonal targeting and the connectivity in general are the most relevant indicators (Markram *et al.* 2004). In case of CR+ neocortical neurons, the axon most typically originates from the body or one of the primary dendrites and then forms the descending main trunk with side branches with descending and ascending projection; the tangentially pointing branches are rare and the whole axonal tree usually does not extend beyond the width of the dendritic tree of that particular neuron. However, in vertical direction the axonal tree of one CR+ neuron often spans significant portion of the cortical thickness, in case of the neurons located in supragranular layers also extending to the layers IV, V and VI. That means that the most often found CR+ cells that possess vertically oriented bipolar or bitufted dendritic branching, also have a similarly oriented axonal arbour

(Conde *et al.* 1994, Fonseca and Soriano 1995, Gabbott and Bacon 1996a, Gabbott *et al.* 1997a, 1997b, Meskenaite 1997, Gonchar and Burkhalter 1999, 2003, Zaitsev *et al.* 2005, Caputi *et al.* 2009). The possible role of these neurons could be therefore to provide the inhibition of projection neurons within the cortical minicolumn and so to synchronize their activity (Gabbott *et al.* 1997b). One special type of vertically oriented bitufted neurons with especially extensive vertical axonal arborisation (so called horse-tail) extending deep to the infragranular layers are the double-bouquet cells. In primates, including human (but not in rodents), some of these neurons were found to express calretinin (Conde *et al.* 1994, del Rio and DeFelipe 1997a, DeFelipe *et al.* 1999).

Besides the CR+ neurons with vertically oriented axonal tree, cells with horizontal axonal arborisation (most often with multipolar dendritic morphology) have also been found (Meskenaite 1997, Gabbott *et al.* 1997b). Recently, two different types of CR+ neurons in layer 2/3 in the mouse neocortex were described (Caputi *et al.* 2009). The first type was the CR+ bipolar neuron with vertically arranged dendritic and axonal arbours, but the second type was multipolar with preferentially horizontal orientation of both the dendritic and the axonal arbours. The bipolar neurons also uniformly co-expressed VIP, which was not found in multipolar CR+ cell in this study. The two types clearly differ in electrophysiological characteristics as well (see below), what infers that at least two significantly different populations of CR+ neurons exist in the neocortex (besides Cajal-Retzius cells, see below).

On the basis of their axon targeting, cortical interneurons can be divided into dendrite-targeting (with many subgroups), soma-targeting and axon-targeting interneurons. The CR+ neurons are mostly dendrite targeting, similarly as CB+ neurons, but unlike PV+ neurons which typically innervate soma or axon initial segment of pyramidal neurons. More precisely, the CR+ axonal boutons that form symmetrical, GABAergic synapses typically innervate dendritic shafts and less often dendritic spines or somata (Meskenaite 1997). There are

noteworthy interareal differences in types of postsynaptic cells innervated by CR+ neurons. While pyramidal neurons (especially their dendrites) were found to represent the main target of CR+ axons in all examined layers in the human temporal neocortex (del Rio and DeFelipe 1997b), the CR+ interneurons innervate mainly pyramidal neurons in infragranular, but preferentially other GABAergic interneurons in supragranular layers of the primary visual cortex (monkey: (Meskenaite 1997), rat: (Gonchar and Burkhalter 1999)). Also in monkey prefrontal cortex, CR+ neurons innervate preferentially other GABAergic neurons in supragranular layers (Melchitzky and Lewis 2008). Therefore, it indicates that at least in some cortical areas and layers the CR+ neurons (by inhibiting other classes of inhibitory interneurons) may exert significant disinhibitory effect on the pyramidal neurons (Meskenaite 1997, Gonchar and Burkhalter 1999). Based on these observations, a "gating cell" function for the CR+ neurons – switching the flow of information between two pathways by inhibiting one of them and disinhibiting the other one – was suggested by (Callaway 2004). Analogous situation was also described in the hippocampus, where CR+ interneurons were found to be specialized to control other interneuronal types (Gulyas *et al.* 1996). Javier de Felipe *et al.* found in the monkey visual areas that especially the CB+ neurons are densely contacted by CR+ axonal terminals (DeFelipe *et al.* 1999). On the other hand, the large PV+ basket cells did not seem to be contacted by CR+ axonal terminals. Further, the connectivity between the interneuronal populations is in no way unidirectional, because also the CR+ neurons were substantially innervated by PV+ and CB+ interneurons.

The CR+ interneurons are also innervated by the pyramidal cortical neurons. Interestingly, in the monkey prefrontal cortex, the density of excitatory inputs on CR+ dendrites in supragranular layers was found to be significantly lower (40-90%) than on the PV+ dendrites (Melchitzky and Lewis 2003). Similarly, in the visual areas of the rat, unlike the PV+ neurons, the CR+ neurons in layers II/III receive only sparse inputs from the feedback and

feedforward excitatory connections (connecting primary and higher-order visual areas). On the contrary, the CR+ neurons in layer I are selectively targeted by feedback connections from higher-order visual areas (Gonchar and Burkhalter 2003).

The synaptic connections between individual interneurons of the same type were described in all interneuronal classes, including CR+ interneurons (Gabbott and Bacon 1996a, Gabbott *et al.* 1997a, 1997b, Meskenaite 1997, Gonchar and Burkhalter 1999, Melchitzky *et al.* 2005, Melchitzky and Lewis 2008, Caputi *et al.* 2009). The inhibitory interneurons also form the so called electrical synapses, or gap junctions. These are preferentially formed between interneurons of the same class and were also found to connect the CR+ interneurons (Caputi *et al.* 2009). Together, the CR - CR chemical and electrical synapses synchronize the activity of interconnected neurons, which then form a neuronal assembly that consequently modulates the activity of other interneuronal classes as well as of the pyramidal neurons in cortical microcircuits.

While the GABAergic cortical interneurons preferentially form connections with neurons in their close proximity, sparse GABAergic cells with long-range cortico-cortical projections were also found in the neocortex (e.g. (Peters *et al.* 1990). Interestingly, 14% of GABAergic long-distance cortico-cortical neurons in the monkey were recently found to be CR+ (none of these neurons were PV+) (Tomioka and Rockland 2007). The exact physiological function of such long-range GABAergic neurons has not been elucidated so far, but various possibly important tasks have been proposed, e.g. synchronization of multiple local neuronal networks with otherwise weakly formed interareal connections (Buzsáki 2006).

In monkey prefrontal cortex it was shown that the CR+ neurons, unlike the other classes of GABAergic interneurons, are not contacted by dopamine terminals (Sesack *et al.* 1995). Nevertheless, they possess the D_5 dopamine receptors (Glausier *et al.* 2009), which infers that the extrasynaptic dopamine transmission may exert influence on CR+ cortical neurons. On the

contrary, the VIP+ and CCK+ interneurons (the majority of which also express CR) were found to be selectively excited by nicotinic receptor stimulation in the rat neocortex (Porter *et al.* 1999).

Besides the above discussed GABAergic CR+ neurons forming inhibitory, symmetric synapses, non-GABAergic CR+ cells and asymmetric excitatory CR+ synapses were also described. In the human temporal neocortex, 26% of CR+ neurons were found to be non-GABAergic (del Rio and DeFelipe 1996) and 31 % of synapses formed by the CR+ axon terminals were of the asymmetrical type and hence presumably excitatory (del Rio and DeFelipe 1997b). Very similarly, 23 % of CR+ cells were GABAergic and 29 % of synapses formed by the CR+ axon terminals were asymmetric in the monkey prefrontal cortex (Melchitzky *et al.* 2005). In the both cited studies, the CR+ axonal terminals which formed asymmetric synapses contacted predominantly dendritic spines.

Electrophysiological properties of calretinin-expressing neurons

The PV+ interneurons are uniformly described as fast-spiking: in response to a depolarizing current injection, they show high-frequency firing of action potentials without marked accommodation (lowering) of firing frequency. On the other hand, the CR+ (and CB+) neurons belong to the non-FS cell types (interneurons exerting other then FS pattern). The non-FS interneurons are quite heterogeneous; various spiking patterns, which do not match exactly with expression of any CaBP, have been described. In the rodent brain, the CR+ neurons were found to belong to the regular-spiking non-pyramidal (RSNP) cells or to burst-spiking non-pyramidal (BSNP) cells (Kawaguchi and Kubota 1997, Kawaguchi and Kondo 2002). Interestingly, in the already mentioned study (Caputi *et al.* 2009), the two different CR+ neuron types (bipolar and multipolar) in the mouse cortical layers II-III also differed in electrophysiological properties: the bipolar neurons showed bursting firing pattern, while the

multipolar neurons had a regular firing pattern. The authors suggest that the multipolar CR+ neurons together with one type of PV+ neurons form an interneuronal network which is able to drive synchronized inhibition of pyramidal neurons. In opposite, the bipolar CR+ neurons could act as "disinhibitory" neurons relieving pyramidal cells from inhibition.

In the rat neocortex, the CR+/VIP+ bipolar interneurons have also been described as irregular spiking (IS): an initial burst of action potentials is followed by intermittent action potentials (Cauli *et al.* 1997, 2000, Porter *et al.* 1998).

Toledo-Rodriguez et al. (2004) described four major clusters of ion channel genes co-expressed in cortical interneurons of the rat. Three of these clusters contained also one of the three CaBP. The "CR cluster" expressed (besides CR) SK2, Kv3.4 and Caα1B ion channel subunits and was associated with accommodation of spiking pattern. The PV and CB clusters differed in both the expression of ion channel subunits and the electrophysiological properties (PV cluster was associated with fast spiking and CB cluster with bursting behaviour).

The CR+ neurons were described as classic accommodating/adapting in the monkey prefrontal cortex, the pattern consistent with the RSNP in the rat (Zaitsev *et al.* 2005, 2009). Also these studies confirmed a marked correlation between the CaBP expression and the electrophysiological properties of interneurons.

Cajal-Retzius Cells

One "special" neuronal population which also expresses CR is formed by the Cajal-Retzius (CjR) cells. These cells differ significantly from the other CR+ neuronal types in various aspects: location exclusively in first cortical layer, their glutamatergic phenotype, specific role in cortical layers patterning, etc.. Interestingly, although the number of CjR neurons

dramatically decreases in the early postnatal period and they probably completely disappear from the rodent cortex, in the higher mammalian orders, including primates a small but significant population of CjR cells can be found also in the adult neocortex (Martin *et al.* 1999, Ábrahám *et al.* 2005). For more information, consult some of the recent reviews on CjR cells, e.g. (Soriano and Del Rio 2005).

Calretinin-expressing neurons in neurologic and psychiatric disorders

The possible changes in expression of calcium binding proteins in various neurological and psychiatric disorders were studied in detail by many authors. Interestingly, while the CB and especially the PV expression or the number of CB and/or PV expressing neurons decreases, the CR expression seems not to be affected in conditions such as schizophrenia, major depression, Alzheimer disease and multiple sclerosis, e.g.(Hof *et al.* 1993, Fonseca and Soriano 1995, Beasley *et al.* 2002, Hashimoto *et al.* 2003, Clements *et al.* 2008). In various epileptic conditions, the CR expression or number of CR+ neocortical neurons was shown to be normal or decreased less prominently than the PV and CB expression (Garbelli *et al.* 1999, 2006, Zamecnik *et al.* 2006, Aronica *et al.* 2007, Barinka *et al.* 2009). Whether this "resistance" of CR+ neurons results from the proposed neuroprotective role of CR, or whether it is the specific position of these neurons in cortical microcircuits that protects them from deterioration, has not been sufficiently elucidated yet.

Conclusions

Calretinin plays a role in modulation of synaptic events and signal transduction in neuronal networks. The role of CR in other intracellular Ca^{2+}- sensitive processes and in neuroprotection is still unclear and remains to be defined.

With some limitations, the calcium-binding proteins CR, PV and CB can be used as convenient markers for different populations of cortical interneurons, which has already been proved by gene expression profiling, electrophysiological as well as developmental studies.

The CR expressing neurons increase in number significantly in primate neocortex, when compared to rodents. In primates, they are the most numerous interneuronal population.

By inhibiting other interneurons, at least some of the CR+ interneurons are capable to exert disinhibitory effect on pyramidal neurons, a property not described in any other interneuronal population.

The CR+ neurons which co-express VIP (and ChAT) probably play an important role in neurovascular coupling.

Besides the majority of CR+ neurons utilizing GABA as a neurotransmitter, smaller proportion of CR+ neurons belongs to the non-GABAergic phenotype.

Finally, the CR expressing neurons seem to be more resistant to various neuropathological conditions then the PV and CB interneuronal populations.

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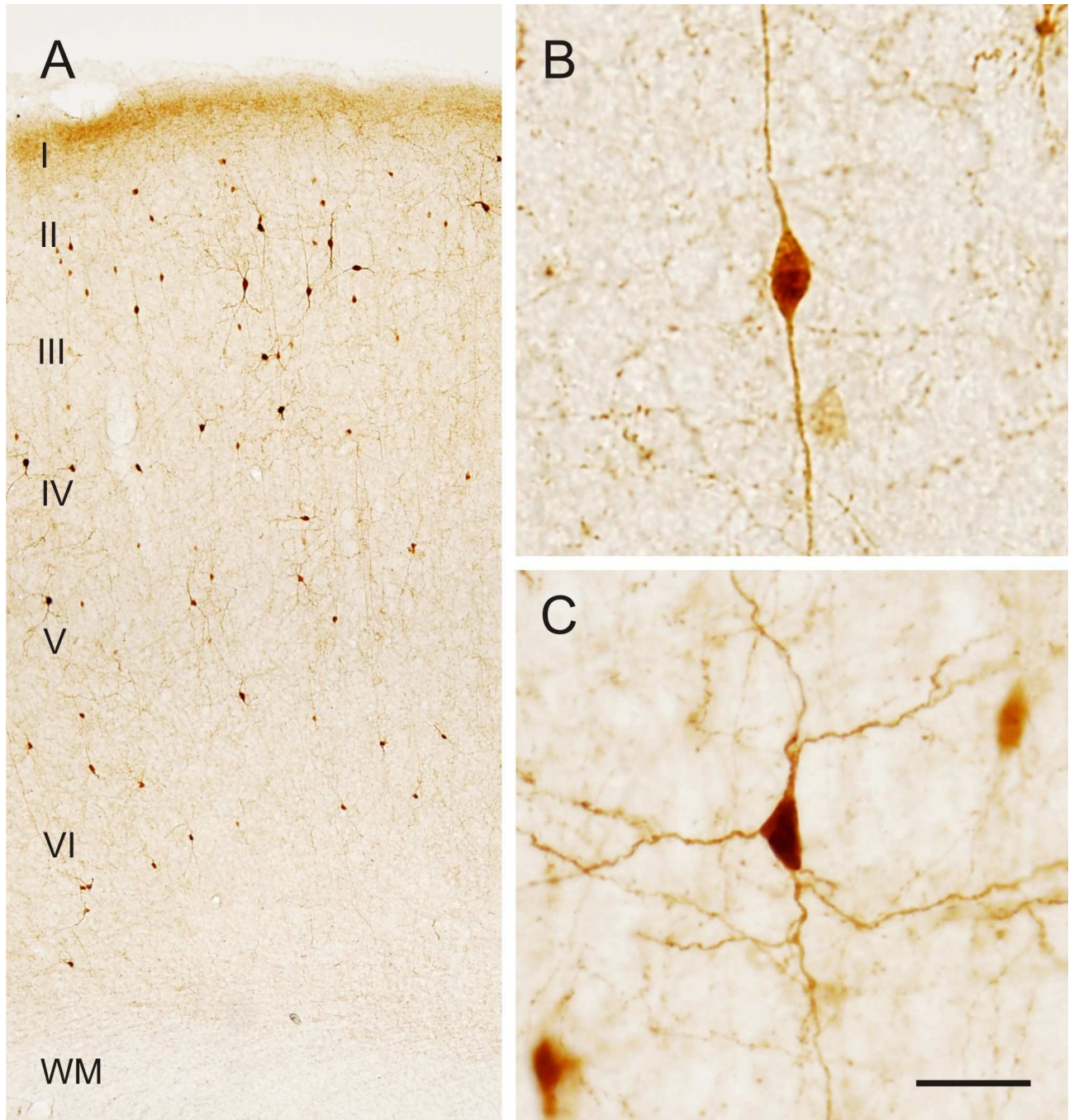
Figure 1.

Representative photomicrograph illustrating the distribution of calretinin immunoreactive

(CR+) neurons in neocortex of the rat (A). Examples of typical bipolar (B) and multipolar (C)

CR+ neurons. Scale bar = 200 µm in A and 25 µm in B and C; I–VI: cortical layers; WM:

white matter.