Research report

Neurocalcin immunoreactivity in the rat main olfactory bulb

Jesús G. Briñón a, Rosario Arévalo a, Carlos Crespo a, Ignacio G. Bravo a, Katsuo Okazaki b, Hiroyoshi Hidaka b, José Aijón a, José R. Alonso a,*

a Dpto. Biología Celular y Patología, Universidad de Salamanca, Salamanca E-37007, Spain
b Department of Pharmacology, Nagoya University School of Medicine, Nagoya 466, Japan

Accepted 17 March 1998

Abstract

The morphological characteristics and distribution of neurocalcin NC-immunoreactive elements were studied in the rat main olfactory bulb (OB) using a polyclonal antibody and the avidin–biotin immunoperoxidase method. NC-positive elements were abundant in the glomerular layer (GL), where numerous immunostained external tufted cells and periglomerular cells were detected. Other less abundant NC-immunolabeled populations included middle and internal tufted cells, Van Gehuchten cells, horizontal cells, vertical cells of Cajal, deep short-axon cells and granule cells. This study demonstrates the presence of NC immunoreactivity in subsets of different neuronal types in the rat main OB. This calcium-binding protein has been found in interneurons, and no evidence of immunoreactivity to NC is detected in projecting neurons. Despite the large population of labeled external tufted cells, most of them belong according to morphological criteria to the local circuit group and some others to those with interbulbar and/or intrabulbar connections. The identification of neuronal subpopulations expressing NC provides a further characterization and shows the existence of biochemical differences within morphologically identical neurons. Thus, this marker may be a useful tool in unravelling the circuitries of the rodent OB in both normal and experimental conditions. The exact physiological function of NC in the olfactory system remains unknown. On the basis of similarities to recoverin, it could be involved in mechanisms responsible for sensory adaptation. Additionally, its calcium-binding abilities may contribute to improve the temporal precision of stimuli transmission, or be concerned with general calcium-related events occurring in specific interneuronal groups. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Calcium-binding protein; Neurocalcin; Olfactory bulb; Specific cell marker

1. Introduction

Neurocalcin (NC) (mol.wt. 23–24 kDa, pI 5.3–5.5) is a calcium-binding protein isolated from bovine brain where four isoforms have been identified [36]. This protein shares similarities with visinin and recoverin, and has been included into the neural calcium-sensor proteins group defined by the photoreceptor cell-specific protein, recoverin [15].

Several types of calcium-binding proteins are present in the nervous system where they mediate the effects of calcium ions in neurons. Their exact function is poorly understood, however, since they are expressed in particular neuronal groups. When immunohistochemically detected, they provide a selective staining of distinct neuronal populations throughout the brain [9,10,30,32]. In addition to this neurochemical characterization, the study of their distribution patterns in anatomically well-known structures provides a basis to interpret their functional roles. The olfactory bulb (OB), with its highly laminated structure, the presence of several types of fully characterized output neurons and interneurons, and its well-known connections is an ideal region to study the general principles of neural and chemical organization.

Immunoblot analysis demonstrated that NC is present in the OB, cerebrum, cerebellum, brainstem, spinal cord, retina, pituitary, and adrenal gland [5,24,25]. Immunohistochemical studies have shown NC-immunoreactivity in amacrine and ganglion cells of the bovine retina [24], and in different regions of the olfactory system, such as the...
receptors of the olfactory epithelium and vomeronasal organ [6,14], and in the main and accessory OBs [5,28]. In the accessory OB, several neuronal types, including tufted cells, periglomerular cells, Van Gehuchten cells and vertical cells of Cajal demonstrated NC-immunoreactivity [28], whereas in the main OB, the NC-immunopositive elements were described as groups of external tufted cells located both in the glomerular layer (GL), and in the external plexiform layer (EPL) [5]. However, in addition to these NC-containing elements, we have found other different NC-immunopositive neuronal types distributed throughout all layers of the rat main OB, except in the olfactory nerve layer (ONL). Therefore, the aim of the present study is to complete the description of NC-containing elements in the rat main OB in order to obtain a full characterization of all neuronal types expressing this calcium-binding protein.

2. Materials and methods

Five adult male Wistar rats, weighing between 200 and 250 g and grown and kept under standard laboratory conditions were used. The animals were deeply anaesthetized with ketamine (Ketolar 50 mg/kg body weight) and perfused through the ascending aorta with 100 ml Ringer solution followed by 400 ml fixative solution made up of 4% paraformaldehyde and 15% saturated picric acid in 0.1 M phosphate buffer, pH 7.3 (PB). After being removed, the OBs were dissected and postfixed at 4°C for two additional hours in the same fixative. After several washes in PB, 35-μm thick vibratome sections were obtained, or, after cryoprotection in 30% sucrose in PB at 4°C, 25-μm thick sections were cut on a cryostat.

2.1. Immunohistochemistry

Free-floating sections were washed in PB and incubated for 30 min in 10% normal goat serum in PB. After being washed in PB they were incubated for 48 h at 4°C in anti-NC primary antibody, raised in rabbit against rat brain NC [24] diluted 1:8000 in PB. Thereafter, the sections were processed according to the avidin–biotin immunoperoxidase method [13]. The sections were washed (3 × 10 min) in PB and incubated for 1 h at room temperature in biotinylated anti-rabbit immunoglobulin (Vector Laboratories, Burlingame, USA) diluted 1:250 in PB. After washing in PB (3 × 10 min), sections were transferred to Vectastain ABC reagent diluted 1:200 in PB for two additional hours. The antibody–enzyme complex was visualized by incubating the sections in a solution of 0.05% 3,3’-diaminobenzidine tetrahydrochloride (Sigma) with 0.003% hydrogen peroxide in 0.2 M Tris–HCl buffer, pH 7.6, for 5–10 min, until the desired staining intensity was reached. Sections were then rinsed in PB, mounted on gelatin-coated slides, air dried, dehydrated through graded ethanol series, cleared in xylene and coverslipped with Entellan (Merck).

2.2. Controls

The primary antibody used has been fully characterized by immunoblotting [24] and does not cross-react with other brain proteins. Its specificity was tested by antibody preadsorption with native NC (2.5 μg/ml), obtaining a complete abolition of the immunohistochemical staining. In addition, three controls of the immunohistochemical procedure were carried out by incubating sections in the same media omitting: (1) the primary antibody; (2) the biotinylated immunoglobulin, and (3) the avidin peroxidase complex. No residual reaction was observed.

2.3. Analysis

Sections were analyzed under a Zeiss III microscope and drawings were made through a camera lucida adapted to this microscope. Photographs were taken on a Zeiss III photomicroscope. Positive neurons were counted with 16 × and 40 × planapochromatic objectives. Quantitative assessments of the relative density of NC-immunoreactive neurons were performed by analyzing coronal sections at nine levels in each animal. The most rostral section was that where all bulbar layers were clearly delineated. Eight additional levels were chosen each 300 μm throughout the rostro-caudal axis of the OB. All labeled neurons, regardless of their staining intensity, were included in the evaluation. The number of immunostained cells for each neuronal type was referred to the total area of the section, and the density is given as number of cells per mm² of fixed tissue. ANOVA was used for the statistical analysis of the results. Fisher PLSD and Sheffe F-tests did not detect statistically significant differences in the density of each neuronal type neither throughout the rostro-caudal axis of the OB nor between animals.

Individual labeled cells with their focusing plane in the middle of the section thickness were drawn with a camera lucida using the 100 × oil immersion objective, and their cell-body diameters were plotted on a digitizer tablet connected to a semiautomatic image analysis system (MOP-Videoplan, Kontron). For each neuronal type, 50 cells from each analyzed section in all five animals were measured, with the exception of those neuronal types which were rarely observed. In this case, all observable cells were measured.

3. Results

After NC-immunohistochemistry, most immunopositive neurons displayed densely stained cell bodies, dendrites and lengthy axons. Weakly labeled neurons were also
observed, mainly located in the inner layers. The results were constant among all animals used and they are summarized in Fig. 1 and Table 1. The different NC-immunopositive neuronal types were identified following the criteria of previous classifications based on Golgi impregnations [20,21,27,33]. The NC-immunostaining was mainly located in the GL and the EPL, although all layers of the OB, except the ONL, demonstrated NC-immunopositive elements (Fig. 2a).

3.1. Olfactory nerve layer

The ONL was completely negative. Olfactory fibers coursing from the olfactory receptor cells and all ONL intrinsic cells were NC-immunonegative (Fig. 2a).

---

Fig. 1. Camera lucida drawings showing the different neuronal types displaying NC-immunoreactivity in the rat OB. Glomeruli and mitral cell profiles are marked with dashed lines. Scale bar: 100 μm. (a–d) Periglomerular cells. (e–h) External tufted cells in the GL. (i) External tufted cell in the EPL. (j) Van Gehuchten cell. (k) Internal tufted cell. (l,m) Displaced granule cells. (n) Horizontal cell. (o,p) Vertical cells in the IPL. (q–u) Granule cells. (v,w) Deep short-axon cells in the innermost region of the GCL and in the WM. ONL: olfactory nerve layer. GL: Glomerular layer. EPL: External plexiform layer. MCL: Mitral cell layer. IPL: Internal plexiform layer. GCL: Granule cell layer. WM: White matter.
Table 1
NC-positive neuronal types in the rat olfactory bulb

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Location</th>
<th>Max. diameter μm (mean ± S.E.M.)</th>
<th>Frequency*</th>
<th>Staining intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periglomerular cell</td>
<td>GL</td>
<td>9.0 ± 0.2</td>
<td>++ ++</td>
<td>low</td>
</tr>
<tr>
<td>External tufted cell</td>
<td>GL/EPL</td>
<td>12.3 ± 0.3</td>
<td>++ ++</td>
<td>high</td>
</tr>
<tr>
<td>Van Gehuchten cell</td>
<td>EPL</td>
<td>13.2 ± 0.5</td>
<td>++ ++</td>
<td>high</td>
</tr>
<tr>
<td>Middle tufted cell</td>
<td>EPL</td>
<td>15.7 ± 0.6</td>
<td>+ +</td>
<td>low</td>
</tr>
<tr>
<td>Internal tufted cell</td>
<td>EPL</td>
<td>18.3 ± 0.5</td>
<td>+</td>
<td>low</td>
</tr>
<tr>
<td>Displaced granule cell</td>
<td>EPL/MCL</td>
<td>9.0 ± 0.3</td>
<td>+ +</td>
<td>medium</td>
</tr>
<tr>
<td>Horizontal cell</td>
<td>MCL/IPL</td>
<td>14.4 ± 0.4</td>
<td>+ +</td>
<td>high</td>
</tr>
<tr>
<td>Vertical cell of Cajal</td>
<td>MCL/IPL</td>
<td>12.0 ± 0.3</td>
<td>+</td>
<td>medium</td>
</tr>
<tr>
<td>Granule cell</td>
<td>GCL</td>
<td>8.6 ± 0.5</td>
<td>+ + +</td>
<td>low</td>
</tr>
<tr>
<td>Deep short-axon cell</td>
<td>GCL/WM</td>
<td>14.9 ± 0.7</td>
<td>+</td>
<td>medium</td>
</tr>
</tbody>
</table>

*Frequency: ++: 1 to 5 cells per section; ++ +: 5 to 20 cells per section; + + +: more than 20 cells per section.

3.2. Glomerular layer

The most prominent staining was obtained in the GL where two different neuronal populations were clustered around the glomeruli (Fig. 1a–h, Fig. 2a). One of these populations was formed by neurons of 10–15 μm of maximum diameter, with spherical or fusiform somata, and strong immunostaining intensity. They surrounded the glomeruli although they were most abundant on their inner half, that is, close to the EPL (Fig. 1e–h, Fig. 2a–c). The external cell pole gave rise to a single, thick primary dendrite that divided close to the soma, and each secondary dendrite subsequently branched again, producing a dense tuft. The terminal dendritic branches lacked spines and varicosities, although because of the strong staining of the glomerular neuropil it was sometimes difficult to observe the complete arborization of each individual dendrite. The tuft originating from each neuron was normally located in the lateral sides of the glomerulus rather than in its central core. In general, the axons of these NC-immunopositive neurons arose from the opposite pole from which the dendrite originated. These thin, finely beaded axons were orientated in two directions. Some of them coursed parallel or slightly obliquely to the OB layering, that is through the GL/EPL boundary, giving collaterals, when observed, at the inner side of neighboring glomeruli. In other cases, the axons were orientated in a descending fashion, and some of them could be followed deep into the EPL and the mitral cell layer (MCL). In accordance with these characteristics, these cells were classified as external tufted cells.

The other NC-immunoreactive neuronal population found in the GL was formed by smaller (6–10 μm), faintly stained neurons with ovoid or round cell bodies (Fig. 2b,c). They were also located around the glomeruli and showed some variability, including monodendritic and bidendritic cells, as well as monoglomerular and biglomerular ones (Fig. 1a–d). The most frequent dendritic pattern consisted of a long spiny primary dendrite that entered and ramified within a single glomerulus forming a wide terminal field. Neurons with this primary dendrite ramifying close to the soma were also observed. More rarely, two primary dendrites arose from different poles of the cell body and entered either a single glomerulus or adjacent glomeruli. A thin axon arose from the opposite side of the cell body coursing towards the EPL. In most cases, the axons did not penetrate deeper into the EPL; but they extended into the superficial region of this layer, around adjacent glomeruli. In accordance with these morphological features, these cells were identified as periglomerular cells.

3.3. External plexiform layer

NC-immunopositive elements appeared throughout the whole extent of this layer. Both NC-immunoreactive neurons and fibers were observed. Fibers displayed variable thicknesses and trajectories, and most of them coursed perpendicular or slightly obliquely to the OB layering (Fig. 2e). NC-immunopositive neurons appeared in all regions of this layer although they were more numerous at the border with the MCL (Fig. 2a). They were morphologically heterogeneous, corresponding to different neuronal types that are described according to their location, from the most external to the most internal ones.

Close to the border with the GL, NC-immunoreactive neurons had fusiform cell bodies ranging in size from 11 to 14 μm. They showed a thick and smooth primary dendrite directed towards the GL that entered into a single glomerulus forming a characteristic tuft of terminal branches (Fig. 1i). Whereas most primary dendrites extended perpendicular to the OB layering (Fig. 2d), in a few cases the main dendrite approached the glomerulus following oblique or almost horizontal trajectories (Fig. 2e). Frequently, the opposite pole of the cell body gave rise to a thinner secondary dendrite that extended parallel to the GL/EPL border for long distances without entering any glomerulus (Fig. 1f). These secondary dendrites did not branch in their initial portion, but only in their distal region where they terminate in several thin branches. In some cases, a short axon-like process emerged close to the base of the secondary dendrite or directly from it, extending...
radially inwards in the EPL. All these morphological features are in accordance with those of tufted cells.

Another distinctive type of NC-immunopositive neuron of the EPL resembled the morphological characteristics and location of Van Gehuchten cells. They had piriform or slightly ovoid somata with sizes between 10 and 15 μm in maximum diameter. The cell body gave rise to a variable number of thick and varicose dendrites that branched elaborately close to it (Fig. 1j, Fig. 2e, Fig. 3a,b). Additional thinner dendrites emerged from variable somatic locations and extended towards superficial regions, branching close to the GL (Fig. 3a). No NC-immunostained axon-like process was observed emerging from these cells.

The most abundant population of NC-positive neurons in this layer was located at the border with the MCL, and they showed the morphological features of granule cells. These neurons had spherical or slightly ovoid cell bodies with maximum diameters ranging from 8 to 11 μm. They exhibited two dendrites arising from opposite poles of the somata that extended radially in opposite directions. One main dendritic trunk coursed towards the external region of the EPL branching before the border with the GL was reached (Fig. 1m, Fig. 3c). The opposite dendritic trunk branched profusely close to the cell body, originating a small dendritic field either in the internal plexiform layer (IPL) or in the granule cell layer (GCL).

In the same region, close to the border with the MCL, a few NC-immunostained internal tufted cells were observed. They showed fusiform, faintly stained, large cell bodies, with maximum diameters ranging from 17 to 20 μm. Two thick smooth dendrites arose from their poles coursing parallel to the MCL for distances longer than 400 μm, branching into the inner half of the EPL (Fig. 1k, Fig. 3d). Another dendrite emerged from one side of the neuronal body coursing perpendicular to the OB layering, reaching and entering one glomerulus (Fig. 1k). The branching pattern of these dendrites, as well as recognizable axon-like processes, were difficult to observe because of the weak labeling.

3.4. Mitral cell layer and internal plexiform layer

The mitral cells did not display NC-immunoreactivity, although their immunonegative cell bodies were frequently distinguishable because they were surrounded by strongly positive profiles (Fig. 2a). The somal staining in the MCL and IPL was classified into two different neuronal populations. Most labeled cells occurred in both the MCL and the IPL, and had morphological characteristics of horizontal cells. The presence of a continuous row of these cells at the level of the MCL was a constant feature in the rat OB after NC-immunostaining (Fig. 2a). They displayed variable morphological shapes (round, piriform, ovoid) and were medium-sized (12–15 μm maximum diameter). Dendrites arose from opposite poles of the somata and extended throughout the MCL, branching into the adjacent regions of this layer (Fig. 1n, Fig. 3e–g).

The other NC-immunopositive neuronal type observed in these layers had the morphological characteristics of the vertical cells of Cajal (Fig. 1o–p). They had spherical, piriform or ovoid neuronal bodies with maximum diameters between 10 and 13 μm. In addition to the variability of the somal shape, variations in the dendritic branching pattern were detected. Most of them exhibited two main dendritic trunks emerging from opposite poles of the somata, and were radially orientated. Frequently, the dendrite that extended towards the inner layers of the OB was thicker than the one that crossed the EPL and branched close to the GL (Fig. 4a). More rarely, only one dendrite directed towards either the EPL or the GCL was observed in these cells (Fig. 4b,c).

3.5. Granule cell layer and white matter

The largest population of NC-immunopositive cells in the GCL was composed of small-sized neurons (8–11 μm) identified as granule cells. They had a segregated distribution, being only observed in the most external region of the GCL (Fig. 1q–u). No immunostained granule cells were detected in the middle or inner region of this layer. NC-immunoreactive granule cells displayed weak staining restricted to the cell body, whereas dendrites lacked immunostaining or were faintly labeled only in their proximal region (Fig. 4d). Although the weak staining in this neuronal group could be interpreted as doubtful, it disappeared when the antibody was preincubated with native NC (not shown).

In the internal region of the GCL and in the white matter, a few NC-immunopositive fusiform cells with maximum diameters ranging from 13 to 16 μm were observed. According to their size, shape and location, they presumably belonged to the deep short-axon cell group, although they were difficult to classify with certainty since only the...
Fig. 3. Photomicrographs of NC-positive neurons in the EPL, MCL and IPL of the rat OB. Scale bar: 50 μm for all figures. (a,b) Van Gehuchten cells in the EPL displaying different branching patterns in their dendrites. (c) Characteristic bipolar cell in the deep region of the EPL with morphological features of granule cells. (d) Internal tufted cell (solid arrow) in the internal region of the EPL, showing thick processes arising from opposite poles of the somata. Note the difference in size related to the horizontal cell beside it (open arrow). (e) Horizontal bipolar cells in the inner region of the EPL with dendrites directed towards the MCL. (f) Fusiform cell in the MCL with a main dendritic trunk running parallel to the OB lamination. (g) Horizontal cells in the MCL showing slightly stained axons (arrows) that can be followed towards superficial layers.
Fig. 4. NC-immunostained neurons in the deep layers of the OB. Scale bar: 50 μm. (a,b) Vertical cells in the MCL with long dendritic branches reaching the superficial EPL (a) or the deep GCL (b). (c) Vertical cell in the IPL arborizing towards both the MCL and the GCL. (d) Weakly stained granule cells with stained apical dendrites (arrows). (e) Deep short-axon cell with varicose dendrite in the GCL. (f) Bipolar short-axon cell in the WM.
cell body and the proximal region of their primary varicose dendrites were stained (Fig. 1v–w, Fig. 4e,f).

4. Discussion

The present study has clearly established the distribution of NC-immunoreactive neurons in the rat main OB. The general distribution pattern of these elements is different from those reported for other calcium-binding proteins, such as calbindin D-28k, calretinin, parvalbumin or calmodulin [7,8,17,30]. Thus, the immunohistochemical detection of NC constitutes an excellent neuroanatomical tool to characterize biochemical subpopulations within groups previously defined on the basis of morphological criteria by using Golgi impregnation [27,33] or immunocytochemistry for other calcium-binding proteins [1,4,7,8,17,30].

4.1. Methodological considerations

Our data confirm and extend previous observations [5], in which only external tufted cells were reported as NC-immunopositive. Both studies have been carried out using the same type of animals (Wistar rats) and the same primary antibody [24]. A possible explanation for the different number and characteristics of the stained structures could be a loss in the protein antigenicity during the chemical treatment of the tissue. In fact, Rogers and Résoibois [32] reported a poor staining for calretinin in Helly-fixed paraffin-embedded brains, the same method used by Bastianelli et al. [5]. Therefore, the paraffin embedding procedure could reduce the sensitivity in the detection of NC in comparison with vibratome or cryostat sections, as used in the present study.

4.2. Neurocalcin-positive elements in the superficial layers

The NC-immunoreactive elements were found in all layers of the rat OB except in the ONL where all olfactory fibers were negative as previously described [5]. Although NC is expressed by numerous olfactory receptor cells [6], their axons are NC-negative when they reach the OB. This lack of NC-immunoreactivity in the distal olfactory axons could indicate a heterogeneous intracellular distribution of this protein.

The largest number of stained elements was observed in the GL, where subpopulations of two different neuronal types displayed NC-immunoreactivity. They were clearly distinguishable by their size and staining intensity. Cells displaying strong immunoreactivity were larger and showed a smooth dendritic branch with a characteristic tuft inside the glomeruli, which are typical features of external tufted cells [27]. The lightly labeled neurons were identified as periglomerular cells and they were differentiated from the external tufted cells on the basis of their smaller cell-body size, the presence of dendritic spines and the different branching pattern of their dendrites, as previously described [27]. A few NC-positive tufted cells were also observed in the EPL. These neurons were identified as middle tufted cells in accordance with their location and the presence of long secondary dendrites extending through the central region of the EPL [20].

Tufted cells differ in several aspects, such as the presence and morphological features of secondary dendrites, the size and density of the intraglomerular tuft, the presence, extent, and distribution of the axonal projection, the degree of responsiveness to electrical stimulation of the olfactory nerve, and in their chemical content [21]. Of particular interest in relation with our observations is the classification established according to their target regions. Internal tufted cells and most middle tufted cells have projections that are largely extrinsic to the OB [35], whereas external tufted cells and the middle tufted cells located near the GL are local circuit neurons or they are involved in intra- or interbulbar connections [21,35]. Although double-labeling studies with retrograde tracers would be necessary to be conclusive, our results suggest that most, if not all, tufted cells projecting into high brain areas are NC immunonegative.

Within the external tufted cell group, neurons located at the GL–EPL boundary project to the contralateral OB, or connect medial and lateral regions of the same OB, whereas cells occurring in the superficial two-thirds of the GL appear to be local circuit neurons [21,34,35]. These groups can be distinguished on the basis of morphological criteria: neurons engaged in interbulbar and intrabulbar circuits have secondary dendrites [34], whereas most local circuit neurons do not [21]. Accordingly, most NC-stained tufted cells are local circuit neurons. Therefore, NC is an excellent and reliable marker of external tufted cells categorized as local circuit neurons, and its immunocytochemical localization provides a method for the identification and demarcation of this group, and the study, through combined techniques, of its hodological and physiological peculiarities.

4.3. Neurocalcin-positive elements in the deep layers

Granule cells were classified into three morphological groups on the basis of the location of their somata and gemmules [22]. This laminar organization offers a basis for differential synaptic interactions with deep vs. superficial principal neurons [16,20,22,26]. The different granule cell types are of functional significance, and each functional group appears to be determined by its position in the GCL [38]. The existence of these distinct groups with particular location in the GCL is suggestive regarding the distribution pattern of those expressing NC. Since, according to their location, most, if not all NC-immunopositive granule cells are able to interact with superficial tufted cells, the expression of this protein could identify neurons that are interconnected as part of a specific circuit, as suggested for other calcium-binding proteins [10]. In addition, the identi-
fication of this biochemically different subset of granule cells could constitute a strategy to differentiate a physiological subtype within the entire granule cell population.

Concerning the second-order interneurons, the expression of NC is much more scarce. The NC-positive Van Gehuchten cells were identified on the basis of their location, somal morphology and size and the characteristi-
cally elaborated branching pattern of their varicose den-
drites [33]. Although initially this neuronal type was only reported in the cat OB [37], subsequent studies have detected the presence of neuronal populations showing the same morphological features in other mammalian species, such as hamster, hedgehog and rat [8,19,33].

Most NC-positive secondary interneurons of the deep layers were identified as horizontal cells, whereas a few neurons showed the characteristics of vertical cells of Cajal [29,33]. A few deep short-axon cells also displayed NC immunoreactivity. Nevertheless, it was not possible to classify them with total certainty within any particular subtype of this group since their dendrites were poorly stained and only their proximal region was observable, whereas this further classification is based upon the com-
plexity and distribution of their arborizations and the density and distribution of dendritic spines [33].

4.4. Functional implications

Our understanding of the possible functions of NC is still limited; therefore, its physiological role in neurons remains speculative. This calcium-binding protein has been categorized as a calcium-sensor protein involved in modu-
ulatory and signal transduction events [15]. Since NC oc-
curred in the vast majority of olfactory receptors, a signifi-
cant function in olfactory signal transduction has been hypothesized [14]. Additionally, sensory adaptation in ol-
factory receptors is thought to be mediated by a calcium-
binding protein similar to recoverin [18]. The similarities
between recoverin and NC, and its predominant expression
by juxtaglomerular neurons, would argue for an involve-
ment of NC in calcium related events concerned with
olfactory sensory adaptation. Nevertheless, the widespread
distribution of NC also support their involvement in general mechanisms rather than, or in addition to, such spe-
cialized functions.

Other mechanisms where NC would be involved may
be those proposed for other calcium-binding proteins that
include short-term calcium buffering, redistributing cal-
cium within the neuron, and protecting the cell against the
damaging effects of excessive calcium influx [2]. Among
them, the prevailing expression of NC in superficial tufted
cells could be related with the control of endogenous
oscillations of cytosolic calcium originated as normal re-
sponses to olfactory stimuli. This would contribute to
shortening action potentials and/or transmitter release,
thus improving the temporal precision of the stimuli trans-
mission. In fact, circuits where superficial tufted cells are
involved tend to preserve temporally distributed informa-
tion across neuronal populations [35].

Considering the remarkable parallels between chem-
and photoreception [3], some points of analogy could be
detected. The amacrine cells, like the granule cells, are
believed to produce prolonged GABA-mediated inhibition
through signals which propagate mainly within local por-
tions of their arborizations [11,12,38]. In these cells, NC,
like other calcium-binding proteins [31] would be needed
to limit substantially the spread of calcium signal produced
by excitatory amino acids [23]. Interestingly, NC has been
detected in amacrine cells [24], thus arguing for a possible
relationship between these similar functional properties
and the expression of NC.

Acknowledgements

The authors express their gratitude to Mr. G.H. Jenkins for revising the English version of the manuscript. This work was supported by grants from the DGICyt (PB94-1388) and the `Junta de Castilla y León'.

References


