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Distribution of calcium-binding proteins in the chick visual system

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Abstract

The calcium-binding proteins calbindin (CB), calretinin (CR), and parvalbumin (PV) have been extensively studied over the last decade since they appear to be important as buffers of intracellular calcium. In the present study we investigated the distribution of these proteins in the chick visual system by means of conventional immunocytochemistry. The results indicated that CB, CR, and PV are widely distributed in retinorecipient areas of the chick brain. In some regions, all three calcium-binding proteins were present at different intensities and often in different neurons such as in the dorsolateral thalamic complex. In other areas, such as the nucleus geniculatus lateralis ventralis, only CB and CR were detected, whereas PV was absent. These results show that these three calcium-binding proteins are differentially distributed in the visual system of the chick, with varying degrees of colocalization.

Key words
- Calbindin
- Calretinin
- Chick
- Immunohistochemistry
- Parvalbumin
- Visual system

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In the nervous system, calcium ions control a myriad of processes, including fast transport of molecules (1,2), synthesis and release of neurotransmitters, and membrane excitability (3,4). These actions depend largely on calcium-binding proteins, which mediate and modulate the actions of calcium ions (5-8). A number of calcium-binding proteins have been characterized as buffering proteins since they are associated with the control of intracellular calcium levels (9), and thus presumably with the protection of the cells against death that might ensue after an excess of intracellular calcium is produced (10-12). Calbindin (CB), calretinin (CR), and parvalbumin (PV) are among the buffer-type calcium-binding proteins and, although their exact function in neurons is far from being elucidated, they have been extensively used as neuronal markers (13,14). In the present study, we used immunocytochemical techniques to map the distribution of CB, CR, and PV in retinorecipient areas of the chick brain, as the first step of a project aimed at characterizing their functional organization in the visual system.

Six 1- to 15-day-old chicks (*Gallus gallus*) were used in this study. The animals were deeply anesthetized with ketamine (1 mg/100 g body weight, *im*) and xylazine (2 mg/100 g, *im*) and perfused through the heart with phosphate-buffered saline and 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB), pH 7.4. After 5 h of postfixation, the brains were transferred to a 30% (w/v) sucrose solution in PB to ensure cryoprotection. Coronal sections (30 µm) of the frozen brains were cut with a sliding microtome. The free-floating brain sections were incubated with monoclonal mouse antisera against CB and PV (Sigma Chemical Co., St. Louis, MO), diluted 1:2,000 in PB containing 0.3%

Triton X-100 (TX-100) and 5% normal goat serum (NGS), for 14-18 h at room temperature. Brain sections were also incubated with a polyclonal rabbit antiserum against CR (Chemicon, Temecula, CA), diluted 1:500 in PB containing 0.3% TX-100 and 10% NGS. After washing 3 times with PB (10 min each) the sections were incubated with a biotinylated goat anti-mouse antiserum (Jackson Labs, West Grove, PA) for CB and PV, and a goat anti-rabbit antiserum (Jackson Labs) for CR, all diluted 1:200 in PB with 0.3% TX-100, for 1 h at room temperature and then washed with PB and incubated with an avidin-biotin-peroxidase complex (ABC Elite, Vector Labs, Burlingame, CA) for 1 h. After incubation with 0.05% 3-3'-diaminobenzidine and 0.3% hydrogen peroxide in PB and intensification with 0.05% osmium tetroxide in water, the sections were mounted on gelatin- and chromoalumen-coated slides, dehydrated, cleared and coverslipped with Permount (Fisher, Pittsburgh, PA). In many instances the sections were counterstained with Giemsa (15). The intensity of immunoreactivity for CB, CR, and PV in the neuropil and perikarya of retinorecipient areas of the chick brain was subjectively rated from absent to very intense. The retinorecipient areas of the chick brain were identified according to a stereotaxic atlas (16). No attempt was made to quantify the intensity of staining in perikarya or the number of stained perikarya.

Table 1 - Distribution of the calcium-binding proteins calbindin (CB), calretinin (CR) and parvalbumin (PV) in retinorecipient areas of the chick brain.

AP, Area pretectalis; GLv, nucleus geniculatus lateralis, pars ventralis; IGL, intergeniculate leaflet; LMmc, nucleus lentiformis mesencephali, pars magnocellularis; nBOR, nucleus of the basal optic root; TeO, tectum opticum; OPT, nucleus opticus principalis thalami.

	CB		CR		PV	
Structures	Perikarya	Neuropil	Perikarya	Neuropil	Perikarya	Neuropil
AP						
GLv						
IGL						
LMmc						
nBOR						
TeO Layer 1						
Layer 2						
Layer 3						
Layer 4						
Layer 5						
Layer 6						
Layer 7						
Layer 8						
Layer 9						
Layer 10						
Layer 11						
Layer 12						
Layer 13						
Layer 14						
Layer 15						
OPT						

Absent
Slight
Moderate
Intense
Very intense

Immunoreactivity to antibodies against CB, CR, and PV was found in almost all retinorecipient structures of the chick brain studied. Table 1 shows the distribution of the three calcium-binding proteins in the visual areas of the chick brain. CB-like immunoreactivity (CB-LI) was observed in each retinorecipient area, and was especially intense in perikarya of the area pretectalis. Most CB-LI cells were small- to medium-sized and labeled perikarya were absent only in layers 11 and 12 and in soma-free layers (1, 7, and 14) of the optic tectum. Marked neuropil staining for CB-LI was found in the area pretectalis and layers 5 and 6 of the optic tectum. Only layers 10-12 of the tectum were devoid of CB-LI in the neuropil (Figure 1).

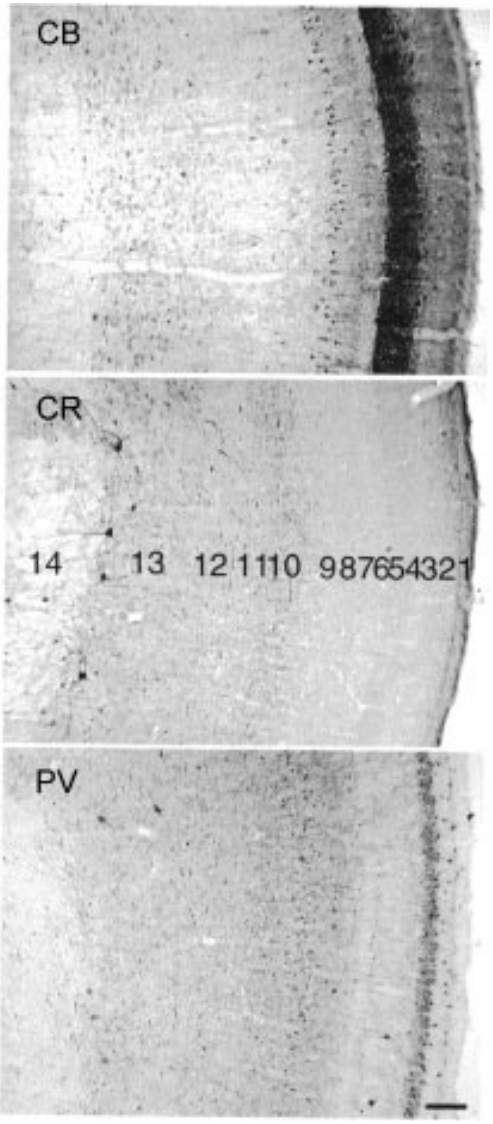

Figure 1 - Digital images of coronal sections of the chick brain illustrating the pattern of immunoreactivity for calbindin (CB), calretinin (CR), and parvalbumin (PV) in the optic tectum. The numbers in the middle image indicate the approximate localization of the different tectal layers. Scale bar: 100 µm.

Perikarya staining for CR-LI was intense or very intense in the nucleus opticus principalis thalami, the nucleus intergeniculatus, the nucleus lentiformis mesencephali, pars magnocellularis, and the nucleus of the basal optic root, whereas neuropil staining for CR-LI was very marked in the last three structures and layer 1 of the optic tectum. Cell bodies exhibiting CR-LI were also usually small- to medium-sized, but a few were large. Such cells were absent only in layers 8 and 10 of the tectum, besides the soma-free layers, and neuropil staining for CR-LI was present in each retinorecipient structure.

Perikarya staining for PV-LI was very pronounced in layers 4 and 10 of the optic tectum, and in the nucleus of the basal optic root. These neurons were small-, medium-, or large-sized, and were not found in the nucleus geniculatus lateralis ventralis or layer 15 of the optic tectum. Neuropil staining for PV-LI was very intense only in the nucleus of the basal optic root and layer 4 of the tectum, and absent only in the nucleus geniculatus lateralis ventralis.

These results indicate that the calcium-binding proteins CB, CR, and PV are extensively distributed in the visual system of the chick, in agreement with an earlier suggestion that these proteins may play a role in cell signaling in sensory systems (6). The fact that all three types of calcium-binding proteins were found in small cells might suggest a possibility of co-localization with the neurotransmitter GABA (6), as in the pigeon visual areas, where CB-LI and PV-LI partially co-localize with GABAergic interneurons (17). However, double-labeling experiments are needed to directly verify this possibility. In some regions, however, neurons staining for PV-LI are clearly projection neurons, such as the CR-LI and PV-LI large neurons in the nucleus of the basal optic root

(18). It should be stressed that the antibodies used in this study may not have been able to recognize the presence of CB, CR and PV in some visual neurons. The richness of staining observed for all three antibodies in retinorecipient areas of the chick brain suggests that the expression of these proteins may be regulated by retinal innervation, as demonstrated for the pigeon tectum in an earlier study (19). Furthermore, the possibility exists that at least part of the neuropil staining in those brain areas is due to staining of retinal axons themselves, as is the case for layer 1 of the tectum, which contains mostly retinal axons. Experiments are underway in our laboratory to address these specific questions.

In summary, the present results indicate that visual neurons of the chick brain might use different calcium-binding proteins in their functional organization and, in some cases, more than one type of buffer-type protein. These findings are probably linked to the intrinsic neuronal organization of each retinorecipient structure, given the fact that the retinal input itself is, at least chemically, rather uniform, with all retinal ganglion cells using glutamate as their central neurotransmitter.

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