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PHYSIOLOGY

Distribution of Calcium-Binding Proteins, Parvalbumin and Calbindin, in the Midbrain Auditory Center (MLd) of a Pigeon

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Abstract—Immunohistochemical distribution of calcium-binding proteins, parvalbumin (PV) and calbindin (CB), has been studied in the mesencephalic auditory center (MLd) of pigeon (*Columba livia*). In the central region of the MLd (core, ICC), an overlap in distribution of the PV- and CB-immunopositive (ip) neurons and neuropil has been observed, with different patterns in the central and peripheral parts. In the peripheral region of the MLd (belt, ICS, and ICX), both neurons and neuropil contained only CB. A selective CB chemospecificity of the belt, ICS, and ICX is an evolutionary conserved feature characteristic of all avian species. Interspecies differences in the distribution of PV and CB immunoreactivity in the ICC are a result of adaptive functional specialization, which provides specific processing of different aspects of the auditory information.

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The midbrain nucleus (nucleus mesencephalicus lateralis pars dorsalis, MLd) is the main relay of the avian auditory pathway ascending to the forebrain, which is homologous to the mammalian colliculus inferior. The MLd perceives information on various parameters of the auditory signals through separate channels from the brainstem auditory nuclei responsible for its functional compartmentalization. The MLd is composed of the central lemniscal (core) region (nucleus centralis, ICC) and peripheral extralemniscal (belt) region (nucleus superficialis, ICS, and nucleus externus, ICX), which are different in morphological, hodological, functional, and neurochemical features [1–6]. The internal organizations of these MLd regions differ in various avian species. Investigation of calcium-binding proteins (CaBPr), which are selective functional markers of different types of neurons, provide information on detailed structure of MLd in finches birds, owls [7–10], and chickens [11, 12]. Data on the content of CaBPr in the MLd of other species are not available.

In this study, five pigeons (*Columba livia*) were used. PV and CB distribution in neuropil and neurons of the MLd was examined on the frozen 40 μm sections using the conventional immunohistochemical avidin–biotin–peroxidase method. We used rabbit polyclonal antibodies against CB (Swant, Switzerland) diluted 1 : 5000 and mouse monoclonal antibodies against PV (Sigma, United States) diluted 1 : 1000. Control sections were stained according to the Nissl protocol.

The central ICC nucleus of birds receives tonotopic projections from the primary and secondary brainstem auditory nuclei. The peripheral nuclei ICS and ICX, which encircle ICC dorsolaterally, receive spatial–topic auditory information, as well as non-auditory information (somatosensory and tectal) from the brainstem nuclei and central ICC nucleus [1, 2, 4–7, 13, 14]. The MLd is surrounded by intercollicular region (ICo), which includes several nuclei. Their identification, relationships with the belt MLd nuclei, periaqueductal gray, and preisthmal nuclei are not fully clarified and differently evaluated [1, 10–12].

Staining of PV- and CB-immunopositive (ip) structures proved to overlap in the central nucleus (ICC). The PV-ip neuropil consisting of the dotted (terminal-resembling) and fibrous structures was located along the entire rostrocaudal axis of the nucleus. Along the upper border of ICC, the neuropil was dense and bounded with PV-ip fibers and a poorly stainable stripe above them. In the rest ICC area, the PV-ip neuropil was almost uniformly distributed with a somewhat higher density in the central region (Figs. 1b, 2a). Numerous intensely stained PV-ip cells were throughout the entire ICC. In the central and ventral ICC regions, these were two major types of neurons: the large and middle-sized cells, multipolar and spindle-shaped with stained proximal parts of dendrites and the smaller cells varying in shape and differently stainable. The peripheral dorsolateral ICC region contained rare and small PV-ip cells (Figs. 1b, 1d, 2a). Distribution of these two types of PV-ip neurons in ICC coincides with the distribution of the neuronal bodies on the Nissl-stained sections: the central

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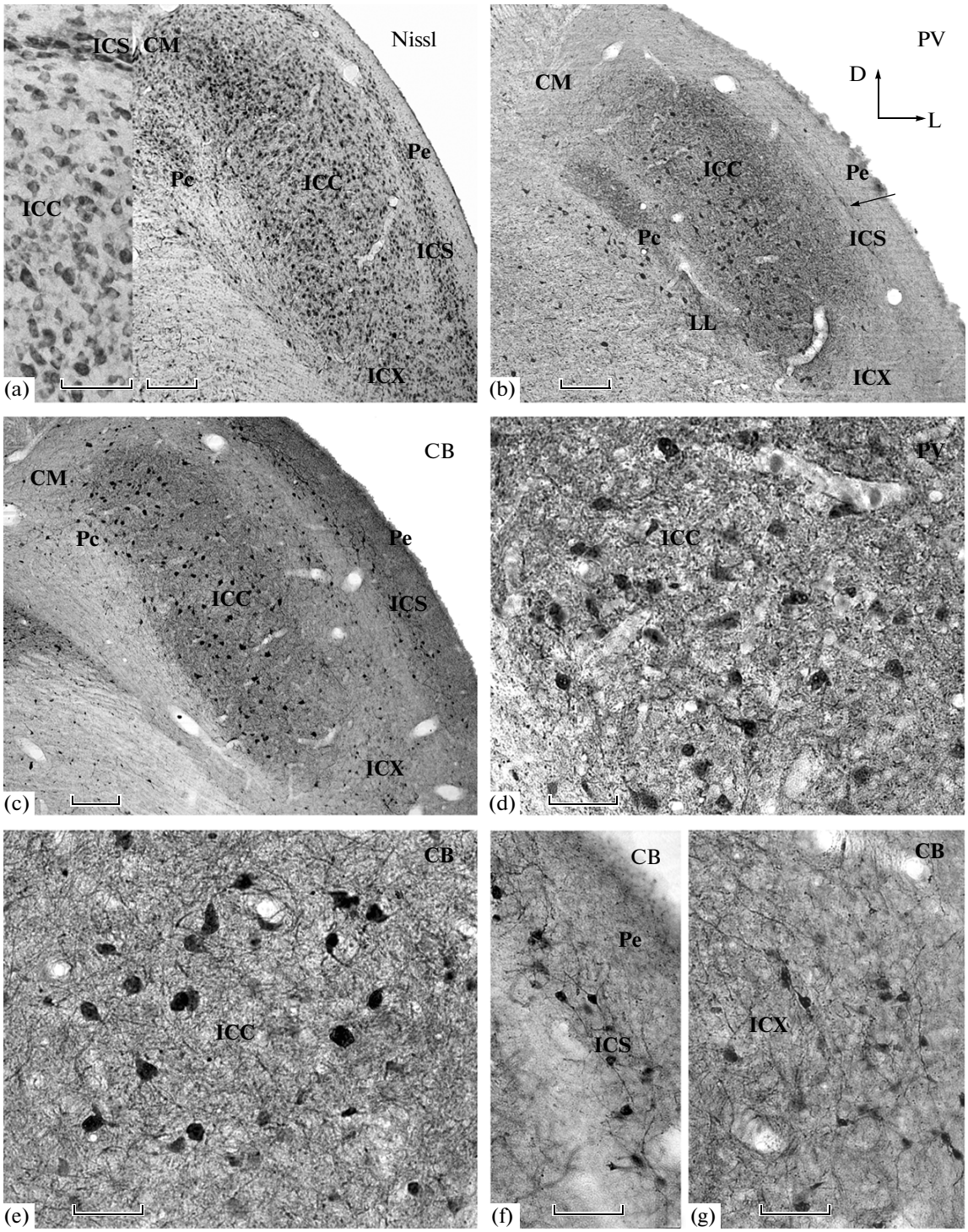

Fig. 1. PV and CB distributions in the pigeon MLd. Micrographs of the midbrain frontal sections through the median region of MLd. (a) Nissl staining with thionine; on the left, ICS and ICC at large magnification; (b) PV, an arrow indicates PV-ip fibers in the internal region of the ICS; (c) CB; (d) PV-ip neurons and neuropil in the ICC; (e) CB-ip neurons and neuropil in the ICC; (f) CB-ip neurons and neuropil in the ICS; (g) CB-ip neurons and neuropil in the ICX. Scale: (a–c) 200 μm; (d–g) 100 μm.

and ventral regions contained a mixed population of the large and middle-sized cells with intensely stained basophilic matter, as well as smaller cells with differently stained cytoplasm; small pale cells predominated in the lateral peripheral ICC region (Fig. 1a).

CB and PV immunoreactivities overlapped in ICC neuropil and neurons. Both dotted and fibrous CB-ip neuropils were located along the entire rostrocaudal ICC axis. Numerous, often intensely stained, CB-ip cells were scattered in the central and ventral regions

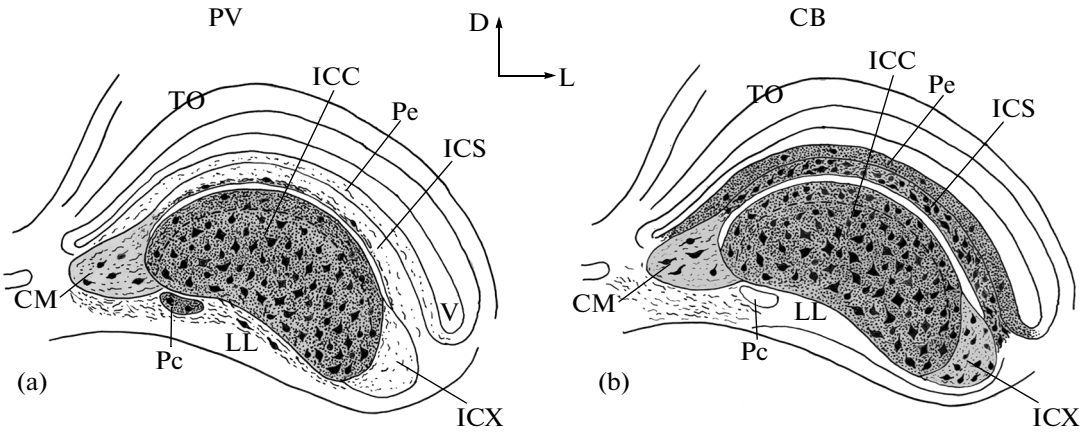

Fig. 2. A scheme of the (a) PV and (b) CB distributions in the frontal section of the pigeon MLd through the median level. Small dots and lines are the immunopositive dotted and fibrous structures, respectively. Black symbols and circles are immunopositive neurons. V, brain ventricle.

of the nucleus (Figs. 1c, 1e). These were neurons of two types morphologically similar to PV-ip neurons situated in the same regions of the nucleus, i.e., the large and middle-sized multipolar and spindle-shaped neurons with stainable dendrites contributing to the neuropil immunoreactivity, as well as small, mostly round and oval cells. In the dorsolateral ICC region, the CB-ip cells varying in shape predominated (Figs. 1c, 1e, 2b).

The medial MLd region (caudomedial nucleus, CM according to [11]) contained a less compact PV- and CB-ip neuropil than other ICC regions, as well as sparse PV- and CB-ip neurons (Figs. 2a, 2b). Some authors attribute this nucleus to the MLd, because it receives projections from the brainstem auditory nuclei and has similar features [1, 11]. According to other authors, this nucleus is a component of the ICo [8, 12].

A small oval nucleus, nucleus paracentralis (Pc), that is adjacent to the ventral region of the MLd, is assigned to the ICo [1]. In our experiments, it was clearly outlined against the background of the dense intensely stainable PV-ip neuropil with sparse PV-ip cells (Figs. 1b, 2a). This nucleus was completely devoid of CB-ip elements (Figs. 1c, 2b). The Pc-nucleus location and cell composition are similar to those of a nucleus of the same shape which is detectable when Nissl staining is used (Fig. 1a). Because of the contradictory identification of the avian ICo complex, the differences in the MLd and ICo immunoreactivities against the studied proteins can't be characterized with certainty. On the ventral side, PV-ip fibers of the lateral lemniscus (LL) run into the caudal MLd and spread along the ventromedial border of the nucleus. It contains interstitial spindle-shaped PV-ip cells with processes along the fiber direction (Figs. 1b, 2a).

The belt MLd nuclei, ICS and ICX, are virtually devoid of PV immunoreactivity with the exception of a bundle of PV-ip fibers with rare interstitial PV-ip neurons in the lower ICS region above the unstainable narrow strip that separates ICC and ICS (Figs. 1b, 2a). On the contrary, CB-ip neurons varying in shape, both small and middle-sized were numerous, closely spaced, and intensely stained throughout the entire ICS. Their dendrites were parallel or vertical with respect to the lateral MLd surface; they attained the periventricular layer (Pe) that contained a dense dotted CB-ip neuropil and sparse CB-ip cells (Figs. 1c, 1f, 2b). In Nissl-stained preparations, they corresponded to a layer of tightly packed cells with an intensely stained cytoplasm, which were separated from ICC by a cell-free stripe (Fig. 1a). An indistinct ICX nucleus adjacent to the caudoventral pole of ICC was also devoid of PV immunoreactivity. It contained the non-compact CB-ip neuropil and diffusely scattered CB-ip cells with long multidirectional dendrites, which were mostly round, small, middle-sized, and moderately or poorly stainable.

Comparison of our results with the data on other avian species revealed both similarities and differences in the PV and CB distributions in MLd. The PV distribution in the pigeon ICC (our data) is similar to that in finches birds [12], in which this nucleus contains an extremely dense PV-ip neuropil and PV-ip neurons varying in size and shape. Their location coincides with the terminal field of the pathways ascending from the brainstem auditory nuclei. At the same time, the CB distribution in ICC varies in different avian species. In owls, with their extremely well-developed auditory system, CB is detectable in all ICC regions [7, 9]. In contrast to owls, the finch central ICC region [8], which corresponds to MLd.I (according to [10]), has a poorly detectable CB immunoreactivity, though in the peripheral ICC regions (MLd.O according to

[10] or the "transitory region" according to [8]), PV- and CB-ip neuropil and neurons overlapped. In the chicken ICC, CB was not detectable at all [12]. According to our data, the intensities of staining of PV- and CB-ip neuropil and neurons in pigeon is similar within the core region of ICC. The neurons are similar in morphology, and most of them coexpress PV and CB proteins (our unpublished data). Nevertheless, different PV and CB concentrations and the metabolic activities (cytochrome oxidase) within the core and peripheral dorsolateral regions suggest that ICC compartmentalization is possible in pigeon [1, 15]. In contrast to ICC, the peripheral ICS and ICX nuclei of all the birds contain exclusively CB-ip cells differing in morphotype from CB-ip cells of ICC ([7–9] and the results of this study).

Our results confirm that the MLd organization in pigeon is based on the core–belt principle, which is typical of MLd of other members of sauropsid amniotes. However, complementarity of PV and CB distributions between the central lemniscal (core) and peripheral extralemniscal (belt) MLd regions varies in different species, primarily because the contents of these proteins in the lemniscal ICC nucleus of various species are different ([7–9] and the data of this study). The interspecific variation of CaBPr specificity of avian ICC is, probably, a result of different functional ICC organization, in particular, of different location of the auditory projection inputs encoding information on the frequency, intensity and spatial localization of the auditory signals in finches birds, chickens, pigeons, and owls, as well as of different locations of the non-auditory, e.g., somatosensory, projections [1, 4, 10]. Interspecific variability of the PV and CB distributions is also characteristic of the mammalian core region of colliculus inferior. The plasticity of the lemniscal region of the auditory system during the vertebrate evolution is a result of progressive development of the peripheral auditory structures and of the adaptive functional specialization of the centers in response to a certain content of the auditory signaling and dependent on its role in behavior of various species.

On the other hand, selective CB chemospecificity of the peripheral extralemniscal MLd region (the ICS and ICX nuclei) in all avian species studied as well as of homologous region of the mesencephalic auditory center of other sauropsid amniotes and mammals suggests its high evolutionary conservativeness. This is related to the fact that the extralemniscal centers of the auditory system have numerous connections with other non-auditory brain centers, which provide involvement of the auditory information into various vitally important brain functions responsible for food-procuring, reproductive, communicative, and other behavioral forms necessary for species survival.

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