Cerebellar Projections of the Lagena (the Third Inner Ear Otolith Endorgan) in the Pigeon

V. I. Khorevin

INTRODUCTION

The cerebellum is one of the most phylogenetically old suprasegmental cerebral structures; it includes the cerebellar cortex and cerebellar subcortical nuclei. The cerebellar cortex is built of three layers, the most superficially localized molecular layer, the intermediate layer with Purkinje cells, and the granular layer [1, 2]. The cerebellar nuclei look like aggregations of the nerve cells and fibers localized in the wall of the cerebral IV ventriculum. In the representatives of different classes of vertebrates, the cerebellum varies in its dimensions and form, from a simple commissure-like structure in Cyclostomata to a very complicate polylobular structure in mammals [3]. Except for the peculiarities of the macroscopic structure of the cortex, the cytoarchitecture of the cerebellum is rather homotypic in the sequence of vertebrate classes [4].

Afferent inputs to the cerebellum are formed by two types of fibers, mossy and climbing ones, and they have different projection targets. Impulsion from the vestibular endorgans and also from other sources comes to the cerebellum via mossy fibers forming numerous synapses on cells of the granular layer. Axons of these cells pass the Purkinje cell layer and, after bifurcation in the molecular layer, form parallel fibers contacting with about 100 to 300 Purkinje cells. In turn, one Purkinje cell obtains inputs from about 500 parallel fibers. In humans, peculiar features of densities of localization of granular cells within the regions of termination of mossy fibers have not been found. The area of synaptic contacts of mossy fibers and the mean length of such contacts per unit of the neuropil volume in the cerebellum of humans, however, are greater than those in the pigeon and rat [5].

According to phylogenetic differences and specificities of termination of mossy fibers, three sections are distinguished in the cerebellum (the archicerebellum, paleocerebellum, and neocerebellum). The first one, or vestibulocerebellum, is the main zone of termination of primary vestibular afferents coming from endorgans of the membranous labyrinth and also of secondary vestibular afferents coming from the vestibular nuclei [6-8]. In fishes, this cerebellar section occupies the main part of the volume of the cerebellum, but its relative dimension decreases in more highly organized vertebrates [1]. In birds and mammals, the vestibulocerebellum looks like a relatively small lobe localized most caudally and ventrally and containing, in turn, two lobules, nodulus (lobule X) and uvula (lobule IX). The paleocerebellum, or spinal cerebellum, is represented by phylogenetically newer...
and more centrally localized parts of the cerebellum, the vermis and intermediate fields of the cortex. The paleocerebellum is the zone of termination of the afferent tracts transmitting information from the spinal cord structures and a few nuclei of the cranial nerves, and also from the eyes and hearing organs. The neocerebellum, or cerebrocerebellum, is most highly developed in humans and anthropoid apes; it occupies lateral fields of the cerebellar cortex. This cerebellar part is supplied with information from the neocortex after synaptic transmission through synapses of the corresponding fibers in the pontine nuclei [2, 3, 5].

In birds, the cerebellum is represented mostly by the vermis, except for the lateral fields of lobules VII and VIII, which obtain inputs mainly from the pontine nuclei [1, 2, 8].

Climbing fibers form the second group of afferent inputs to the cerebellum these fibers come from the nucleus and dorsomedial cellular column of the oliva inferior and through the inferior cerebellar pedunculi go toward Purkinje cells of the contralateral nodulus and uvula [4, 6, 7]. A climbing fiber forms synaptic contacts on the neighboring Purkinje cell within the projection zone of this fiber, and, at the same time, the same climbing fiber synthetically terminates on inhibitory Golgi cells [4]. As is supposed, functioning of the olivo-cerebellar system in birds is based on principles similar to those in mammals, because the ratio of numbers of inferior oliva cells/Purkinje cells in birds (1:16) is comparable with the respective ratio in different mammals belonging to different subclasses (1: 4-1:17) [8].

The cerebellar cortical output is represented exclusively by the axons of Purkinje cells projecting to the cerebellar nuclei. In fishes and amphibians, there is only one cerebellar nucleus; reptilians and birds possess two nuclei (medial and lateral); in lower mammals, three nuclei have been identified, while there are four nuclei in humans and other primates [1, 2]. The dimension of laterally localized cerebellar nuclei increases in a parallel manner with increase in the dimension of the cerebellar cortex, and 90% of neurons of all cerebellar nuclei belong to the laterally positioned nucl. dentatum [1].

Overlapping of the zones of termination of primary afferents coming from the otolith maculae and cristae of the canali semicirculare is a special feature of organization of the vestibular inputs to the cerebellum in birds. In pigeons, when a radioactive marker (14H-leucine) was used, similar patterns of distribution of labelled fibers in the cerebellum were observed in the cases of both global application of this marker to the vestibular nerve and its local applications to the cristae of each semicircular canal and utricular macula. Labelled fiber rosettes were found to be concentrated within the granular cell layer in lobules X and IX (their ventral parts); some (not numerous) fibers were also observed in the dorsal part of lobule IX and in the anterior lobe [9].

As is known, the cerebellar cortex functions, in a certain sense, as a coordination system for regulation of posture and initiation of movements [6]. At the same time, the lagena, being the third otolith organ localized in birds in the cochlear canal distally with respect to the hearing organ [10], can be involved in the detection of displacements of the body in the vertical direction. In addition, according to recently proposed hypotheses, the lagena can be closely related to the navigation of birds in accordance with the magnetic field of the Earth (due to magnetic properties of the lagenar otoconia) [11-13]. The question on the lagenar projections to the cerebellum in birds is complicated by the fact that the lagena is localized in close proximity to the hearing organ formed by a much greater number of the cells [14]. Such topographic proximity can result in the action of the markers on the cochlear nerve in the respective experiments [15-17].

In this study, we examined cerebellar projections of the lagena in birds (pigeon) using anterograde axonal transport of biotinylated dextran amine (BDA) in order to provide the most accurate local application of the marker to the lagenar region.

METHODS

Experimental data were obtained on pigeons (Columbia livia), on the group used earlier in the study of other central projections of the lagena [18]. In the respective publication, we described in detail the methods used; thus, here we only briefly describe the techniques of surgery, application of the marker, identification of the sites of its accumulation, and plotting of the pattern of distribution of labelled fibers in the cerebellum.

Under endotracheal anesthesia with a mixture of isoflurane and oxygen, we opened the labyrinth by removing the temporal bone below the horizontal semicircular canal. The lagena was visually identified by drilling two openings. The first one was initially made in the lateral wall of the bony labyrinth rostrally from the utriculus; then, after opening of the vestibulum, the second opening was made in the bottom of the latter. The lagena was opened using a