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ORIGIN OF EFFERENT FIBERS OF THE VESTIBULAR APPARATUS IN GOLDFISH. A HORSERADISH PEROXIDASE STUDY

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SUMMARY

After injecting a horseradish peroxidase (HRP) solution into the vestibular apparatus in goldfish (*Carassius auratus*), cells in the medulla oblongata exhibited retrogradely transported HRP reaction product. These labeled cells were confined to 3 nuclei, i.e. the nucleus motorius tegmenti of Bartelmez (with the most prominent labeled cell groups), the ventral vestibular nucleus (Deiter's nucleus) and the medial vestibular nucleus. We consider these labeled cells to be the origin of the efferent innervation to the vestibular apparatus in goldfish. Neurons providing efferent innervation to the sensory periphery in the nucleus motorius tegmenti have, as yet, not been described.

Electrophysiological recordings from the labyrinth of the frog suggested an inhibitory, centrifugal pathway to the sensory receptor organs of the vestibular apparatus [5, 14]. Electron microscopical studies on the receptor cell layer of the gold fish labyrinth revealed axon terminals synapsing on the hair cells. These terminals are assumed to be inhibitory [10]. The cells of origin of an efferent pathway to the vestibular apparatus have anatomically been identified in guinea pigs and cats [4, 12]. However, these cells have not been described in lower vertebrates.

In our experimental approach in goldfish (*Carassius auratus*), we injected unilaterally 0.18–0.38 μl of a 30% horseradish peroxidase (HRP) solution (in 1% poly-L-ornithine [7]), into the labyrinth of 7 specimens. HRP was injected into the cupula of the horizontal semicircular canal in 5 animals, and into the utriculus in two. At 24 h after the injection, the animals were perfused with saline transcardially

and then with a mixture of 1.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Frozen frontal sections of the brain, 40 μm thick, were treated for the demonstration of HRP according to the tetramethylbenzidine (TMB) technique [9]. In two cases we slightly damaged the cupula and the injections of 0.38 μl and 0.35 μl produced an accumulation of HRP reaction product in the surrounding meningea. Besides this, and the retrograde labeling of the neurons in the medulla, no other structures exhibited HRP reaction product and no indication for HRP diffusion was found.

In all animals we found cells labeled by the granular reaction product of retrogradely transported HRP [8] in three different nuclei of the medulla oblongata: the nucleus motorius tegmenti of Bartelmez [2] and the ventral and medial vestibular nuclei. The nucleus motorius tegmenti (NMT) lies in the ventromedial region of the medulla oblongata along the whole rostrocaudal extent. According to Bartelmez [2]: 'the NMT serves to coordinate the activities of the motor nuclei of the brain and cord'. The most significant cells belonging to the NMT are the two giant Mauthner cells in its rostral part, the Mueller cells, and 'large cells' [2]. The large cells are confined to 3 subdivisions of NMT: the pars rostralis at the level of the vestibular nuclei, the pars medialis and the pars inferior at the level of the facial and vagal lobe.

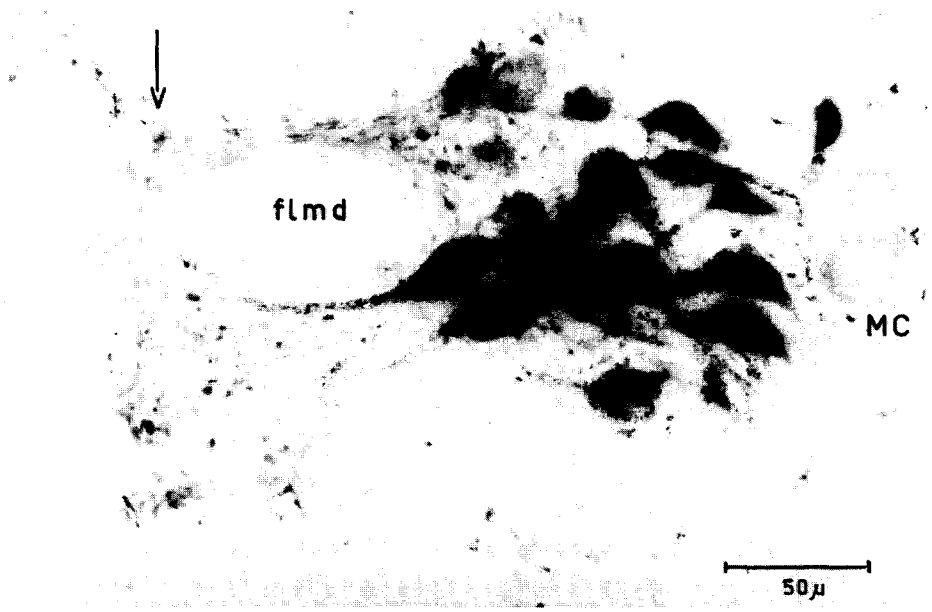

Fig. 1. Transverse section through the NMT of goldfish G 8. Most of the large cells are labeled by retrograde transport of HRP. Arrow indicates midline. MC, unlabeled Müller's cell; flmd, fasciculus longitudinalis medialis, pars dorsalis. TMB reaction, neutral red counterstain.

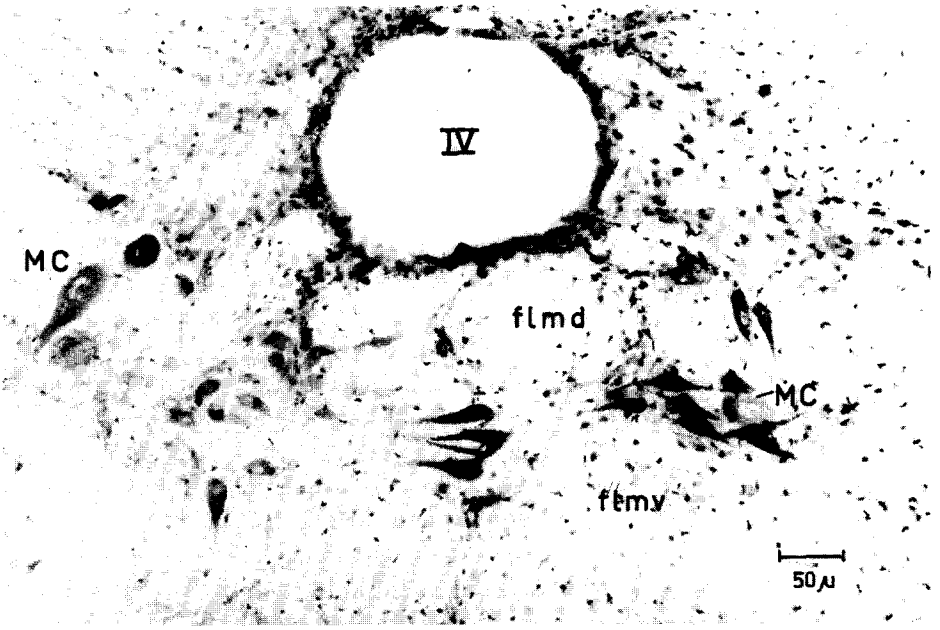

Fig. 2. Transverse section through the NMT of goldfish G 6 at the level of the vestibular nuclei. Most of the large cells of the right (ipsilateral) NMT are labeled. The group of labeled large cells at the midline was seen only in this specimen. Müller's cells (MC) are always unlabeled; flmv, fasciculus longitudinalis medialis, pars ventralis; IV, IV ventricle. TMB reaction, neutral red counterstain.

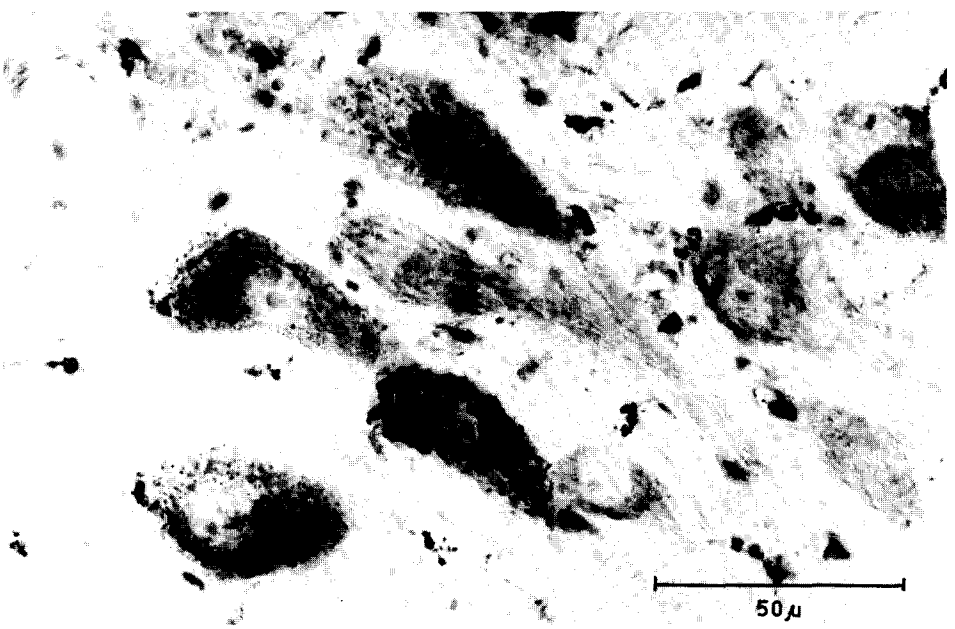

Fig. 3. Transverse section of the ventral vestibular nucleus (Deiter's nucleus) ipsilateral to the HRP injection showing labeled and unlabeled Deiter cells. TMB reaction, neutral red counterstain.

After unilateral HRP injection into the vestibular apparatus, 70% of these large cells are labeled ipsilaterally and less than 1% contralaterally. The maximum number of labeled cells in the NMT was 40. Of these 40 cells, 39 were located ipsilateral and one contralateral to the injection site. This was found in goldfish G 8, after an injection of only 0.18 μl HRP into the utriculus (Fig. 1).

In cross-sections the large cells appear arranged along the lateral rim of the fasciculus longitudinalis medialis (flm), and between pars dorsalis and pars ventralis of the flm. The large cells, about 30 μm in diameter, exhibit a typical fusiform shape, giving off one or two dendrites. These dendrites extend medially or ventrolaterally. In one case, the medial cells lie on the midline and their dendrites cross to the contralateral side (Fig. 2). We consider these fusiform large cells as the origin of efferent innervation to the vestibular apparatus. Neurons providing efferences to the labyrinth have previously not been described in the NMT.

We also found some HRP-labeled cells in the ventral vestibular nucleus ipsilaterally and contralaterally to the injection site. These neurons belong to the so-called 'Deiter cells' [11]. Ipsilaterally, 18% of the Deiter cells were labeled and contralaterally less than 2%. However, compared to the neurons of the NMT the granule density in Deiter cells is rather sparse, suggesting that this efference is less prominent (Fig. 3). This observation is compatible with an autoradiographic study of Alvarez and Püschel [1] in which tritiated amino acids were injected into the medulla oblongata at the level of the VII motor nucleus, effecting grain accumulations on axonal terminals close to the sensory hair cells of the vestibular apparatus. Alvarez and Püschel [1] consider these terminals as efferent axons. These axons might be identical to terminals which in our case pick up the injected HRP for retrograde axonal transport to the original cell body.

In addition to the nucleus motorius tegmenti and ventral vestibular nucleus, there is strong evidence for a further efferent origin in the medial vestibular nucleus. Some small-sized neurons of the nucleus, ipsilateral to the injection site, appear to contain granular HRP reaction products. However, due to strong afferent innervation by the vestibular nerve and interaction with anterograde-transported HRP reaction products, we were not able to classify these neurons as being labeled by retrograde transport. Electrophysiological recordings of cells in the medial vestibular nucleus suggest their efferent innervation of the vestibular apparatus [6].

The efferent innervation of the vestibular apparatus in goldfish takes its origin mainly from the large fusiform neurons in the nucleus motorius tegmenti and, to a lesser degree, from the ventral and, probably, from the medial vestibular nucleus.

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