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Origin of centrifugal fibers to the labyrinth in the frog (*Rana esculenta*). A study with the fluorescent retrograde neuronal tracer 'Fast blue'

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After injecting a solution of a fluorescent retrograde neuronal tracer (Fast blue, Diamidino compound 253/50) into the perilymphatic space of the frog labyrinth (*Rana esculenta*), labeled cells were found in the ventral and dorsal nuclei of the VIIIth nerve and in the nucleus reticularis medius. We consider these labeled cells to be the origin of the efferent innervation of the frog labyrinth. No evidence was found for the existence of a direct cerebello-labyrinthine connection.

Electrophysiological studies of the labyrinth of the frog demonstrated an inhibitory centrifugal pathway leading to the sensory receptor organs of the inner ear[7,12,17]. Light and electron microscopic studies on the receptor cell layer of the frog labyrinth revealed axonal terminals synapsing on the hair cells of all vestibular end-organs as well as on those of the amphibian papilla[4,11,14]. However, no efferent fibers were found at the level of the basilar papilla[5,15].

The cells of origin of the centrifugal pathways to the labyrinth have anatomically been identified in the goldfish, bird, guinea pig, cat and squirrel monkey[6,8,18,20–22]. In the frog, parent cells of this efferent innervation have been located in the ventral nucleus of the VIIIth nerve. Purkinje cells in the cerebellar auriculum have also been reported to project to the labyrinth[11,12].

Preliminary experiments with application of HRP to the labyrinth failed to demonstrate any labeled cells due to heavy precipitate of HRP reaction product in the entire brain stem. Therefore, we used Fast blue (FB, diamidino compound 253/50), a new fluorescent retrograde neuronal tracer[2].

Aliquots of 0.2 μl of a 4% solution of FB were unilaterally injected into the labyrinth of 7 young frogs (*Rana esculenta*). The animals were anesthetized with MS 222 Sandoz (aethyl-m-aminobenzoat), the tympanic membrane was opened and the plectum and columella were removed. FB was injected through the oval window into the perilymphatic cistern. After four days survival, the animals were perfused transcardially with saline followed by 10% formalin. The brains were soaked for 24 h in 30% sucrose-solution. Frozen frontal sections of the brain, 40 μm thick, were collected in distilled

water and then mounted and air dried but not coverslipped. The sections were studied with a Reichert UnivaR fluorescence microscope with filter mirror systems 49 and 4°9, which provide exitation light at 490 nm. After documentation of the fluorescence, the sections were stained with cresyl violet. As a control for the fluorescence, the brain of one unoperated frog was processed as above. The nomenclature of Opdam et al.[13] was followed for the nuclei of the brain stem.

In all animals which had received an FB injection, labeled cells were discernible not only by their blue-green fluorescence but also by fine brilliant blue fluorescent granules in the cytoplasm of the somata and proximal dendrites. The nucleus and surrounding glial cells showed no fluorescence. A low and diffuse fluorescence was however, consistently seen in the neuropil in the nuclei of the VIIIth nerve, probably caused here by anterograde transport of FB through the primary afferent fibers. Labeled neurons were found in 3 different nuclei of the medulla oblongata: the nucleus ventralis nervi octavi (VIIIv), the nucleus dorsalis nervi octavi (VIIId), and the nucleus reticularis medius (Rm, Fig. 1). No fluorescent neurons could be found in these 3

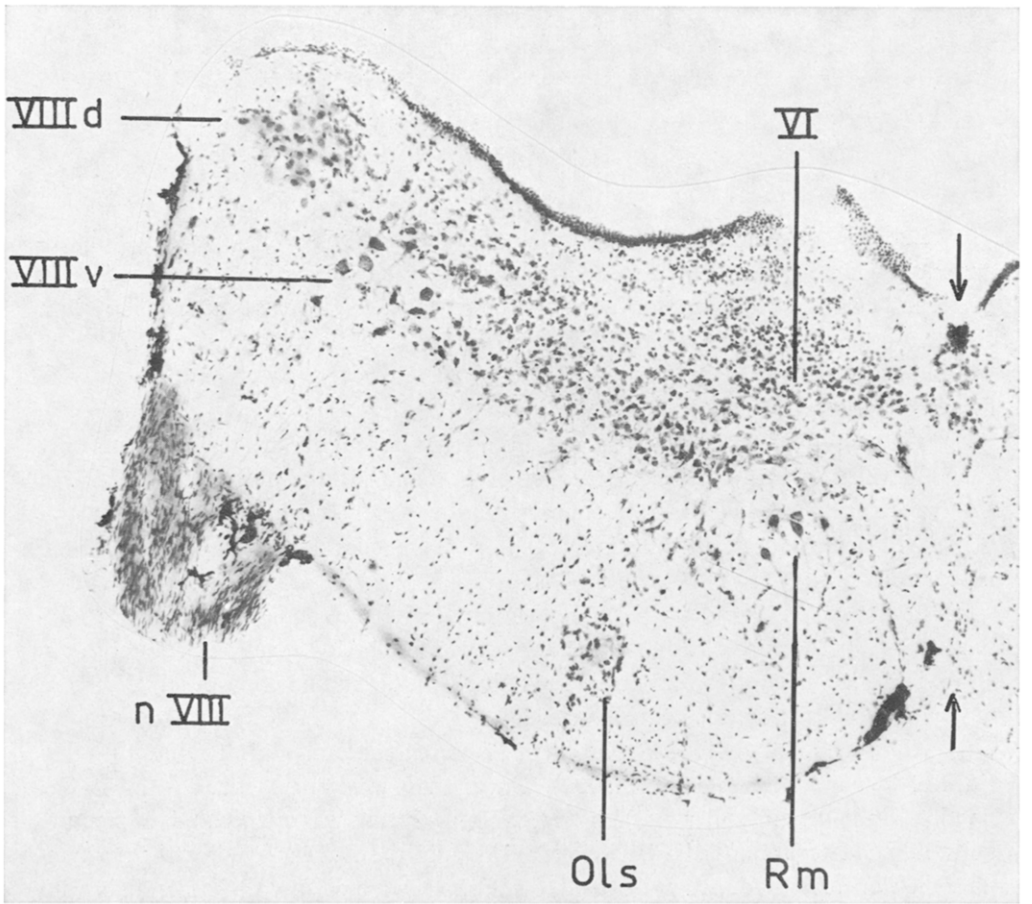

Fig. 1. Transverse section through the left half of the frog medulla oblongata to show the position of the nuclei of the VIIIth nerve (VIIIv, VIIId) and the nucleus reticularis medius (Rm). nVIII = nervus octavus, Ols = oliva superior, VI = nucleus abducentis. Arrow indicates midline. Cresyl violet stain.

nuclei in the unoperated frog. The most prominent group of retrogradely labeled cells (about 75% of all labeled cells) was found in the nucleus ventralis nervi octavi (VIIIv, Fig. 2A). This nucleus consists of loosely arranged large and medium sized polygonal cells which are contacted by the vestibular component of the VIIIth nerve[9].

Up to 300 labeled large and medium sized cells were found bilaterally in the VIIIv, i.e. about 80% of the VIIIv cells were labeled without a demonstrable difference between the two sides. Staining the sections with cresyl violet did not reveal any distinction between labeled and unlabeled cells (Fig. 2B). These findings are in agreement with published data. In the frog the existence of efferent neurons in the VIIIv were demonstrated electrophysiologically and by means of retrograde transport of Procion yellow through the anterior branch of the VIIIth nerve[14]. In an HRP study in the goldfish, efferent vestibular cells were found bilaterally in the corresponding nucleus (ventral vestibular nucleus), though more cells were labeled ipsilateral to the injection site[21].

The nucleus dorsalis nervi octavi (VIIId), in which the auditory component of the VIIIth nerve terminates[9] contained about 20% of the total number of labeled brain stem cells. The labeled neurons occurred bilaterally and were mostly accumulated in the dorsomedial parts of the nucleus. Again, labeled and unlabeled cells — relatively small spherical or fusiform cells — could not be differentiated in sections stained with cresyl violet. In the goldfish evidence for efferent vestibular neurons in the corresponding nucleus (medial vestibular nucleus) was found by HRP transport from the labyrinth, but here only in the ipsilateral nucleus[21]. In pigeon, by contrast, no efferent cells were positive identified in the vestibular nuclei in an HRP and [^{3}H]adenosine study[18]. In mammals, no efferent neurons originate in the vestibular nuclei[6,8,22].

Five per cent of the total of labeled brain stem neurons occurred in the nucleus reticularis medius (Rm). The Rm is represented by medium sized cells and a few large cells. It is located in the ventromedial region of the medulla oblongata, ventrolateral to the fasciculus longitudinalis medialis and the abducens nucleus, at the level of the octavus nuclei (Fig. 1). Bilaterally only medium sized fusiform cells were labeled (Fig. 3A). These were more labeled cells ipsilateral to the injection site. One or two large dendrites of these cells jut out after the staining with cresyl violet. The one runs in a ventral direction, the other more laterally (Figs. 1, 3). This finding indicates the close correspondence of the frog Rm ('motorische Schaltzellen' of Röthig[16]) with that of the nucleus motorius tegmenti (NMT) of Bartelmex[1] of the fish, since in the goldfish NMT similar fusiform neurons could be labeled with HRP from the labyrinth while the large Müller cells remained unlabeled[21]. In the goldfish however, in contrast to the frog, only a single labeled cell was found in the contralateral nucleus; the medial dendrite of the cells extended straight medialwards and sometimes crossed the midline, instead of running ventrally. In the pigeon, a similar group of fusiform efferent vestibular neurons was described bilaterally in a corresponding region medioventral to the abducens nucleus[18]. In mammals, efferent vestibular neurons were located lateral to the abducens nucleus, interposed between the abducens and the superior vestibular nucleus[6,8,22].

Sections through the cerebellum revealed a slight fluorescence of the Purkinje

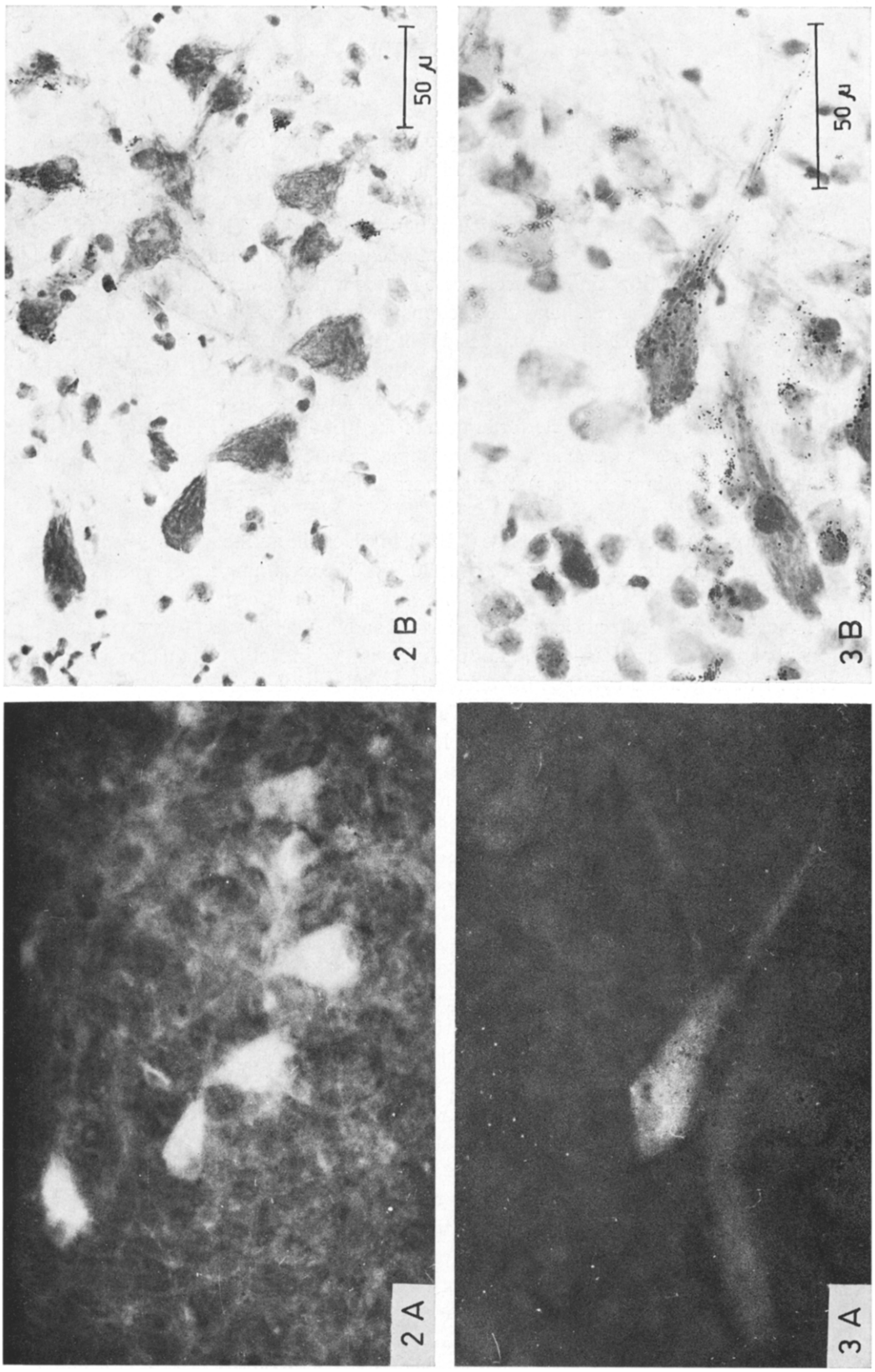

Fig. 2. A: fluorescent efferent neurons of the ventral nucleus of the VIIIth nerve. Note fluorescence in the neuropil due to labeling of afferent vestibular fibers. Same magnification as in B. B: cresyl violet stain of the same section to show the distribution of labeled and unlabeled cells.

Fig. 3. A: two labeled fusiform neurons from the right nucleus reticularis medius. B: the same cells in cresyl violet stain.

cells throughout the entire region. The same kind of fluorescence, however, was found in the unoperated frog, and was therefore interpreted as autofluorescence. This negative finding is in agreement with degeneration studies[9,10,19] which were unable to demonstrate the origin of labyrinth efferent fibers from cerebellar neurons.

A comparison of the efferent vestibular systems in the goldfish, frog, pigeon, cat and monkey indicates that efferent vestibular fibers originate from neurons of the brain stem reticular formation. Provided that this nucleus is identical in the species studied, it gradually shifts from a caudal position in goldfish to a more rostral position in mammals, and from near the midline in goldfish to a position ventral, ventrolateral and lateral in relation to the abducens nucleus in frog, pigeon and mammals, respectively. Furthermore, it gradually consolidates from an elongated row of groups of neurons to a compact nucleus. In the goldfish and frog efferent neurons are located in the nuclei of the VIIIth nerve in addition to those in the reticular formation.

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1 Bartelmez, G. W., Mauthner's cell and the nucleus motorius tegmenti, *J. comp. Neurol.*, 25 (1915) 87–126.
2 Bentivoglio, M., Kuypers, H. G. J. M., Catsman-Berrevoets, C. E., Loewe, H. and Dann, O., Two new fluorescent retrograde neuronal tracers which are transported over long distances, *Neurosci. Lett.*, 18 (1980) 25–30.
3 Corvaja, N., Grofova, I. and Pompeiano, O., The origin, course and termination of vestibulospinal fibers in the toad, *Brain Behav. Evol.*, 7 (1973) 401–423.
4 Flock, Å. and Flock, B., Ultrastructure of the amphibian papilla in the bullfrog, *J. acoust. Soc. Amer.*, 40 (1966) 1262.
5 Frishkopf, L. S. and Flock, Å., Ultrastructure of the basilar papilla. An auditory organ in the bullfrog, *Acta Otolaryng.*, 77 (1974) 176–184.
6 Gacek, R. R. and Lyon, M., The localization of vestibular efferent neurons in the kitten with horseradish peroxidase, *Acta Otolaryng.*, 77 (1974) 92–101.
7 Gleissner, L. and Henriksson, N. G., Efferent and afferent activity pattern in the vestibular nerve of frog, *Acta Otolaryng.*, Suppl. 192 (1963) 90–103.
8 Goldberg, J. M. and Fernández, C., Efferent vestibular system in the squirrel monkey: anatomical location and influence on afferent activity, *J. Neurophysiol.*, 43 (1980) 986–1025.
9 Gregory, K. M., Central projections of the eighth nerve in frogs, *Brain, Behav. Evol.*, 5 (1972) 70–88.
10 Gregory, K. M., The stato-acoustic nuclear complex and the nucleus cerebelli of the frog, *Brain, Behav. Evol.*, 10 (1974) 146–156.
11 Hillman, D. E., Light and electron microscopical study of the relationships between the cerebellum and the vestibular organ of the frog, *Exp. Brain Res.*, 9 (1969) 1–15.
12 Llinas, R. and Precht, W., The inhibitory vestibular efferent system and its relation to the cerebellum in the frog, *Exp. Brain Res.*, 9 (1969) 16–29.
13 Opdam, P., Kemali, M. and Nieuwenhuys, R., Topological analysis of the brain stem of the frogs *Rana esculenta* and *Rana catesbeiana*, *J. comp. Neurol.*, 165 (1976) 307–332.
14 Precht, W., Richter, A., Ozawa, S. and Shimazu, H., Intracellular study of frog's vestibular neurons in relation to the labyrinth and spinal cord, *Exp. Brain Res.*, 19 (1974) 377–393.
15 Robbins, R. G., Bauknight, R. S. and Honrubia, V., Anatomical distribution of efferent fibers in the VIIIth cranial nerve of the bullfrog *(Rana catesbeiana)*, *Acta Otolaryng.*, 64 (1967) 436–448.
16 Röthig, P., Beiträge zum Studium des Zentralnervensystems der Wirbeltiere. 11. Über die Faserzüge im Mittelhirn, Kleinhirn und der Medulla oblongata der Urodelen und Anuren, *Z. Mikr.-anat. Forsch.*, 10 (1927) 381–472.

17 Schmidt, R. S., Frog labyrinthine efferent impulses, *Acta Otolaryng.*, 56 (1963) 51–64.
18 Schwarz, D. W. F., Schwarz, I. E. and Tomlinson, R. D., Avian efferent vestibular neurons identified by axonal transport of [^{3}H]adenosine and horseradish peroxidase, *Brain Research*, 155 (1978) 103–107.
19 Stern, T. A. and Rubinson, K., Efferent projections of the cerebellar cortex of Rana Pipiens, *Anat. Rec.*, 169 (1971) 438.
20 Strutz, J. and Spatz, W. B., Superior olivary and extraolivary origin of centrifugal innervation of the cochlea in guinea pig. A horseradish peroxidase study, *Neurosci. Lett.*, 17 (1980) 227–230.
21 Strutz, J., Schmidt, C. L. and Stürmer, C., Origin of efferent fibers of the vestibular apparatus in goldfish. A horseradish peroxidase study, *Neurosci. Lett.*, 18 (1980) 5–9.
22 Warr, W. B., Olivocochlear and vestibular efferent neurons of the feline brain stem: their location, morphology and number determined by retrograde axonal transport and acetylcholinesterase histochemistry, *J. comp. Neurol.*, 161 (1975) 159–182.