

Electron Microscopic Study of the Termination of the Centrifugal Fibers in the Goldfish Olfactory Bulb

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Summary. The terminals of centrifugal fibers to the olfactory bulbs of goldfish were studied by electron microscopy after transection of the medial, lateral or entire olfactory tract. The centrifugal fibers originate in the telencephalic hemisphere, pass through both the medial and the lateral olfactory tract, and form synaptic contacts with dendrites in the granule cell layer.

Key words: Centrifugal fibers – Olfactory bulb – Goldfish – Degenerating synapses – Electron microscopy.

The fine structure of the olfactory bulb has been studied mainly in mammals and the results have provided morphological bases for an understanding of the neural mechanisms in olfactory function (Rall et al., 1966; Nicoll, 1969, 1971; Shepherd, 1972). Andres (1970, 1975) and Ichikawa (1976) have reported that the fine structure of the olfactory bulb of teleosts is fundamentally similar to that of mammals. Sheldon (1912) and Holmgren (1920), on the basis of Golgi observations, have reported that centrifugal fibers, passing through the olfactory tract, terminate in the olfactory bulb in teleosts. However, it has not been established from where the centrifugal fibers arise and with what neuronal elements in the olfactory bulb they make synapses.

In the present experiment, the terminations of the centrifugal fibers to the goldfish olfactory bulb were studied by electron microscopy.

Materials and Methods

Thirty-two adult goldfish, *Carassius auratus* (7–10 cm in standard length) were used. The fish were operated under 0.03% MS222 (tricaine methanesulfonate) anaesthesia. Sixteen fish were allowed to

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survive for 2, 4, 6, or 8 days in tank water at 20–24°C after the unilateral transection of the entire olfactory tract between the olfactory bulb and the telencephalic hemisphere. In addition, the medial olfactory tract was cut unilaterally in six fish and the lateral olfactory tract in four, and these ten fish were allowed to survive for 4 days after transection. In three other fish, the connection between the telencephalon and the diencephalon was cut; these animals were allowed to survive for 4 days. Three unoperated fish were used as controls. At autopsy, all operated and control fish were anaesthetised with MS222, and then perfused through the arterial trunk with a fixative containing 4% formaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2–7.4). The olfactory bulbs were excised, cut into blocks, and immersed in the same fixative for about 2 h. The blocks were then washed in 0.1 M sodium cacodylate buffer, postfixed with 0.1% OsO₄ in 0.1 M sodium cacodylate buffer for 2 h, dehydrated and embedded. Ultrathin sections were cut with a Porter-Blum MT-2 ultramicrotome and mounted on mesh grids (each grid square was 45 × 45 μm²). The ultrathin sections were stained with uranyl acetate and lead citrate and observed with a Hitachi HS-9 electron microscope. Degenerating synapses were counted directly under the electron microscope.

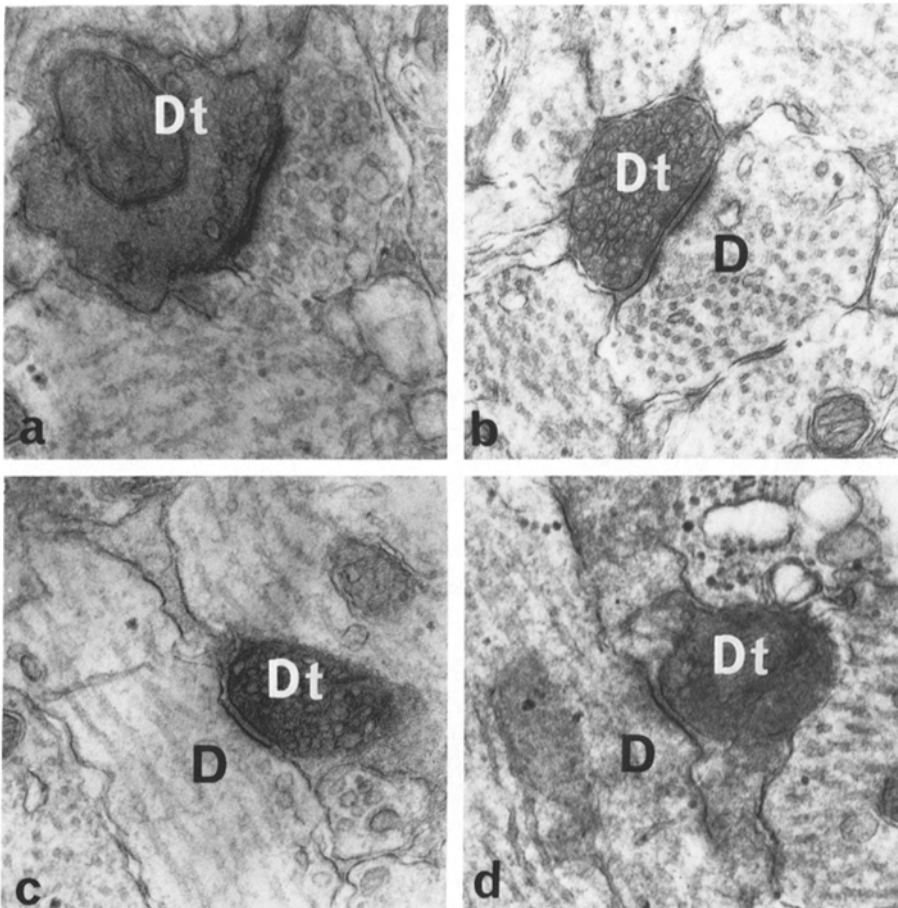


Fig. 1. Electron micrographs of degenerating synapses in the granule cell layer of the olfactory bulb. **a and b** Asymmetrical type of degenerating synapses 4 days after the transection of the olfactory tract. **c and d** Symmetrical type of degenerating synapse 4 days after the transection of the olfactory tract. *Dt* degenerating axon terminal; *D* dendrite. Asymmetrical synapses are characterized by a dense thickening of the postsynaptic membrane; symmetrical synapses show symmetrical thickening of their pre- and postsynaptic membranes. × 60,000

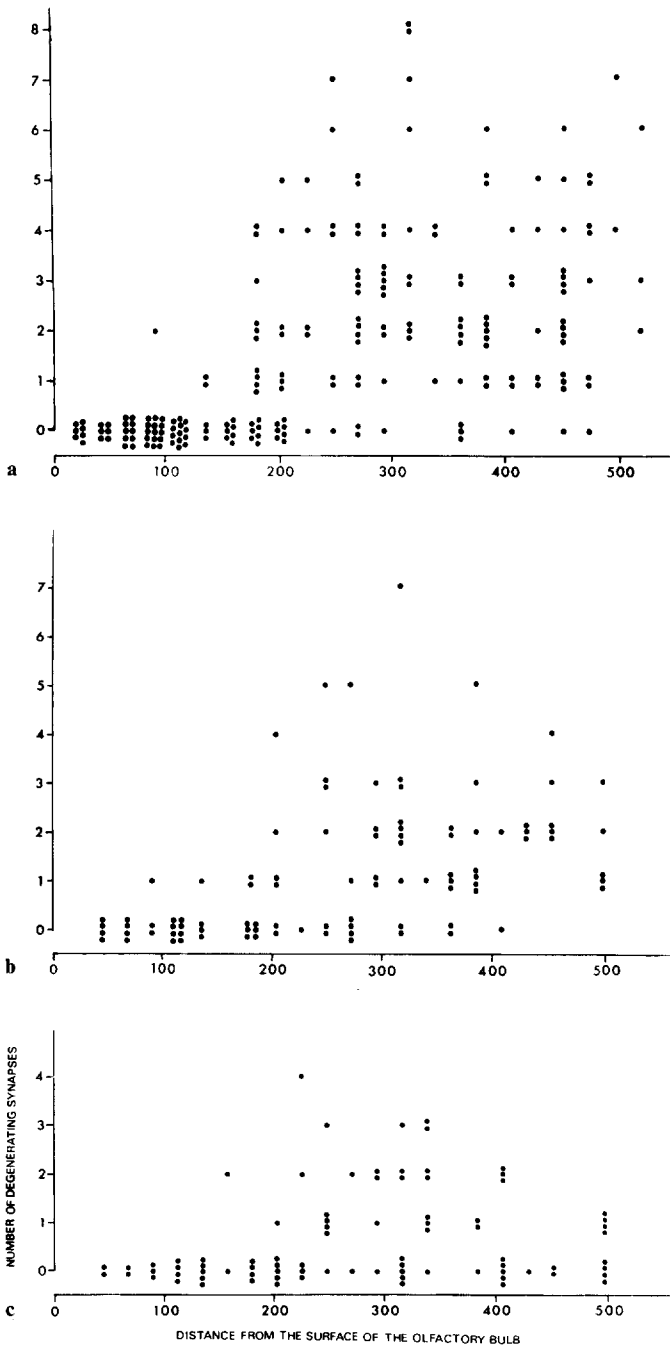


Fig. 2. Distribution of degenerating synapses 4 days after transection of the entire olfactory tract (**a**), the medial olfactory tract (**b**), and the lateral olfactory tract (**c**). The ordinate indicates the number of degenerating synapses per grid square. The abscissa indicates the distance from the surface of the olfactory bulb (μm)

Results

Degenerating axon terminals were characterized by an electron-dense axoplasm, irregularly shaped mitochondria, and synaptic vesicles, and established synaptic contact. Degenerating synapses were observed in the olfactory bulb 2, 4, 6, and 8 days after transection of the olfactory tract, but were most numerous at 4 days. Most were of the asymmetrical type (Fig. 1 a, b) although degenerating symmetrical synapses (Fig. 1 c, d) were observed occasionally. At 2–8 days after the operation, intact asymmetrical and symmetrical synapses were also observed. In unoperated fish, no degenerating synapses were found.

The goldfish olfactory bulbs comprise four layers (from superficial to deep): 1) olfactory nerve layer (approximately between 0 and 50 μm from the surface), 2) glomerular layer (50–100 μm), 3) mitral cell layer (100–200 μm), and 4) granule cell layer (200–500 μm). The distribution of degenerating synapses was examined in relation to the laminar organization of the olfactory bulb. After transection of the entire olfactory tract, degenerating synapses were mostly observed in the granule cell layer (average number of degenerating synapses 2.6 per grid square) (Fig. 2a). No degenerating synapses were found in the olfactory nerve layer or in the glomerular layer. Degenerating synapses were rarely observed in the mitral cell layer. After transection of the medial olfactory tract, degenerating synapses were observed mainly in the granule cell layer (average number 1.6 per grid square) (Fig. 2b), and were rarely found in the other layers. After transection of the lateral olfactory tract, degenerating synapses were also found in the granule cell layer (average number 0.9 per grid square) (Fig. 2c). These results showed that most of the centrifugal fibers to the olfactory bulb pass through the medial and lateral

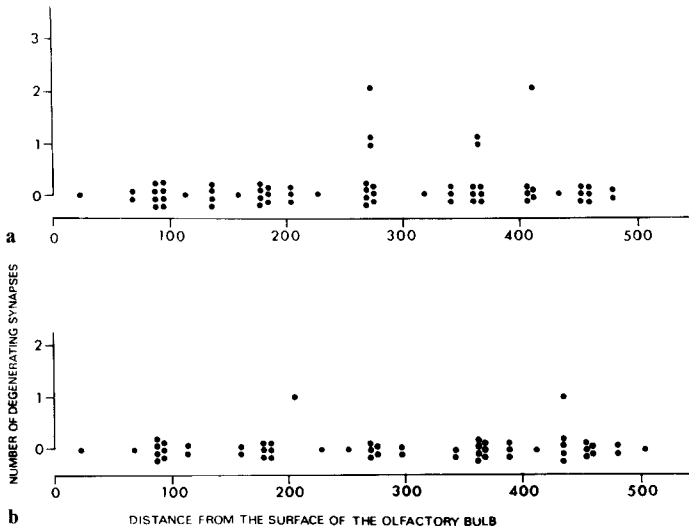


Fig. 3. Distribution of degenerating synapses 4 days after transection of the contralateral olfactory tract (**a**), and of the connection between the telencephalic hemisphere and diencephalon (**b**). The ordinate indicates the number of degenerating synapses per grid square. The abscissa indicates the distance from the surface of the olfactory bulb (μm)

olfactory tracts and terminate in the granule cell layer. After transection of the connection between the telencephalon and the diencephalon, degenerating synapses were observed scarcely in the olfactory bulb (Fig. 3a). Degenerating synapses were rarely found in the contralateral olfactory bulb after transection of the olfactory tract (Fig. 3b). These results suggest that the centrifugal fibers originate chiefly from the telencephalic hemispheres.

Discussion

Several investigators (Döving, 1966; Satou, 1971; MacLeod, 1976) have provided electrophysiological evidence that the fish olfactory bulb receives centrifugal fibers, and Ichikawa (1976) reported the existence of centrifugal fibers by electron microscopy. Light microscopic observations on the teleost have suggested that the centrifugal fibers to the olfactory bulb originate in the telencephalic hemisphere, the diencephalon, and the contralateral olfactory bulb, pass via the medial olfactory tract, and terminate in the granule cell layer (Sheldon, 1912; Holmgren, 1920). The present study has demonstrated that the centrifugal fibers originate mainly in the telencephalic hemispheres, pass via both the medial and lateral olfactory tracts and terminate in the granule cell layer. As for the centrifugal fibers to the olfactory bulb in mammals, it has been reported by several anatomists (Price, 1968, 1969; Price and Powell, 1970b, d; Pinching and Powell, 1972; Broadwell, 1975, 1976) that the centrifugal fibers originate in the anterior olfactory nuclei on both sides, and in the ipsilateral horizontal limb of the diagonal band, pass through both the medial and lateral olfactory tract, and terminate upon the dendrites of granule cells and periglomerular cells. The site of origin of the centrifugal fibers of teleosts has yet to be defined more precisely. The terminals of the centrifugal fibers were mostly found in the granule cell layer, presumably on the dendrites of the granule cells and/or of the short axon cells (Ichikawa, 1976).

Many asymmetrical and symmetrical synapses in the granule cell layer were unaffected by transection of the olfactory tract. Price and Powell (1970a, c) also observed these intact synapses in mammalian olfactory bulb after transection of the olfactory tract. They suggested that the intact synapses were made between the axon collaterals of the mitral cells and the granule cell dendrites (asymmetrical synapses), or between the axons of the short axon cells and the granule cell dendrites (symmetrical synapses). It has been suggested that synaptic arrangements in the fish olfactory bulb are similar to those in the mammals (Andres, 1975; Ichikawa, 1976), but further studies will be necessary to elucidate the fine details of the connections in the fish olfactory bulb.

References

- Andres, K.H.: Anatomy and ultrastructure of the olfactory bulb in fish, amphibia, reptiles, birds, and mammals. In G.E.W. Wolstenholme and J. Knight (Eds.), *Ciba Found Symp., Taste and Smell in Vertebrates*, pp. 177-194. London: Churchill 1970
- Andres, K.H.: Neue morphologische Grundlagen zur Physiologie des Riechens und Schmeckens. *Arch. Oto-Rhino-Laryng.* **210**, 1-41 (1975)

- Broadwell, R.D.: Olfactory relationships of the telencephalon and diencephalon in the rabbit. II. An autoradiographic and horseradish peroxidase study of the efferent connections of the anterior olfactory nucleus. *J. Comp. Neurol.* **164**, 389–410 (1975)
- Broadwell, R.D., Jacobowitz, D.M.: Olfactory relationships of the telencephalon and diencephalon in the rabbit. III. The ipsilateral centrifugal fibers to the olfactory bulbar and retrobulbar formations. *J. Comp. Neurol.* **170**, 321–346 (1976)
- Döving, K.B.: Comparative electrophysiological studies on the olfactory tract of some teleosts. *J. Comp. Neurol.* **131**, 365–370 (1966)
- Holmgren, N.: Zur Anatomie und Histologie des Vorder- und Zwischenhirns der Knochenfische. *Acta Zool.* **1**, 137–315 (1920)
- Ichikawa, M.: Fine structure of the olfactory bulb in the goldfish, *Carassius auratus*. *Brain Res.* **115**, 43–56 (1976)
- MacLeod, N.K.: Field potentials in the olfactory bulb on the codfish (*Gadus morhua*). *Comp. Biochem. Physiol.* **55A**, 297–299 (1976)
- Nicoll, R.A.: Inhibitory mechanisms in the rabbit olfactory bulb: dendrodendritic mechanisms. *Brain Res.* **14**, 157–172 (1969)
- Nicoll, R.A.: Physiological evidence for dendrodendritic synaptic interactions in the rabbit's olfactory glomerulus. *Brain Res.* **32**, 212–217 (1971)
- Pinching, A.J., Powell, T.P.S.: The termination of centrifugal fibres in the glomerular layer of the olfactory bulb. *J. Cell Sci.* **10**, 621–635 (1972)
- Price, J.L.: The termination of the centrifugal fibres in the olfactory bulb. *Brain Res.* **7**, 483–486 (1968)
- Price, J.L.: The origin of the centrifugal fibers to the olfactory bulb. *Brain Res.* **14**, 542–545 (1969)
- Price, J.L., Powell, T.P.S.: The synaptology of the granule cells of the olfactory bulb. *J. Cell Sci.* **7**, 125–155 (1970a)
- Price, J.L., Powell, T.P.S.: An electron microscopic study of the afferent fibres to the olfactory bulb from the cerebral hemisphere. *J. Cell Sci.* **7**, 157–187 (1970b)
- Price, J.L., Powell, T.P.S.: The mitral and short axon cells of the olfactory bulb. *J. Cell Sci.* **7**, 631–651 (1970c)
- Price, J.L., Powell, T.P.S.: An experimental study of the origin and the course of the centrifugal fibres to the olfactory bulb in the rat. *J. Anat.* **107**, 215–237 (1970d)
- Rall, W., Shepherd, G.M., Reese, T.S., Brightman, M.W.: Dendrodendritic synaptic pathway for inhibition in olfactory bulb. *Exp. Neurol.* **14**, 44–56 (1966)
- Satou, M.: Electrophysiological study of the olfactory system in fish. I. Bulbar response with special reference to adaptation in the carp, *Cyprinus carpio* L. *J. Fac. Sci. Univ. Tokyo. Sec. IV.* **12**, 183–218 (1971)
- Sheldon, R.E.: The olfactory tracts and centers in teleosts. *J. Comp. Neurol.* **22**, 117–340 (1912)
- Shepherd, G.M.: Synaptic organization of the mammalian olfactory bulb. *Physiol. Rev.* **52**, 864–917 (1972)