Brain Research, 335 (1985) 148–152
Elsevier

BRE 20805

Ganglion cells of the terminal nerve: morphology and electrophysiology

ICHIRO FUJITA, MASAHIKO SATOU and KAZUO UEDA

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113 (Japan)

(Accepted December 18th, 1984)

Key words: nervus terminalis — carp — intracellular recording — horseradish peroxidase — hyperpolarizing potential

The ganglion cells of the terminal nerve (TN cells) in the carp were identified using intracellular recording and staining techniques. The TN cells showed characteristic location and morphology as well as characteristic responses after electrical shocks to the olfactory nerve and tracts. These features of the TN cells were distinct from those of the mitral or other cells in the olfactory bulb.

The terminal nerve (nervus terminalis) is found in various species of vertebrate classes but not in birds. Its ganglion cells (TN cells) and fibers are distributed within or along the olfactory pathway (see references in ref. 25). In teleosts, the terminal nerve projects to some telencephalic areas[1,26,27], which seem to be involved in sexual behavior[5,14,15,24], as well as to the retina[6,17,18,28]. It has immunoreactivity to the luteinizing hormone–releasing hormone[11,17,18,30] and to the molluscan cardioexcitatory peptide[30]. Lesion and stimulation studies have raised the possibility that the terminal nerve transmits sexually relevant chemosensory information[4,6,7,29]. Thus, the terminal nerve seems to be a biologically significant system.

Electrophysiological studies of the terminal nerve have been very scarce[3]. Recording from the TN cells has not been made in any vertebrates. In addition, the description of the morphology of the TN cells in teleosts is quite incomplete even at the light microscopic level, because previous anatomical studies are focused on the location, projection and immunocytochemistry of the TN cells[1,6,17,18,20,26–28,30]. Recently, we succeeded in recording intracellularly from the TN cells in the common carp[8,10]. We report here the results of an intracellular recording and staining of the TN cells and demonstrate the characteristic features of the TN cells in terms of morphology and electrophysiology.

Fourteen carp (*Cyprinus carpio*) of either sex, weighing 680–820 g, were used. The general experimental procedure was the same as that described elsewhere[21]. Electrical shocks of 100 μs were applied to the medial (MOT) or lateral (LOT) olfactory tract or to the olfactory nerve (ON). The strength of the stimuli was adjusted to elicit the maximal field potentials in the olfactory bulb[21]. Glass micropipettes filled with a 2 M potassium citrate or a 3 M potassium chloride solution were used for the intracellular recording. For the intracellular staining, glass micropipettes filled with a 10% solution of horseradish peroxidase (HRP; Sigma type VI, dissolved in a 0.05 M Tris-HCl buffer containing 0.5 M potassium acetate, pH 7.6) were used. HRP was iontophoresed into the cell with depolarizing current pulses of 500 or 600 ms duration, repeated once every second. The total amount of the injected HRP ranged from 3 to 24 nA $\times$ min. After fixation, the olfactory bulb was embedded in gelatin, and serial frozen sections were cut frontally or horizontally at 100 μm. The sections were then reacted according to the modified Hanker–Yates method[2,12].

In cyprinids, the TN cells are located in the medial part of the ON at the rostral level of the olfactory bulb[20,26–28], and so intracellular recordings were made from cells in this region. Field potentials evoked by ON shocks were monitored as the record-

Correspondence: I. Fujita. Present address: Department of Biological Control System, National Institute for Physiological Sciences, Okazaki 444, Japan.

0006-8993/85/$03.30 © 1985 Elsevier Science Publishers B.V. (Biomedical Division)

ing electrode was advanced vertically into the olfactory bulb, so that the electrode traversed the olfactory nerve and/or the glomerular layers of the olfactory bulb exclusively: when the electrode traversed the olfactory nerve and/or glomerular layers at the most rostral level of the olfactory bulb, the compound action potential of the olfactory nerve and the negative slow wave (C2 wave), which represents a depolarization in peripheral dendrites of the granule cells, were exclusively recorded at any depth. In a more caudal track, the compound action potential of the olfactory nerve disappeared, and the C2 wave reversed its polarity to positive, when the electrode traversed the mitral cell layer[21,22]. However, it was still possible that electrodes might impale mitral cell dendrites which extend into the glomerular layer[9,13,19]. To check the location and morphology of the impaled cells, 9 cells were stained by intracellular injection of HRP. An example of the labeled cells is shown in Fig. 1. The large soma (75 × 50 μm) was located among the ON fibers in the rostromedial part of the ventral olfactory bulb (Fig. 1A). Several thick and many thin dendrites arose from the soma (Fig. 1B). The dendrites ramified near the soma. Some thin branches took a wavy course. The dendrites did not make up glomerular tufts like those of mitral cells. In another labeled cell, a dendrite ran rostrally among the ON fibers over 800 μm. However, we have not yet observed any dendrites entering the olfactory epithelium. Although the axon could not be identified in the cell shown in Fig. 1, we could identify the axons in 5 other cells. The axons arose from the somata and projected caudally through the olfactory nerve and/or glomerular layers along the ventromedial surface of the olfactory bulb to the MOT. In two cells, an intrabulbar axon collateral was observed. The axons were thicker (2.5 μm) than those of mitral cells (about 1 μm). Thus, the labeled cells were morphologically distinct from the mitral or other cells in the olfactory bulb[9,13,19]. Because there are no other cells of comparable size and shape in this region[20,26–28], we identified the labeled cells as the TN cells. We further concluded that all of the analyzed cells described below were TN cells because the cells which were not injected with HRP showed responses identical to those of the HRP-labeled cells.

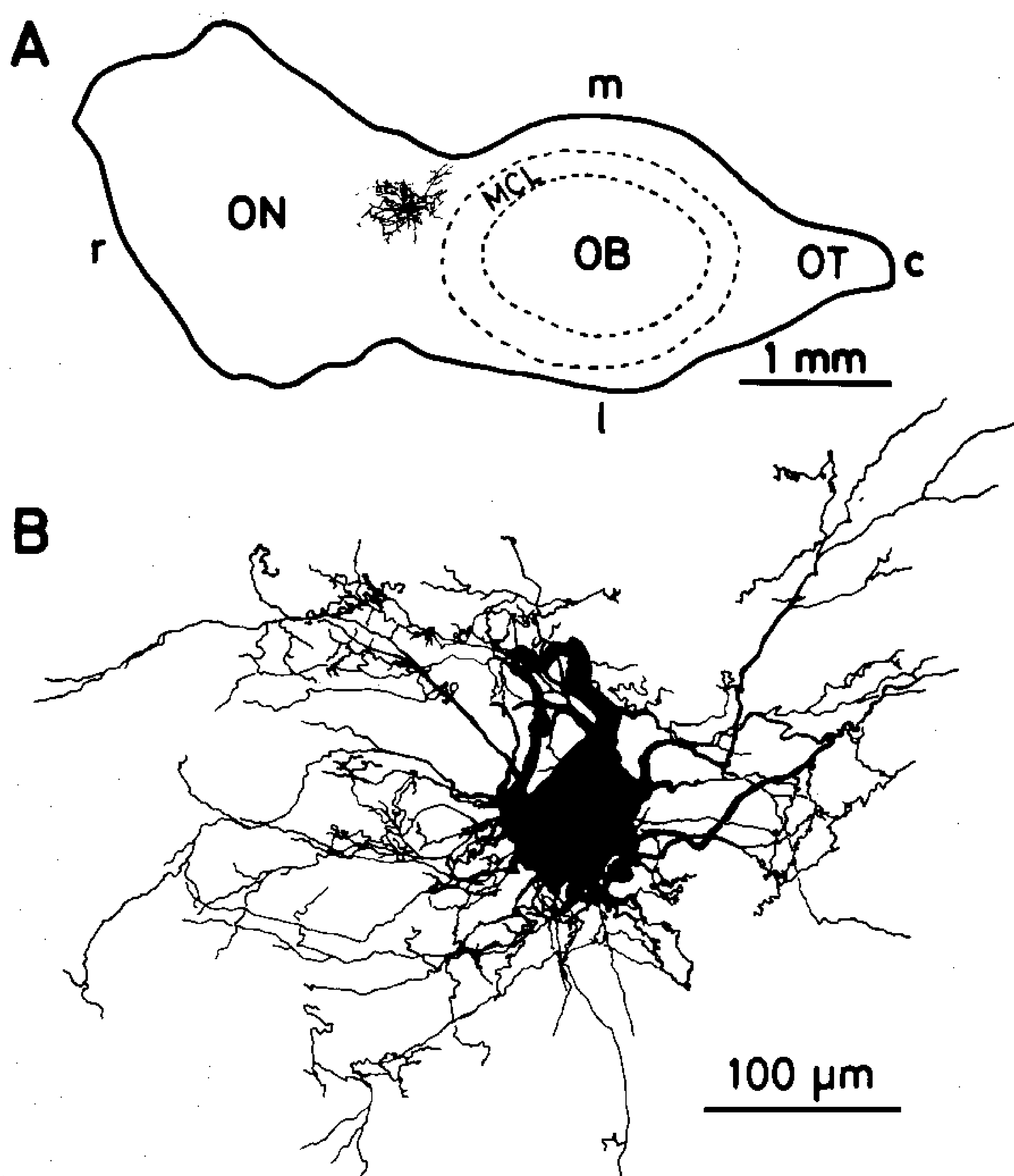

Fig. 1. Reconstruction of a TN cell intracellularly labeled with HRP. The TN cell is located in the medial part of the olfactory nerve (A) and has characteristic morphology (B). MCL, mitral cell layer; OB, olfactory bulb; ON, olfactory nerve; OT, olfactory tract; m, l, r, c, medial, lateral, rostral and caudal directions, respectively.

Thirty-eight TN cells whose resting potentials ranged from −35 to −70 mV were analyzed. MOT shocks elicited an action potential in 18 of the 38 cells, and there was a long-lasting hyperpolarizing potential in all of the cells (Fig. $2A_a$). The amplitude and duration of the MOT-evoked hyperpolarizing potentials ranged from 5 to 20 mV and from 950 to 1900 ms, respectively. LOT shocks elicited no response in any cell. The spikes seen in Fig. $2B_a$ were spontaneous ones. In 29 (76%) of the 38 cells, single ON shocks also elicited a long-lasting hyperpolarizing potential (5–23 mV in amplitude, 650–1400 ms in duration) (Fig. $2C_a$), while in the other 9 cells, ON shocks elicited no response. ON shocks also elicited an action potential in some cells (Fig. $2C_a$, arrow). The ON-evoked action potentials cannot be regarded as antidromic ones because they showed a considerable fluctuation in the latency and did not follow the repetitive stimuli at 3 Hz. In mitral cells, ON shocks elicit large excitatory postsynaptic potentials (EPSPs) on which action potentials are superimposed[21]. In the TN cells no such potentials with comparable size to the EPSPs in the mitral cells were observed. However, the properties of the ON-evoked

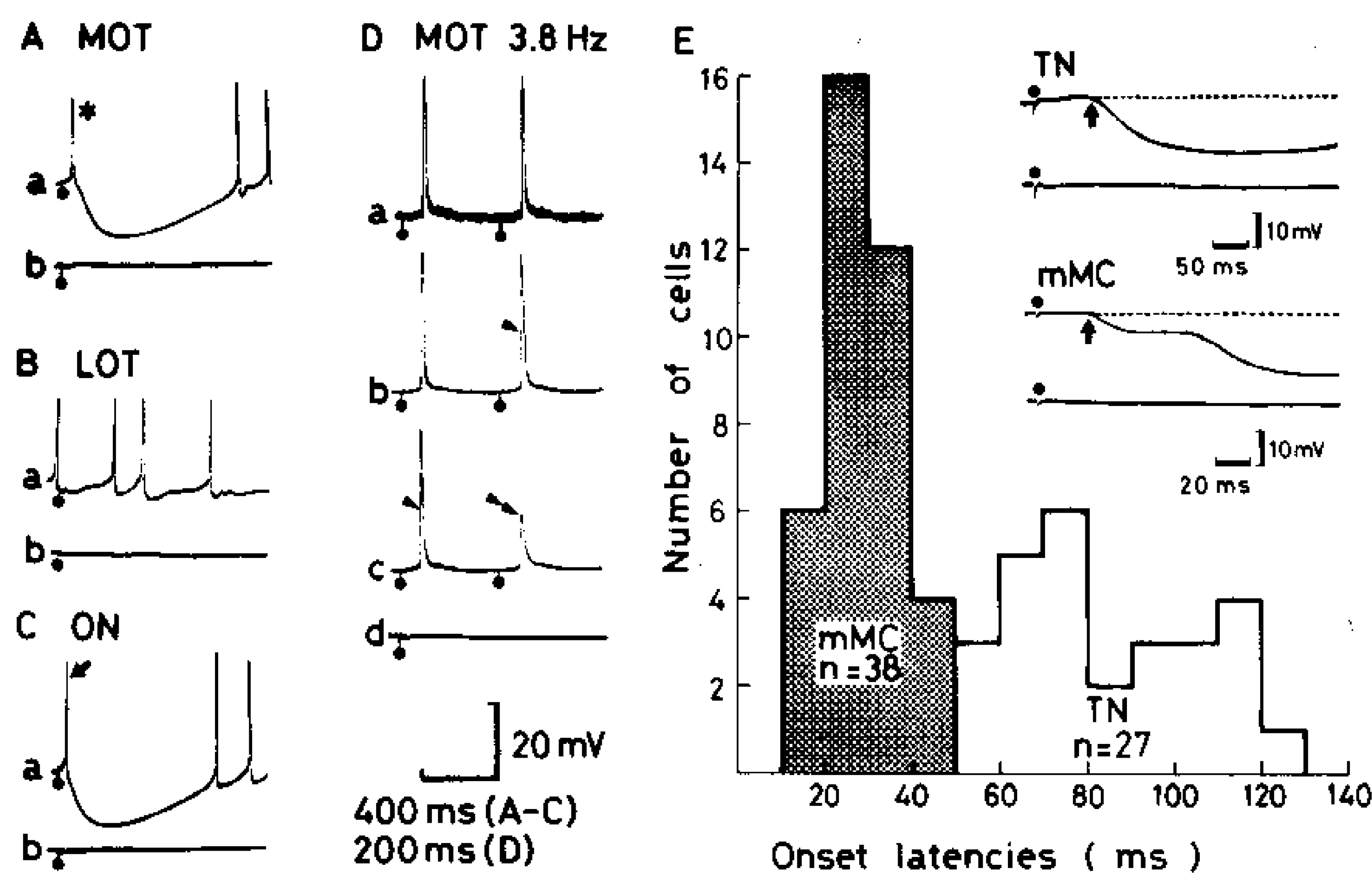

Fig. 2. Intracellular responses of a TN cell to MOT (A_a), LOT (B_a), ON (C_a) and repetitive MOT ($D_{a,b,c}$) shocks. Lower traces (A_b, B_b, C_b, D_d) are extracellular field potentials just outside the cell. Asterisk in A_a indicates an antidromic potential. ON shocks also elicited an action potential in this cell (arrow in C_a, see text). Arrowheads in $D_{b,c}$ indicate a notch in the rising phase of action potentials. Double arrowheads in D_c indicate an initial segment spike. Dots indicate time of stimulation. E: histograms of onset latencies of IPSPs of mitral cells in the medial part of the olfactory bulb (mMC, shaded columns) and those of hyperpolarizing potentials of TN cells (TN, blank columns). Inset shows intracellular responses of a TN cell and mMC to MOT shocks (upper traces) and extracellular potentials just outside the impaled cells (lower traces). The onset time was determined as the divergence point (arrows) of the superimposed tracings of the hyperpolarizing potentials and the extracellular potentials recorded just outside the cells (dotted lines in the upper traces). Note the different time scales.

action potentials were not examined further in the present study.

The responses to repetitive MOT shocks (3.8 Hz) are shown in Fig. 2D. The membrane potential in this cell was shifted by 14 mV to a more hyperpolarized level during the repetitive shocks, which resulted in the disappearance of the MOT-evoked hyperpolarizing potential. The action potentials were elicited at a constant latency of 55 ms. As the stimulation was continued, an inflection of about 15 mV appeared in the rising phase of the spikes (arrowheads in Fig. $2D_{b,c}$). In some cases, a partial spike, which appears to be an initial-segment component of the spike, was elicited in isolation (double arrowheads in Fig. $2D_c$). These driven spikes collided with the preceding spontaneous spikes (not shown). These results indicate that these spikes are antidromically elicited. The latency ranged from 48 to 96 ms. The axonal conduction velocities, which were estimated from the latency and the distance between the recording site and the MOT-stimulating site in each preparation, ranged from 0.27 to 0.53 m/s (0.44 ± 0.08 m/s, mean ± S.D., n = 18).

The intracellular responses of the TN cells were similar to those of the mitral cells in the medial part of the olfactory bulb (mMC) in that both showed long-lasting hyperpolarizing potentials [inhibitory postsynaptic potentials (IPSPs) in the case of the mitral cells] after MOT or ON shocks but no response after LOT shocks[21]. However, the hyperpolarizing potentials in the TN cells had properties different from the IPSPs in the mMC. First, MOT-evoked IPSPs in the mMC usually have two phases[21], while no such phases were observed in the hyperpolarization of the TN cells (inset in Fig. 2E). Second, repetitive MOT shocks at a frequency higher than 0.5 Hz caused the potentiation of the IPSPs in the mMC[21], while the hyperpolarizing potentials in the TN cells were not potentiated by repetitive MOT shocks. Third, the onset latencies of the MOT-evoked hyperpolarizing potentials in the TN cells (55–125 ms; blank columns in Fig. 2E) were longer than those of the MOT-evoked IPSPs in the mMC (12–43 ms; shaded columns in Fig. 2E). The latencies of the ON-evoked hyperpolarizing potentials of the TN cells also tended to be longer than those of the ON-evoked IPSPs in the mMC, although the onset latencies of the ON-evoked IPSPs in the mMC could not be determined accurately because of the preceding EPSPs.

The present study shows that one can identify the TN cells by two lines of criteria. The first is their location among the ON fibers in the rostromedial part of the olfactory bulb. Monitoring the ON-evoked potentials facilitates the electrode penetration of the TN cells in this region. The second criterion is the characteristic responses of the TN cells to MOT and ON shocks, as has been descibed above. The slow axonal conduction velocity (0.27–0.53 m/s) is another characteristic feature of the TN cells. However, it cannot serve alone as a criterion to identify TN cells because the conduction velocity of some mitral cells is in the same range (0.14–3.89 m/s)[23].

From the results of anatomical studies, it has been suggested that: (1) some TN cells project to the retina through the MOT but others do not[6,17,18,28], and (2) the distal processes of the TN cells project to the olfactory epithelium[6,26]. The present results are consistent with the former suggestion in that only half of the TN cells were activated antidromically by MOT

volleys, although we cannot rule out another possibility — that the failure of the antidromic activation of half of the TN cells is attributable to some technical problems. We could not confirm the latter suggestion since we have not yet observed any HRP-labeled fibers entering the olfactory epithelium or any antidromic action potential after ON shocks.

From the properties of the hyperpolarizing potentials of the TN cells described below, we suggest that these potentials are IPSPs: (1) spontaneous firing (2.2–4.7 Hz) was suppressed during the hyperpolarizing potentials, (2) the amplitude was reduced when the membrane potential was hyperpolarized, and (3) in a few cells tested, the membrane conductance increased during the hyperpolarizing potentials (unpublished observation).

It has been suggested that the terminal nerve has a sensory and/or visceral motor function[6,16,20]. However, no direct evidence for either of these hypotheses has yet been obtained. Recently, though, Bullock and Northcutt examined the effect of the sensory stimulation of various modalities on the neuronal discharge of the terminal nerve, which contain both afferent and efferent fibers (cf. ref. 20), in the dogfish. The discharge rate of the efferent fibers was decreased by certain mechanical stimuli applied to the head region, while the discharge rate was not affected by the stimulation of the other modalities[3]. In their study, they could record only the efferent impulses of thicker fibers, probably because they used gross recording electrodes. They suggested that 'microelectrodes will succeed in recording from afferent units in the nerve or from cell bodies in its ganglion, permitting direct search for the adequate stimuli'. The present study is the first study to record from the TN cells and provides a means of identifying the TN cells electrophysiologically. Considering the possible role of the terminal nerve in olfaction, sexual behavior, reproduction and the efferent modulation of the retinal function[30], we can say that the carp is a preferable material, for the above problems have been intensively studied in cyprinid fishes. Thus, the present results may serve as a basis for future studies to elucidate the function of the terminal nerve system.

We thank Drs. Y. Koyama and Y. Oka for their helpful comments on the manuscript, and Miss H. Tanaka for typing the manuscript. This work was supported by a grant from the Ministry of Education, Science, and Culture of Japan.

1 Bass, A. H., Olfactory bulb efferents in the channel catfish, *Ictalurus punctatus*, *J. Morphol.*, 169 (1981) 91–111.
2 Bell, C. C., Finger, T. E. and Russell, C. J., Central connections of the posterior lateral line lobe in mormyrid fish, *Exp. Brain Res.*, 42 (1981) 9–22.
3 Bullock, T. H. and Northcutt, R. G., Nervus terminalis in dogfish (*Squalus acanthias*, elasmobranchii) carries tonic efferent impulses, *Neurosci. Lett.*, 44 (1984) 155–160.
4 Demski, L. S. and Dulka, J. G., Functional–anatomical studies on sperm release evoked by electrical stimulation of the olfactory tract in goldfish, *Brain Research*, 291 (1984) 241–247.
5 Demski, L. S. and Hornby, P. J., Hormonal control of fish reproductive behavior: brain–gonadal steroid interactions, *Canad. J. Fish. aquat. Sci.*, 39 (1982) 36–47.
6 Demski, L. S. and Northcutt, R. G., The terminal nerve: a new chemosensory system in vertebrates?, *Science*, 220 (1983) 435–437.
7 Døving, K. B. and Selset, R., Behavior patterns in cod released by electrical stimulation of olfactory tract bundlets, *Science*, 207 (1980) 559–560.
8 Fujita, I., Satou, M. and Ueda, K., The nervus terminalis system in the carp (*Cyprinus carpio* L.): analysis of intracellular potentials in the ganglion cells, *Proc. Jap. Soc. gen. comp. Physiol.*, 5 (1983) 78.
9 Fujita, I., Satou, M. and Ueda, K., Morphology of carp mitral cells intracellularly labelled with horseradish peroxidase, *Proc. Jap. Symp. Taste Smell*, 17 (1983) 5–8.
10 Fujita, I., Satou, M. and Ueda, K., Hyperpolarizing potentials in the ganglion cells of the nervus terminalis in the carp., *J. physiol. Soc. Jap.*, 46 (1984) 464.
11 Halpern-Sebold, L. R. and Schreibman, M. P., Ontogeny of centers containing luteinizing hormone–releasing hormone in the brain of platyfish (*Xiphophorus maculatus*) as determined by immunocytochemistry, *Cell Tiss. Res.*, 229 (1983) 75–84.
12 Hanker, J. S., Yates, P. E., Metz, C. B. and Rustioni, A., A new specific, sensitive and non-carcinogenic reagent for the demonstration of horseradish peroxidase, *Histochem. J.*, 9 (1977) 789–792.
13 Kosaka, T. and Hama, K., Structure of the mitral cell in the olfactory bulb of the goldfish (*Carassius auratus*), *J. comp. Neurol.*, 212 (1982) 365–384.
14 Koyama, Y., Satou, M., Oka, Y. and Ueda, K., Involvement of the telencephalic hemispheres and the preoptic area in sexual behavior of the male goldfish, *Carassius auratus*: a brain-lesion study, *Behav. Neural Biol.*, 40 (1984) 70–86.
15 Kyle, A. L. and Peter, R. E., Effects of forebrain lesions on spawning behaviour in the male goldfish, *Physiol. Behav.*, 28 (1982) 1103–1109.
16 Larcell, O., Studies on the nervus terminalis: mammals, *J. comp. Neurol.*, 30 (1918) 3–68.
17 Münz, H., Claas, B., Stumpf, W. E. and Jennes, L., Centrifugal innervation of the retina by luteinizing hormone releasing hormone (LHRH)-immunoreactive telencephalic

neurons in teleostean fishes, *Cell Tiss. Res.*, 222 (1982) 313–323.
18 Münz, H., Stumpf, W. E. and Jennes, L., LHRH systems in the brain of platyfish, *Brain Research,* 221 (1981) 1–13.
19 Oka, Y., Golgi, electron-microscopic and combined Golgi–electron-microscopic studies of the mitral cells in the goldfish olfactory bulb, *Neuroscience,* 8 (1983) 723–742.
20 Rossi, A., Basile, A. and Palombi, F., Speculations on the function of the nervus terminalis system in teleosts, *Riv. Biol.,* 65 (1973) 401–409.
21 Satou, M., Fujita, I., Ichikawa, M., Yamaguchi, K. and Ueda, K., Field potential and intracellular potential studies of the olfactory bulb in the carp: evidence for a functional separation of the olfactory bulb into lateral and medial subdivisions, *J. comp. Physiol.,* 152 (1983) 319–333.
22 Satou, M., Fujita, I. and Ueda, K., Responses of olfactory bulb to olfactory nerve volleys in the carp, *Proc. Jap. Symp. Taste Smell.,* 15 (1981) 52–55.
23 Satou, M., Ichikawa, M., Ueda, K. and Takagi, S. F., Topographical relation between olfactory bulb and olfactory tracts in the carp, *Brain Research,* 173 (1979) 142–146.
24 Satou, M., Oka, Y,., Kusunoki, M., Matsushima, T., Kato, M., Fujita, I. and Ueda, K., Telencephalic and preoptic areas integrate sexual behavior in hime salmon (landlocked red salmon, *Oncorhynchus nerka*): results of electrical brain stimulation experiments, *Physiol. Behav.,* 33 (1984) 441–447.
25 Schwanzel-Fukuda, M. and Silverman, A. J., The nervus terminalis of the guinea pig: a new luteinizing hormone–releasing hormone (LHRH) neuronal system, *J. comp. Neurol.,* 191 (1980) 213–225.
26 Sheldon, R. E., The nervus terminalis in the carp, *J. comp. Neurol. Psychol.,* 19 (1909) 191–201.
27 Sheldon, R. E., The olfactory tracts and centers in teleosts, *J. comp. Neurol.,* 22 (1912) 177–339.
28 Springer, A. D., Centrifugal innervation of goldfish retina from ganglion cells of the nervus terminalis, *J. comp. Neurol.,* 214 (1983) 404–415.
29 Stacey, N. E. and Kyle, A. L., Effects of olfactory tract lesions on sexual and feeding behavior in the goldfish, *Physiol. Behav.,* 30 (1983) 621–628.
30 Stell, W. K., Walker, S. E., Chohan, K. S. and Ball, A. K., The goldfish nervus terminalis: a luteinizing hormone–releasing hormone and molluscan cardioexcitatory peptide immunoreactive olfactoretinal pathway, *Proc. nat. Acad. Sci. (U.S.A.),* 81 (1984) 940–944.