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Author: Matsuzaki, Osamu

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Numbers of Olfactory Receptor Cells and Fine Structure of Olfactory Nerves in Various Birds

OSAMU MATSUZAKI

*Institute of Biological Sciences, University of Tsukuba Tennodai,
Tsukuba, Ibaraki 305, Japan*

ABSTRACT—The numbers of olfactory receptor cells from electron micrographs in various species of birds were counted and the fine structure of their olfactory nerves was observed using electron micrographs. The birds were domestic ducks, a slay-backed gull, quails, budgerigars and bengalees. Data from pigeons obtained from a previous study were also included for comparison. The approximate numbers of olfactory cells on each side were 5,800,000 in the duck, 2,700,000 in the gull, 570,000 in the quail, 130,000 in the budgerigar, and 110,000 in the bengalee. From a cross section of an olfactory nerve, the nerve was observed to be divided roughly into several fascicles by perineurium. Each fascicle was composed of many small bundles which were surrounded by endoneurium. Each small bundle was separated into several divisions by mesaxon which originated from Schwann cells. In the anterior part of the nerve, the number of axons surrounded by mesaxon ranged between one and several dozen. The number in the middle part was much larger than in the anterior part. In the posterior part the number decreased again. The diameter of an axon was 0.21–0.26 μm on average. The axon contained neurotubules, neurofilaments, mitochondria and axonal smooth ER. The fine structure of the olfactory nerve and the numbers of olfactory cells in these birds are discussed and compared with those of other vertebrates.

INTRODUCTION

Shibuya and Tucker [11] proved electrophysiologically that vultures have a keen sense of smell. Bang and Cobbs [4] subsequently showed anatomically that many kinds of birds have large olfactory bulbs. The distribution pattern of the olfactory mucosa in birds is simpler than that in mammals. Accordingly birds provide a comparatively simple model suitable for investigating the mechanisms of odor discrimination at the peripheral and central levels of the olfactory nervous system in warm blooded animals. For this purpose quantitative studies are indispensable. Therefore it is important to determine the approximate number of olfactory receptor cells as a first step in elucidating the mechanism of odor discrimination at the peripheral level. Since Allison and Warwick [2] calculated the number of olfactory receptor cells in the rabbit, the only other vertebrates in which these cells have been counted are fish [5–7] and the pigeon [9]. The present study was undertaken to determine the number of olfactory receptor cells and to observe the structure of the olfactory nerves in various species of birds, and to compare these with data from earlier reports [8, 9].

MATERIALS AND METHODS

Materials

Five species of bird were used in this study:

- Domestic duck (*Anas platyrhynchos*)
- Slay-backed gull (*Larus argentatus*)
- Quail (*Coturnix coturnix*)
- Budgerigar (*Melopsittacus undulatus*)

Bengalee (*Lonchura topela*)

The birds were purchased from a dealer, except for the slay-backed gull which was raised by the late Dr. Kano (Akkeshi Marine Biological Station, Hokkaido University) and was generously offered for this study. Apart from the slay-backed gull, three of each species were used.

The domestic ducks were hybrids between a domesticated duck and a wild duck. These birds are omnivorous, migrate, and have well developed olfactory organs. The slay-backed gull eats fish and also has well developed olfactory organs. It is a colony-making species, and seagull olfaction has already been studied electrophysiologically [10]. Quails, on the other hand, seldom fly and do not move far, the adults eating grass seeds and buds. Budgerigars are small birds, but are gregarious and drift for food, which is mainly grass seeds. Bengalees are also small birds, and the adults feed mainly on grass seeds.

The above birds including the pigeons [9] were selected because of their modes of life. However, since it is important to conduct further studies, their easy commercial availability was also taken into consideration.

Methods

The birds were anesthetized with 50% ethyl urethane (3 ml/kg). The fixative was 2.5% glutaraldehyde solution in 0.05 M sodium cacodylate buffer. This was perfused through the carotid artery into the head. After perfusion the olfactory nerves were isolated and immersed in the fixative solution at 4°C for 6 hr. The tissues were rinsed in a 10% sucrose solution of the sodium cacodylate buffer at 4°C for 12 hr, and fixed again in 2% osmium tetroxide solution at 4°C for 2 hr. They were then dehydrated using a series of ethanol solutions of increasing concentrations, and embedded in epoxy resin. Ultrathin sections for electron microscopy were subjected to double staining with uranyl acetate and lead citrate. Sections for light microscopy were stained with toluidine blue.

Each olfactory cell, which is a primary sensory cell, has one unmyelinated axon extending to the olfactory bulb. It has already

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been shown that this axon neither branches on its course to the olfactory bulb, nor forms a synapse [6]. Therefore, three electron micrographs ($\times 1,000$) were taken at three different sites from a cross section of the olfactory nerve. These were enlarged so that the number of cross-sectioned axons could be easily counted. The number of axons per unit area was calculated from the mean of the three totals counted in these three micrographs. A light microscopic photograph was taken and enlarged, and the outlines of the parts surrounded by perineurium were traced onto a sheet of graph paper, so that the area could be readily measured. The number of axons per unit area, that is, the density of axons, was multiplied by the area

of the cross section of the olfactory nerve.

The diameter of each axon was determined in the following manner. As nearly all the axons had elliptical cross sections, the major and minor axes were measured in each axon. These were multiplied and the square root of the result was regarded as the diameter of the respective axon. A similar method was applied to estimate the diameter of small bundles surrounded by endoneurium.

RESULTS

On leaving the olfactory mucosa, the axons of the

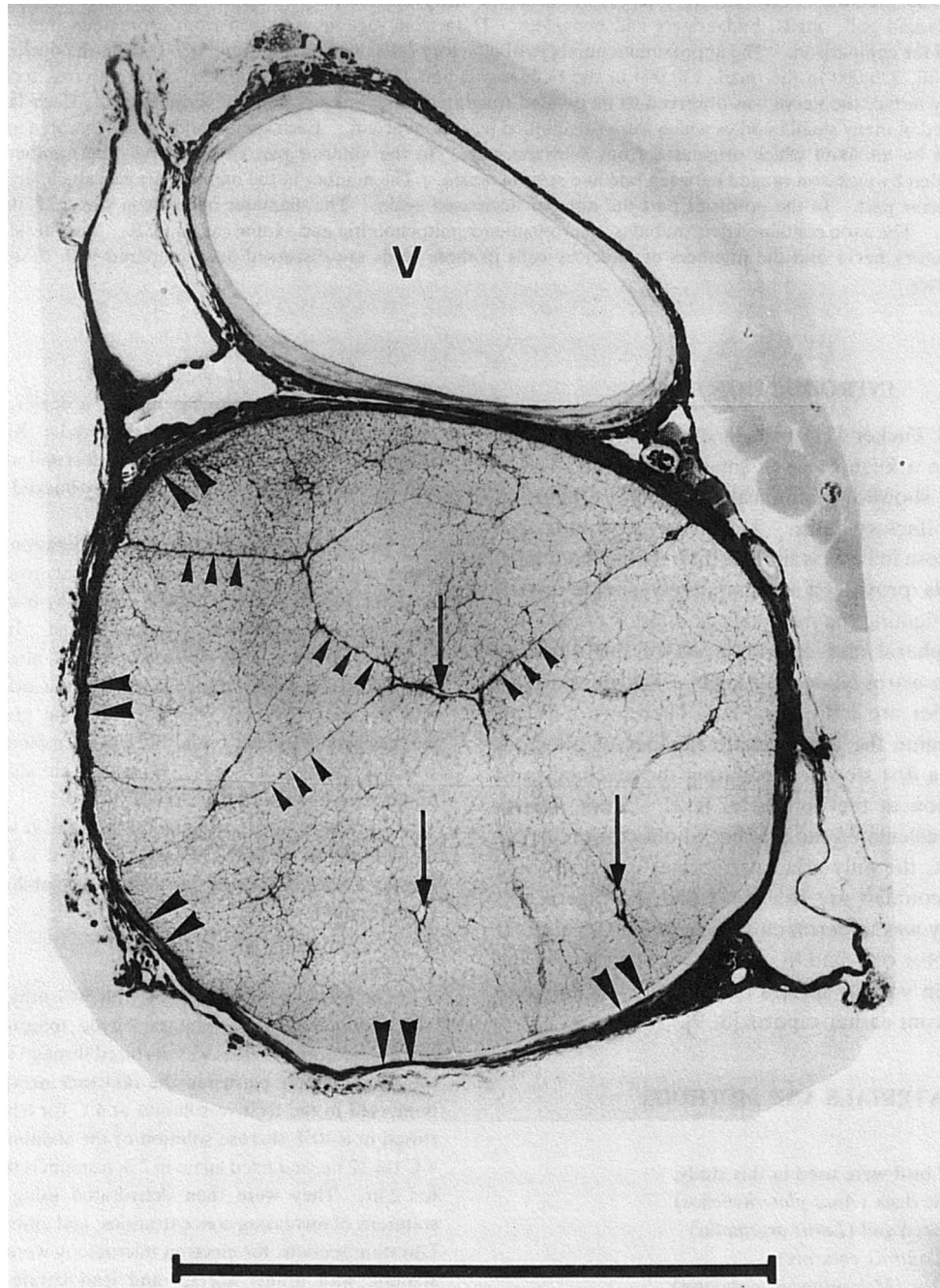


FIG. 1. A cross section of the olfactory nerve of the pigeon. There was a thick vein (V) on the nerve. The nerve wrapped in epineurium (large arrow heads) was divided into several divisions (fascicles) by perineurium (small arrow heads). Perineurium surrounded the fascicles containing many small bundles. There were many capillaries (arrows) between the fascicles. Bar: 1,000 μm .

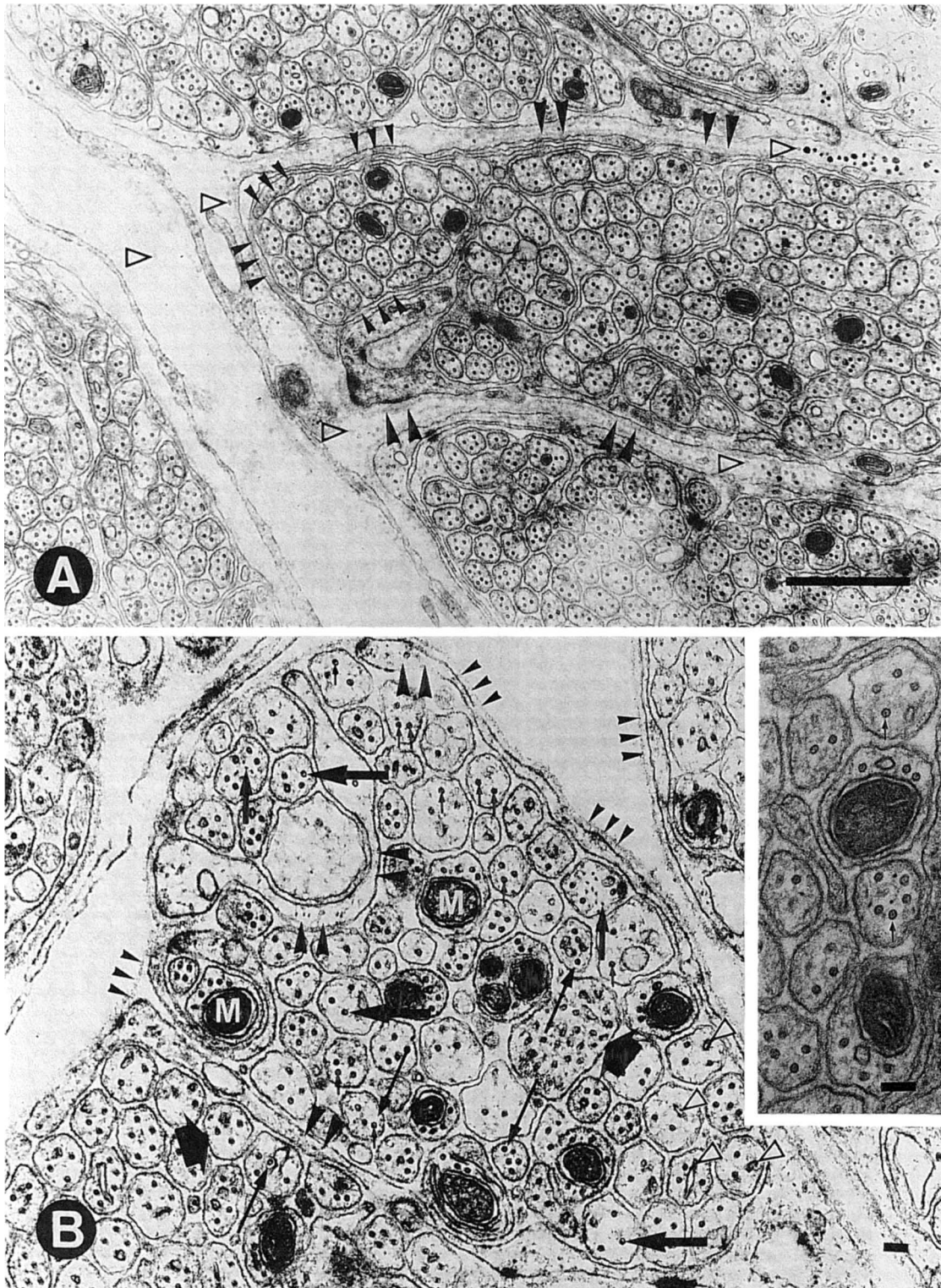


FIG. 2. Cross sections of the anterior part of the olfactory nerves in the pigeon (A) and the slay-backed gull (B). The surface of each Schwann cell was covered with basal lamina. There were numerous collagenous fibers between the small bundles. A: large arrow heads; basal lamina, small arrow heads; mesaxon, triangles; collagenous fibers, bar; 1 μ m. B: large arrow heads; mesaxon, small arrow heads; basal lamina, fatty arrow; axon, M; mitochondria, thin and long arrows; transparent zones around the neurotubules, thin and short arrows; electron dense points in the centers of the neurotubules, large arrows; neurotubules, small arrows; neurofilaments, triangles; axonal smooth ER, bar; 0.1 μ m. In the inlet the arrows show electron dense points in the centers of the neurotubules.

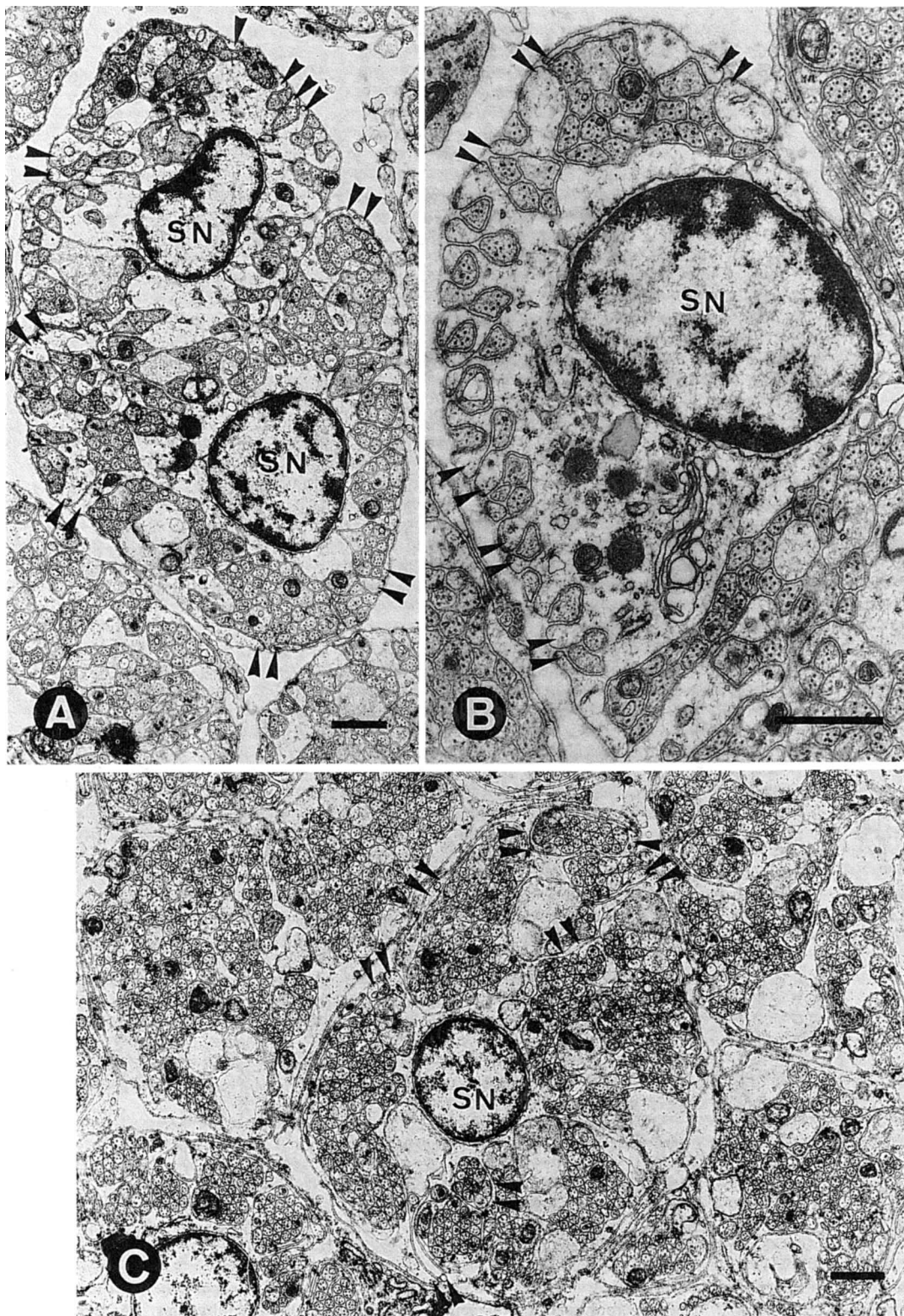


FIG. 3. Cross sections of the anterior part of the olfactory nerves in the slay-backed gull (A and B) and the pigeon (C). A portion of the Schwann cell (SN: nucleus of Schwann cell) formed mesaxon (arrow heads), and the axons were packed into this. In the gull, mesaxon which contained only one axon was often observed in the nerve within 1 mm after the olfactory mucosa. Bar: 1 μ m.

olfactory receptor cells, which are unmyelinated, were formed into bundles with glial cells. The axons, in the form of a pair of olfactory nerves, ran between both eye-sockets to reach the olfactory bulbs. The olfactory nerves in the pigeon [9], the duck and the gull were 800 to 1,500 μm in diameter, and 8 to 12 mm in length. The nerves in the quail, the budgerigar and the bengalee were 100 to 300 μm in diameter, and 4 to 7 mm in length.

A thick vein ran in parallel with the olfactory nerve (Fig. 1). The outermost part of the olfactory nerve was wrapped in epineurium. The nerve contained several (sometimes more than 10) fascicles, each of which was surrounded by perineurium. Each fascicle contained many small bundles of axons enclosed by endoneurium to form small units of axons. Collagenous fibers and fibroblasts formed endoneurium between these small units (Fig. 2). The surface of each Schwann cell was covered with basal lamina. Schwann cells were observed singly or in multiples within the area surrounded loosely by endoneurium. A portion of the Schwann cell formed mesaxon, and the axons were packed into this (Fig. 3).

The sizes and estimated numbers of the small bundles, the mean number of axons contained in a small bundle, and the intervals between them are shown in Table 1. These values were measured in the middle part of the olfactory nerve. The number of axons contained in one small bundle ranged from several dozen to a few thousand. The range of diameters of the small bundles was much smaller than that observed in the four-toed tortoise [8].

The number of axons in one section enclosed by mesaxon varied with the part of the olfactory nerve from which the sample was taken. It ranged from one to several dozen in the anterior (peripheral) part of the nerve, close to the olfactory mucosa (Fig. 3). The farther the origin of the nerve sample was from the mucosa, the larger the number of axons in each section. In the anterior part of the nerve, this number was limited and the nuclei of the Schwann cells were found almost in the center of the small bundles (Fig. 3). The space between the small bundles was filled with collagenous fibers and fibroblasts. Blood vessels were found to run along the epineurium and perineurium, and some of them were distributed within the endoneurium.

The approximate numbers of olfactory receptor cells in the six species of birds were calculated as shown in Table 1. These numbers were smaller than in the rabbit [2] and the four-toed tortoise [8].

Each axon contained neurotubules, neurofilaments, mitochondria and axonal smooth endoplasmic reticulum (ER) (Figs. 2 and 4). The numbers and sizes of the neurotubules and neurofilaments are shown in Table 2. Some of the thicker axons observed in the budgerigar contained a few mitochondria and numerous neurofilaments, while others contained a lot of neurotubules. An electron dense point in the center of each neurotubule was frequently observed in each type of bird (Fig. 4). Electron lucent transparent zones around the neurotubules were also clearly observed (Fig. 4).

Table 2 also shows the diameters of the axons. The mean diameters of the axons in each type of bird were almost the same.

TABLE 1. Numbers of small bundles, estimated value of axons contained in each mean small bundle, intervals between small bundles, and approximate numbers of olfactory receptor cells of six species of bird on each side

	Small bundle		Number of axons contained in a mean small bundle	Interval between small bundles (μm)	Number of olfactory receptor cells on one side
	Size (μm) (mean \pm SD)	Number			
Pigeon	11.0 \pm 3.7	1,300	2,000	0.2–1.0	3,000,000
Domestic duck	4.6 \pm 1.5	17,000	400	1.0–4.6	5,800,000
Slay-backed gull	12.5 \pm 4.4	4,900	2,100	0.3–1.5	2,700,000
Quail	10.9 \pm 4.0	500	2,100	0.3–1.4	570,000
Budgerigar	3.3 \pm 1.0	1,900	100	0.3–0.8	130,000
Bengalee	7.9 \pm 2.9	200	1,100	0.2–0.6	110,000

TABLE 2. Sizes and numbers of neurotubules and neurofilaments, and diameters of axons in each type of bird

	Neurotubule (mean \pm SD)		Neurofilament (mean \pm SD)		Diameter of axon (μm) (mean \pm SD)
	Size (nm)	Number	Size (nm)	Number	
Pigeon	27.3 \pm 2.3	3.0 \pm 1.1	12.0 \pm 1.1	2.1 \pm 3.3	0.22 \pm 0.03
Domestic duck	30.6 \pm 2.5	2.1 \pm 1.0	12.4 \pm 3.2	2.7 \pm 2.5	0.21 \pm 0.04
Slay-backed gull	25.6 \pm 2.2	3.1 \pm 0.9	12.6 \pm 1.1	1.8 \pm 1.9	0.24 \pm 0.05
Quail	33.6 \pm 2.2	3.1 \pm 1.0	13.8 \pm 3.6	2.1 \pm 1.8	0.21 \pm 0.03
Budgerigar	26.4 \pm 1.1	5.5 \pm 2.0	11.2 \pm 3.1	3.0 \pm 2.8	0.26 \pm 0.06
Bengalee	26.4 \pm 1.1	3.2 \pm 1.2	9.6 \pm 2.5	2.0 \pm 1.9	0.21 \pm 0.05

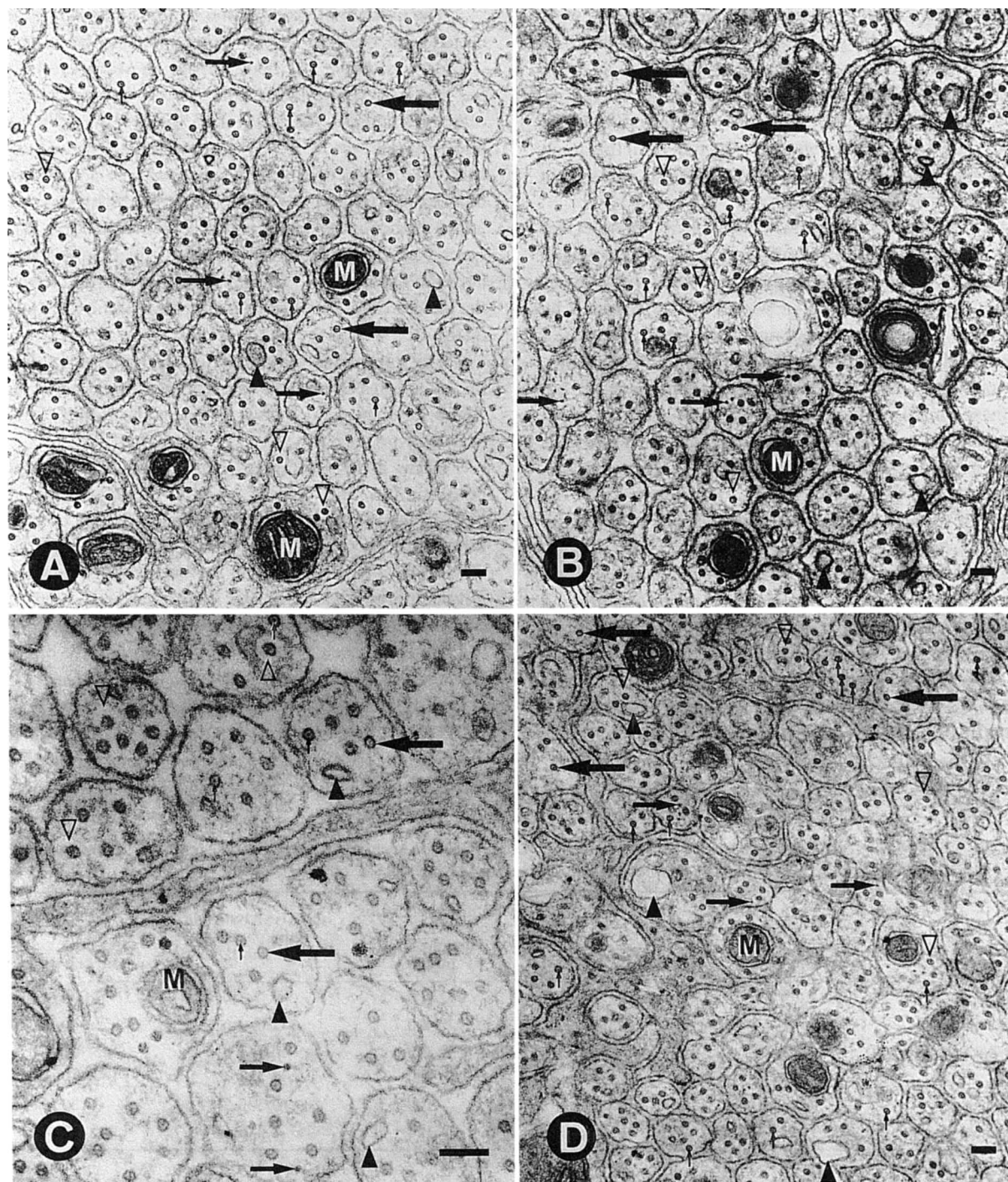


FIG. 4. Cross sections of the middle part of the olfactory nerves in the pigeon (A and B), the budgerigar (C) and the bengalee (D). Each axon contained neurotubules (large arrows), neurofilaments (middle arrows), mitochondria (M) and axonal smooth ER (filled triangles). Electron dense points (small arrows) in the centers of neurotubules were frequently observed in each type of bird. Electron lucent transparent zones (open triangles) around the neurotubules were also observed. Bar: 0.1 μ m.

DISCUSSION

The unmyelinated axons forming the olfactory nerve were roughly divided into several fascicles by perineurium. Each fascicle was composed of many small bundles surrounded by endoneurium. Each small bundle was separated

into several divisions by mesaxon which originated from Schwann cells. Similar findings have been reported in fish [5, 7].

The membranous systems surrounding the axons were relatively well developed in the pigeon, the gull and the quail when compared with those of amphibians (Unpublished

data). In the duck and the bengalee, however, these systems were not so well developed and showed similarities with those of the frog (Unpublished data). The membrane systems of the budgerigar resembled those of the newt (Unpublished data). The number of Schwann cells was also very small, as in the newt. The vascular systems in the birds were well developed as compared with amphibians. Many thin blood capillaries were clearly observed to be distributed between the small bundles.

Some of the axons had a localized, enlarged, nodular area. This did not seem to be an artefact, because neither the intracellular organelles nor the plasmalemma were destroyed, and because it appeared even when the type of buffer or the concentration of fixative was changed.

There seemed to be no particular correlation between the diameter of the axon and the number of either neurotubules or neurofilaments contained therein; there was also no notable correlation between the numbers of neurotubules and neurofilaments.

No neurofilaments were detected in about half of the axons examined. According to Kreuzberg and Gross [7], such a fluctuation in the number of neurofilaments within the axon might indicate that these filaments were not continuous throughout its whole length.

The function of the transparent zone around the neurotubule has not been verified.

The axons of olfactory receptor cells are unmyelinated C fibers [6]. The axons run closely against each other so that interaction may occur between neighboring axons [6, 12]. Thus a small bundle surrounded by a Schwann cell probably conducts signals as a unit [12]. This small bundle seems to correspond to the smallest branch of the olfactory nerve [9]. Adrian (1956) speculated that the axons running in one sheath might be able to act in unison [1]. If it were true, this means that, although an odor stimulus may excite only a few olfactory receptor cells with high sensitivity, the full effect would occur because of the dispersion of this excitation over the small bundle.

Shibuya *et al.* [10], who recorded the responses from the olfactory nerve of the sea-gull (*Larus crassirostris*) using nerve branches of 20 to 40 μm in diameter, suggested that there might be differences in the threshold values for odor concentration between the small branches of the olfactory nerve. If there are specific differences in the kinds of odors and their concentrations to which particular axons within the branches respond, it is possible that these branches may constitute some kind of functional unit for the discrimination of odor at the peripheral level.

Based on the values from Table 1, the duck may be a

good subject from which to isolate branches of the olfactory nerve for further research, because the mean size of the small bundles in the duck was small, and the mean interval between the small bundles was especially large. If a small bundle is some kind of functional unit for odor discrimination, the duck might most effectively discriminate between many kinds of odors.

Bang [3] reported that birds which form colonies, birds prey and those which migrate have well developed olfactory organs. The pigeon, the duck and the gull had a large number of olfactory receptor cells and small bundles. Although budgerigars had a small number of olfactory receptor cells, they had many small bundles.

More studies will be necessary to elucidate odor discrimination at the peripheral level in birds.

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