FULL PAPER *Anatomy*

Ultrastructural and Histochemical Properties of the Olfactory System in the Japanese Jungle Crow, *Corvus macrorhynchos*

Daisuke KONDOH[1,2], Mai NASHIMOTO[1], Shunsaku KANAYAMA[1], Nobuaki NAKAMUTA[1,2] and Kazuyuki TANIGUCHI[1,2]*

[1]*Laboratory of Veterinary Anatomy, Faculty of Agriculture, Iwate University, 3–18–8 Ueda, Morioka, Iwate 020–8550 and* [2]*Department of Basic Veterinary Science, The United Graduate School of Veterinary Science, Gifu University, 1–1 Yanagido, Gifu, Gifu 501–1193, Japan*

(Received 24 December 2010/Accepted 28 March 2011/Published online in J-STAGE 11 April 2011)

ABSTRACT. Although it has been commonly believed that birds are more dependent on the vision and audition than the olfaction, recent studies indicate that the olfaction of birds is related to the reproductive, homing, and predatory behaviors. In an attempt to reveal the dependence on the olfactory system in crows, we examined the olfactory system of the Japanese jungle crow (*Corvus macrorhynchos*) by histological, ultrastructural, and lectin histochemical methods. The olfactory epithelium (OE) of the crow occupied remarkably a small area of the nasal cavity (NC) and had the histological and ultrastructural features like other birds. The olfactory bulb (OB) of the crow was remarkably small and did not possess the olfactory ventricle. The left and right halves of the OB were fused in many cases. In the lectin histochemistry, soybean agglutinin (SBA) and *Vicia villosa* agglutinin (VVA) stained a small number of the receptor cells (RCs) in the OE and the olfactory nerve layer (ONL) and glomerular layer (GL) on the dorsocaudal region of the OB. *Phaseolus vulgaris* agglutinin-E (PHA-E) stained several RCs in the OE and the ONL and GL on the ventral region of the OB. These results suggest that 1) the crow has less-developed olfactory system than other birds, and 2) the dedicated olfactory receptor cells project their axons to the specific regions of the OB in the crow.

KEY WORDS: bird, lectin, nasal cavity, olfactory bulb, olfactory epithelium.

J. Vet. Med. Sci. 73(8): 1007–1014, 2011

In tetrapods, the olfactory system is divided into the main olfactory system and the vomeronasal system. The main olfactory system senses millions of volatile substances received by the olfactory epithelium (OE) in the nasal cavity (NC), and processes the information in the main olfactory bulb. The vomeronasal system detects species-specific substances, such as pheromones, received by the vomeronasal epithelium in the vomeronasal organ, and processes the information in the accessory olfactory bulb. In birds, however, the olfactory system consists of the OE and the olfactory bulb (OB), and completely lacks the components of the vomeronasal system [7, 27].

In birds, the NC is divided into three regions: vestibular region covered with the squamous epithelium, respiratory region covered with the respiratory epithelium, and olfactory region covered with the OE [4]. Although the OE in birds covers the posterior concha (PC) found as a prominence in the olfactory region [4], the OE occupies a relatively smaller area of the NC in birds than in mammals. In the past, therefore, it has been widely believed that birds are more independent of the olfaction than mammals and are dependent on the vision and audition in most behaviors. In several avian species, however, the olfaction is related to the reproductive, homing, and predatory behaviors [2, 13, 20, 28]. In addition, birds have high proportion of functional genes to total olfactory receptor genes [26] to indicate their dependence on the olfaction. For these reasons, the olfactory system appears to play important roles in birds.

It is not clear to what degree the crows depend on the olfactory system in their lives. The common raven (*Corvus corax*) is reported to use the olfactory system to distinguish their feeds [14]. On the other hand, it has been suggested that the American crow (*Corvus brachyrhynchos*) and the Japanese jungle crow (*Corvus macrorhynchos*) have poorly-developed olfactory system because the size of the OB is smaller in comparison with that of the cerebral hemisphere in these crows than in other birds [3, 30]. The morphological and histochemical studies on the olfactory system are essential to reveal the degree of development of the olfactory system in crows. However, the histological and histochemical features of the OE have not been reported in crows. In this study, therefore, the OE of the Japanese jungle crow was examined by histological and histochemical methods and electron microscopes. In addition, the OE and OB of the Japanese jungle crow was examined by lectin histochemistry to reveal the binding patterns with the glycoconjugates in the olfactory receptor cells.

MATERIALS AND METHODS

Eight male Japanese jungle crows, with body weight ranging from 524 to 630 g, were used as materials. All animals were trapped in the rural area in Iwate Prefecture. Permission to capture the crows was obtained from Iwate Prefecture (No. 15–20), as stipulated by Paragraph 2, Arti-

* CORRESPONDENCE TO: TANIGUCHI, K., Laboratory of Veterinary Anatomy, Department of Veterinary Science, Faculty of Agriculture, Iwate University, 3–18–8 Ueda, Morioka, Iwate 020–8550, Japan.
e-mail: anatomia@iwate-u.ac.jp

cle 9 of the Law Concerning the Appropriate Conservation and Hunting of Birds. All procedures were approved by the local animal ethical committee of Iwate University.

Animals and tissue processing: Five animals were anesthetized by injection of pentobarbital (40 mg/kg) and euthanized by cardiac perfusion with Ringer's solution followed by Zamboni's fixative. After decapitation, brains were removed from heads. The nasal tissues were decalcified in Plank-Rychlo's solution [22] overnight, embedded in paraffin and cut frontally at 5 μm. Some of these sections were stained with hematoxylin-eosin (HE), periodic acid/Schiff (PAS), or alcian blue (pH 2.5), and other sections were processed for anti-protein gene product 9.5 (PGP 9.5) immunohistochemistry or lectin histochemistry. Brains were embedded in paraffin and cut frontally at 5 μm. Some of these sections were stained with HE or luxol fast blue/cresyl violet (staining of Kluver-Barrera), and other sections were processed for lectin histochemistry. Three animals were anesthetized by injection of pentobarbital and euthanized by exsanguination. After decapitation, the olfactory mucosae were removed and processed for scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

Anti-PGP 9.5 immunohistochemistry: After deparaffinization and rehydration, sections were incubated with 0.3% H_2O_2 in methanol at room temperature (RT) for 30 min to eliminate endogenous peroxidase. Sections were rinsed in 0.01 M phosphate buffered saline (PBS, pH 7.4) and incubated with PBS containing 2% normal donkey serum at RT for 30 min to block nonspecific reactions. After rinsing, sections were incubated with rabbit anti-PGP 9.5 antibody (1:250, UltraClone Ltd., Isle of Wight, U.K.) at 4°C overnight, and incubated with biotinylated anti-rabbit IgG antibody (1:500, Jackson ImmunoResearch Laboratories, West Grove, PA, U.S.A.) at RT for 30 min. Sections were rinsed, reacted with the avidin-biotin complex (ABC) reagent (Vector Laboratories, Burlingame, CA, U.S.A.) at RT for 30 min, and colorized with 0.05 M Tris-HCl (pH 7.4) containing 0.006% H_2O_2 and 0.02% 3-3'-diaminobenzidine tetrahydrochloride. Control staining was made by the use of PBS to replace ABC reagent.

SEM: The olfactory mucosae were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (PB, pH 7.4) at 4°C for 24 hr. After rinsing in PB, specimens were postfixed in 1% osmium tetroxide in PB at 4°C for 2 hr, dehydrated, and frozen at –20°C. Specimens were dried in the freeze drier (ES-2030, Hitachi, Tokyo, Japan), coated with gold by ion sputter (E-1020, Hitachi), and examined with scanning electron microscope (JSM-6510, JEOL, Tokyo, Japan).

TEM: The olfactory mucosae were fixed in 2.5% glutaraldehyde in 0.2 M PB at 4°C for 24 hr. After rinsing in PB, specimens were postfixed in 1% osmium tetroxide in PB at 4°C for 2 hr, dehydrated, and embedded in epoxy resin. Ultrathin sections (thickness approximately 80 nm) were cut by diamond knife, stained with uranyl acetate and lead citrate, and examined with transmission electron microscope (H-800, Hitachi).

Lectin histochemistry: Several sections of the nasal tissues and brains were processed for histochemistry with the ABC method using 21 biotinylated lectins (Table 1) in the lectin screening kits I-III (Vector). After deparaffinization and rehydration, sections were incubated with 0.3% H_2O_2 in methanol to eliminate endogenous peroxidase. Sections

Table 1. Binding specificities of lectins used in the present study

Lectins	Abbreviation	Concentration (mg/m*l*)	Binding specificity
Wheat germ agglutinin	WGA	1.0×10^{-2}	GlcNAc, NeuAc
Succinylated-wheat germ agglutinin	s-WGA	1.0×10^{-2}	$(GlcNAc)_n$
Lycopersicon esculentum lectin	LEL	2.0×10^{-3}	$(GlcNAc)_{2-4}$
Solanum tuberosum lectin	STL	1.0×10^{-2}	$(GlcNAc)_{2-4}$
Datura stramonium lectin	DSL	4.0×10^{-3}	$(GlcNAc)_{2-4}$
Bandeiraea simplicifolia lectin-II	BSL-II	4.0×10^{-3}	GlcNAc
Dolichos biflorus agglutinin	DBA	1.0×10^{-2}	Gal, GalNAc
Soybean agglutinin	SBA	1.0×10^{-2}	Gal, GalNAc
Bandeiraea simplicifolia lectin-I	BSL-I	4.0×10^{-3}	Gal, GalNAc
Vicia villosa agglutinin	VVA	4.0×10^{-3}	Gal, GalNAc
Sophora japonica agglutinin	SJA	2.0×10^{-2}	Gal, GalNAc
Ricinus communis agglutinin-I	RCA-120	2.0×10^{-3}	Gal, GalNAc
Jacalin		5.0×10^{-4}	Gal, GalNAc
Peanut agglutinin	PNA	4.0×10^{-3}	Gal
Erythrina cristagalli lectin	ECL	2.0×10^{-2}	Gal, GalNAc
Ulex europaeus agglutinin-I	UEA-I	2.0×10^{-2}	Fuc
Concanavalin A	ConA	3.3×10^{-3}	Man, Glc
Pisum sativum agglutinin	PSA	4.0×10^{-3}	Man, Glc
Lens culinaris agglutinin	LCA	4.0×10^{-3}	Man, Glc
Phaseolus vulgaris agglutinin-E	PHA-E	5.0×10^{-3}	Oligosaccharide
Phaseolus vulgaris agglutinin-L	PHA-L	2.5×10^{-3}	Oligosaccharide

Fuc, fucose; *Gal*, galactose; *GalNAc*, N-acetylgalactosamine; *Glc*, glucose; *GlcNAc*, N-acetylglucosamine; *Man*, mannose; *NeuAc*, N-acetylneuraminic acid.

were rinsed in PBS and incubated with 1% bovine serum albumin to block nonspecific reactions. After rinsing, sections were incubated with biotinylated lectins at 4°C overnight, reacted with ABC reagent (Vector) at RT for 30 min, and colorized with 0.05 M Tris-HCl (pH 7.4) containing 0.006% H_2O_2 and 0.02% 3–3'-diaminobenzidine tetrahydrochloride. The optimal concentration for each lectin (Table 1) was determined by preliminary experiments based on the concentrations applied in our previous study [16] to obtain high-contrasted images. Control slides were made (a) by the preabsorption of lectins with each specific sugar residue (Table 1) or (b) by the use of PBS to replace ABC reagent.

RESULTS

Topographical features of the NC: In the Japanese jungle crow, the NC was completely separated into the left and right cavities by the nasal septum. Three nasal conchae (anterior, middle and posterior concha) were distinguished in each NC. The anterior concha protruded from the lateral wall nearby the naris (Fig. 1A), was divided into dorsal and ventral branches, and covered with the keratinized squamous epithelium. The middle concha protruded from the lateral wall over a large area of the NC (Fig. 1A) and mainly formed a scrolled structure. The posterior concha was distinguished as a small prominence of the lateral wall dorsal to the choana (Fig. 1B). Although a large area of the NC was covered with the respiratory epithelium, the posterior concha and the posterior roof of the NC were covered with the OE (Fig. 1C).

Histological and immunohistochemical features of the OE: Histologically, the OE of the Japanese jungle crow was composed of the supporting cells (SCs), receptor cells (RCs), and basal cells (BCs). Nuclei of the SCs, RCs, and BCs were situated in the apical, middle, and basal regions of the OE, respectively (Fig. 1D). The SCs had oval nuclei arranged in two to three layers and elongated cell bodies to reach the basal lamina. The RCs had round nuclei arranged in one to two layers and pear-shaped cell bodies, and extended their dendrites to the luminal surface and their axons toward the basal lamina. The BCs had scanty cytoplasm and irregular nuclei arranged sparsely just above the basal lamina. In the lamina propria, there were several nasal glands opening into the NC (Fig. 1C, arrow), and they were positive to both PAS (Fig. 1F) and alcian blue (Fig. 1G). In contrast, the SCs, RCs, and BCs were negative to both PAS and alcian blue.

To examine the distribution of neuronal cells in the crow nasal mucosa, the nasal mucosa was immunostained with anti-PGP 9.5 antibody, which is used as neuronal marker because PGP 9.5 is present in most neurons of all vertebrate species including birds [6]. In the OE of the Japanese jungle crow, the RCs were positive to anti-PGP 9.5 antibody, and the SCs and BCs were negative to this antibody (Fig. 1E). In addition, all cells in the respiratory epithelium were negative to anti-PGP 9.5 antibody (data not shown). No spe-

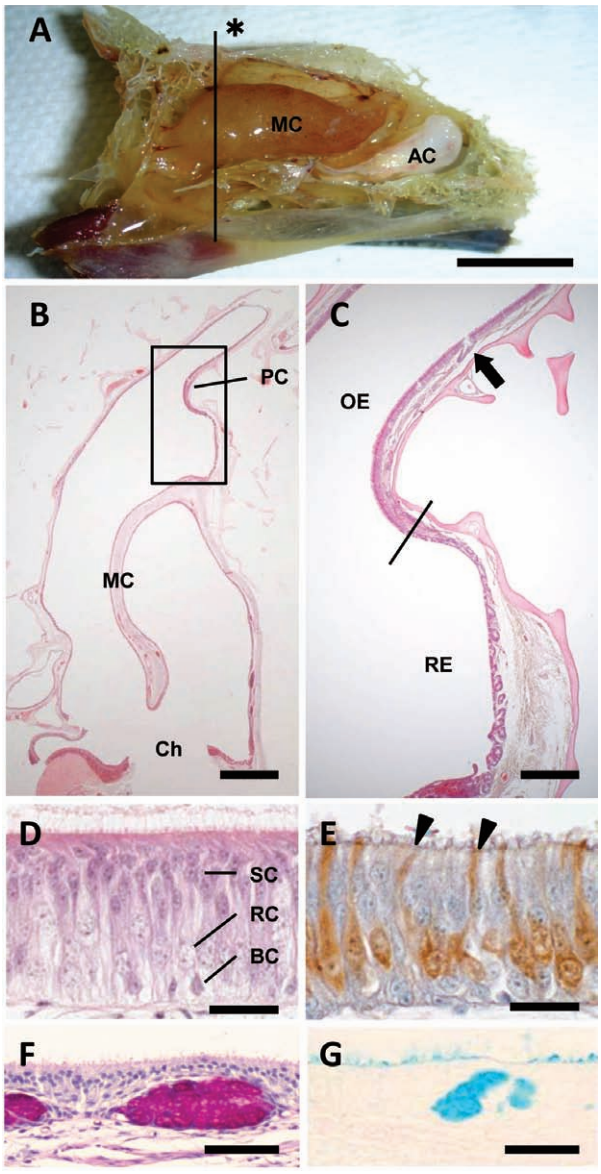

Fig. 1. Topographical and histological features of the nasal cavity in the Japanese jungle crow. A: Topographical view of the left half of the nasal cavity after removal of the nasal septum. The left side of the figure is caudal, and the upper, dorsal. The solid line indicated by asterisk corresponds to Fig. 1B at frontal section. AC, anterior concha; MC, middle concha. Bar=10 mm. B: Frontal section in the caudal region of the nasal cavity. The left side of the figure is medial, and the upper, dorsal in (B) and (C). Boxed area corresponds to Fig. 1C at higher magnification. Ch, choana; MC, middle concha; PC, posterior concha. Bar=1,000 μm. C: Higher magnification view around the posterior concha. The solid line indicates the border between the olfactory epithelium (OE) and the respiratory epithelium (RE). Arrow indicates the nasal gland in the lamina propria. Bar=400 μm. D: Histological features of the olfactory epithelium. BC, nuclei of basal cells; RC, nuclei of receptor cells; SC, nuclei of supporting cells. Bar=20 μm. E: Anti-PGP 9.5 immunostaining in the olfactory epithelium. Arrowheads indicate the dendrites of the receptor cells. Bar=20 μm. F: PAS staining in the olfactory epithelium. Bar=50 μm. G: Alcian blue staining in the olfactory epithelium. Bar=50 μm.

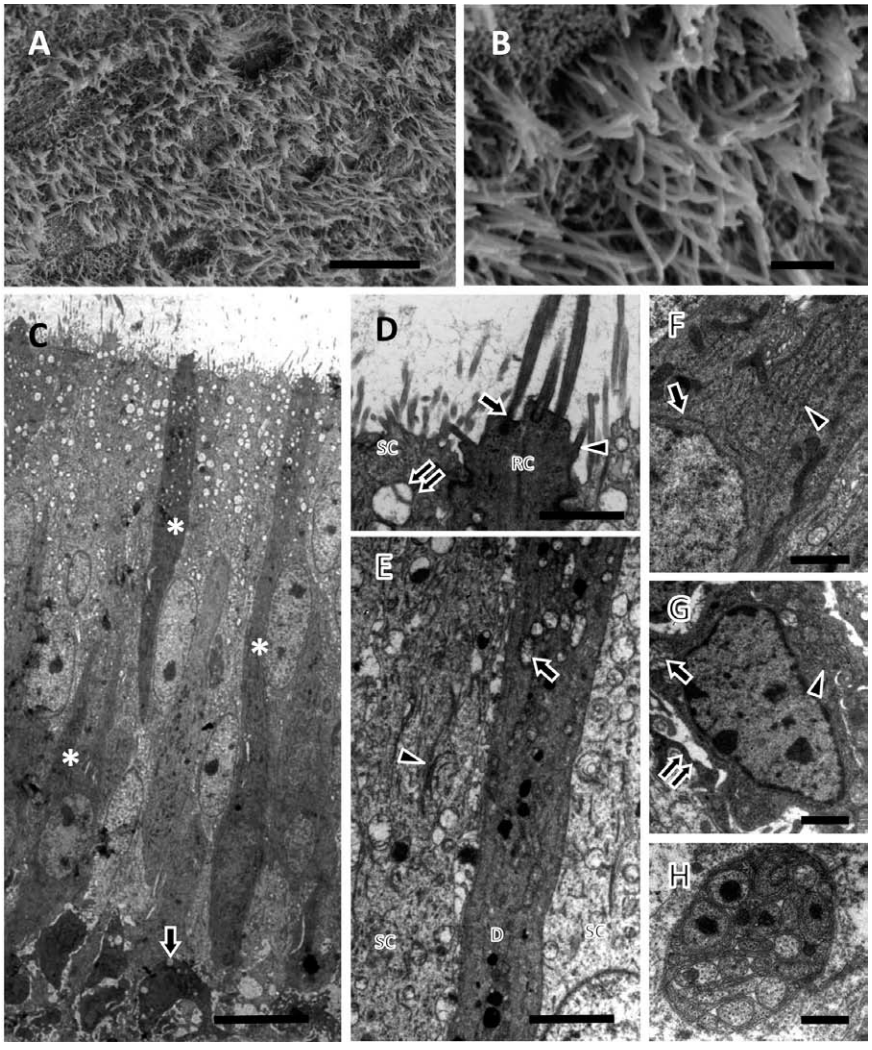

Fig. 2. Scanning (A and B) and transmission (C to H) electron microscopy of the olfactory epithelium in the Japanese jungle crow. A: Surface of the olfactory epithelium covered by cilia and microvilli. B: Higher magnification view on the surface of the olfactory epithelium. C: Ultrastructural features of the olfactory epithelium. Asterisks indicate the receptor cells and arrow indicates the basal cell. D: Higher magnification view around the free border. Arrow indicates the cilium of the receptor cell, arrowhead indicates the microvillus of the receptor cell, and double arrow indicates the secretory granule in the supporting cell. RC, receptor cell; SC, supporting cell. E: Higher magnification view in the apical region. Arrow indicates mitochondria in the dendrite and arrowhead indicates Golgi apparatus in the supporting cell. D, dendrite; SC, supporting cells. F: Higher magnification view in the perinuclear region of the receptor cells. Arrow indicates rough endoplasmic reticulum and arrowhead indicates Golgi apparatus. G: Higher magnification view of the basal cells. Arrow indicates mitochondria, arrowhead indicates rough endoplasmic reticulum, and double arrow indicates the basal lamina. H: Ultrastructural features of the axonal bundle in the lamina propria. Bars=10 μm in (A) and (C), 2 μm in (B), (D) and (E), and 1 μm in (F) to (H).

cific staining was observed in the control slides.

Ultrastructural features of the OE: By SEM analysis, the OE of the Japanese jungle crow was equipped with both cilia and microvilli on its surface (Fig. 2A). The olfactory cilia extended almost vertically and were not intertwined with each other (Fig. 2B). By TEM analysis, the RCs of the Japanese jungle crow had elongated dendrites (Fig. 2C, asterisk), whose terminals formed the dendritic bulbs extending cilia (Fig. 2D, arrow) and microvilli (Fig. 2D, arrowhead) to the luminal surface. The dendrites of the RCs contained numerous mitochondria (Fig. 2E, arrow), rough endoplasmic reticulum (rER), small vesicles, and neurofilaments, although the dendritic bulbs did not contain these structures (Fig. 2D). The perinuclear regions of the RCs contained well-developed rER (Fig. 2F, arrow), Golgi apparatus (Fig. 2F, arrowhead), and numerous ribosomes. The

SCs extended numerous microvilli to the luminal surface (Fig. 2D) and contained Golgi apparatus (Fig. 2E, arrowhead), rER, and mitochondria throughout their cytoplasm, in addition to numerous secretory granules in the apical regions (Fig. 2D, double arrow). The BCs were situated on the basal lamina (Fig. 2G, double arrow), and contained mitochondria (Fig. 2G, arrow) and rER (Fig. 2G, arrowhead). In the lamina propria, there were axonal bundles, and several nonmyelinated axons surrounded by mesaxon (Fig. 2H). The axons contained neurofilaments and mitochondria.

Topographical and histological features of the OB: In the Japanese jungle crow, the OB was a remarkably small structure located on the rostroventral surface of the telencephalon. In 4 among 5 crows examined in the present study, the left and right halves of the OB were fused both topographically and histologically (Fig. 3A). On the other hand, in 1 crow, the left and right halves of the OB were separated completely (Fig. 3B). Histologically, the crow OB did not possess the olfactory ventricle in all crows examined. According to the staining of Kluver-Barrera, the crow OB was divided into 5 layers: olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer, mitral cell layer, and granule cell layer (Fig. 3C).

Lectin histochemistry in the OE and OB: Lectins are proteins binding with glycoconjugates, have specificity to terminal carbohydrates of sugar chains, and are extensively used for the differentiation of cells on the histological sections [17]. In many species, such as the newt [25], frog [23], toad [24], snake [16], lizard [8, 9], and mallard duck [15], it has been shown that various glycoconjugates are expressed in the olfactory system by lectin histochemistry. However, many lectins of the same monosaccharide specificity show different binding patterns for the same cell types or tissue structures in histochemical preparations [5] because of several reasons such as existence of the binding ability with internal components of sugar chains.

Among 21 lectins used in the present study, 11 lectins, WGA, s-WGA, LEL, STL, DSL, RCA-120, Jacalin, PNA, ConA, LCA and PHA-L, stained the RCs of the OE and the ONL and GL of the OB with varying intensities (Fig. 4A, 4D and 4G). SBA and VVA stained a small number of the RCs, and the ONL and GL on the dorsocaudal region of the OB (Fig. 4B, 4E and 4H). In addition, PHA-E stained several RCs, and the ONL and GL on the ventral region of the OB (Fig. 4C, 4F and 4I). In any of these cases, lectins stained the dendrites, somata, and axons (Fig. 4A-C, arrowheads) of the positive RCs. Seven lectins, BSL-II, DBA, BSL-I, SJA, ECL, UEA-I, and PSA did not stain the RCs, ONL, and GL at all. No specific staining was observed in the control slides.

These findings are summarized in Table 2.

DISCUSSION

In the Japanese jungle crow, the OE was restricted to the posterior concha and the posterior roof of the NC. Yokosuka *et al.* [30] reported that the posterior concha is quite indistinct in the NC of the Japanese jungle crow. In addition, by anti-PGP 9.5 immunohistochemistry in the present study, neuronal cells in the NC of the crow are confined to the OE covering the posterior concha and the posterior roof of the NC. Since Adrian [1] showed that the size and complexity of the posterior concha reflect the variety of odoriferous substances discriminated, it is estimated that the Japanese jungle crow has low ability to discriminate odoriferous substances. In the Japanese jungle crow, the OE was thinner than the OE in mammals, composed of the SCs, RCs, and BCs, and possessed the nasal glands in the lamina propria. These histological features of the OE are similar to those in the ordinary birds [3, 27], except for the domestic duck which has the goblet cells in the OE [12]. By PAS and alcian blue staining, it was shown that the nasal glands of the Japanese jungle crow contain a large amount of neutral and acid mucopolysaccharides. These mucopolysaccharides appear to be secreted to the surface of the OE, form the mucous layer, and play important roles in the dissolution of odoriferous substances and the protection of the OE.

By TEM analysis, it was shown that the ultrastructural features of the RCs and BCs are similar between the Japanese jungle crow and other birds reported previously [12, 19, 21]. The ultrastructural aspect of the axons in the axonal bundles was also similar between the crow and other birds [18]. However, the SEM analysis showed that the running pattern of the olfactory cilia is different between the crow and the domestic fowl [21]. Although the olfactory cilia of the crow extended vertically to the surface of the OE, the cilia of the fowl extend vertically and horizontally in parallel with the surface of the OE to form the mat of olfactory cilia [21]. These observations suggest that the RCs of the Japanese jungle crow have well-developed organelles as in other birds but have less-developed olfactory cilia than the fowl. In addition, the present TEM analysis showed that the SCs contain serous secretory granules negative to PAS and alcian blue. In general, non-mammalian SCs have the ability for secretion [10]. Among birds, the SCs of the domestic fowls [21], domestic pigeons [19], and vulture [11] contain secretory granules, although those of the domestic duck [12] do not contain secretory granules. Therefore, it is indicated that the SCs of the Japanese jungle crow is similar to the SCs of the ordinary birds.

In the Japanese jungle crow, the OB was a remarkably small structure located on the rostroventral surface of the telencephalon as previously reported [30]. Although the left and right halves of the OB of the crow are fused in general [3, 30], the left and right halves of the OB were separated completely in 1 among 5 crows examined in the present study. The fusion of the left and right halves of the OB may be affected by several factors such as the age or living environment. However, there were no significant differences in their body weights and the environments of the points where they were trapped among 5 crows used in the present study. Therefore, the differences between the crows with the fused OB and the crow with the separated OB were not deter-

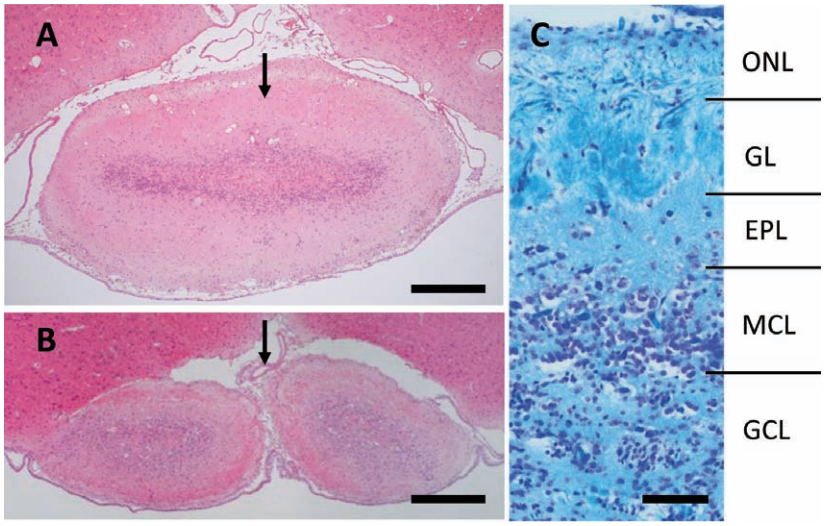

Fig. 3. Topographical and histological features of the olfactory bulb in the Japanese jungle crow. A: Frontal section in the crow with the fused left and right halves of the olfactory bulb. Arrow indicates the midline and the upper side of the figure is dorsal in (A) and (B). Bar=200 μm. B: Frontal section in the crow with the separated left and right halves of the olfactory bulb. Bar=200 μm. C: Kluver-Barrera's staining in the olfactory bulb. EPL, external plexiform layer; GCL, granule cell layer; GL, glomerular layer; MCL, mitral cell layer; ONL, olfactory nerve layer. Bar=50 μm.

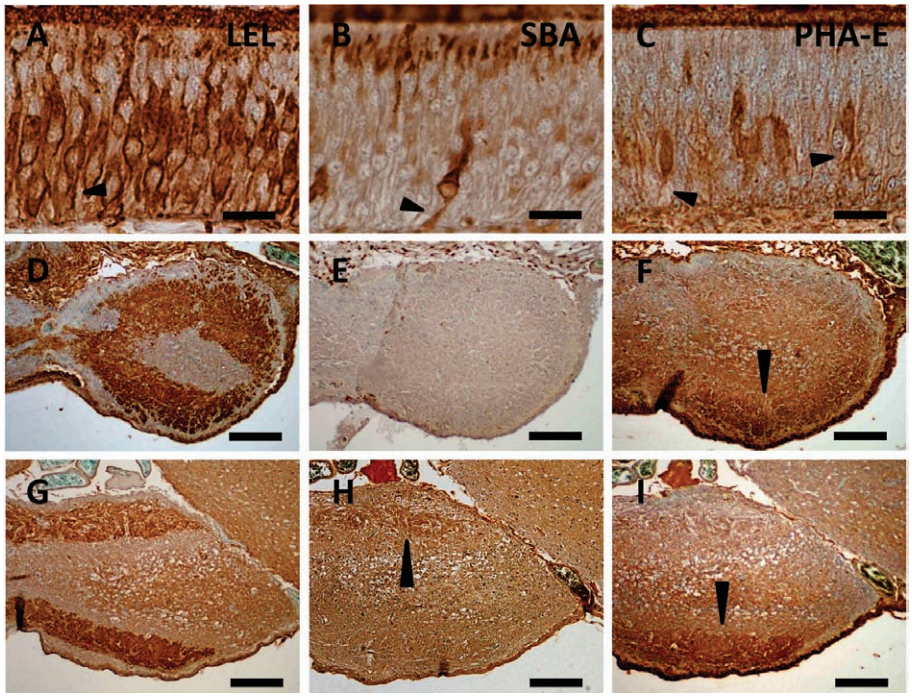

Fig. 4. Binding patterns of lectins in the olfactory epithelium (A to C) and olfactory bulb (D to I) in the Japanese jungle crow. A: LEL staining in the olfactory epithelium. Arrowheads in (A), (B), and (C) indicate the axons of the positive receptor cells. B: SBA staining in the olfactory epithelium. C: PHA-E staining in the olfactory epithelium. D: LEL staining in the rostral region of the olfactory bulb. The left side of the figure is medial, and the upper, dorsal in (D) to (I). E: SBA staining in the rostral region of the olfactory bulb. F: PHA-E staining in the rostral region of the olfactory bulb. Arrowheads in (F), (H), and (I) indicate the positive glomerular layer. G: LEL staining in the caudal region of the olfactory bulb. H: SBA staining in the caudal region of the olfactory bulb. I: PHA-E staining in the caudal region of the olfactory bulb. Bar=20 μm in (A) to (C) and 100 μm in (D) to (I).

Table 2. Lectin binding patterns in the olfactory system of the Japanese jungle crow

Lectin	Olfactory epithelium	Olfactory bulb	
	Receptor cells	Olfactory nerve layer	Glomerular layer
WGA	++	++	++
s-WGA	±	+	+
LEL	++	++	++
STL	++	++	++
DSL	++	++	++
BSL-II	–	–	–
DBA	–	–	–
SBA	++[a)]	++[b)]	++[b)]
BSL-I	–	–	–
VVA	+[a)]	+[b)]	+[b)]
SJA	–	–	–
RCA -120	+	+	+
Jacalin	++	++	++
PNA	+	±	±
ECL	–	–	–
UEA-I	–	–	–
ConA	++	++	++
PSA	–	–	–
LCA	++	++	++
PHA-E	+[c)]	++[d)]	++[d)]
PHA-L	++	++	++

–, negative staining; ±, faint staining; +, moderate staining; ++, intense staining. a) A small number of receptor cells are positive. b) Dorsocaudal region of olfactory bulb is positive. c) Several receptor cells are positive. d) Ventral region of olfactory bulb is positive.

mined. As for the cytoarchitecture, the crow OB in the present study was divided into 5 layers by the staining of Kluver-Barrera and did not possess the olfactory ventricle. Although, the OB shows the multilayered structure possessing the olfactory ventricle in many birds like that in mammals [4], the OB do not possess the olfactory ventricle in the species with the fused OB (for example, the house sparrow and bulbul) [4, 29]. Topographically and histologically, the OB of the Japanese jungle crow were similar to the OB of the house sparrow and bulbul which are highly-dependent on the vision. For this reason, it is indicated that the OB of the crow is less-developed than the OB of many other birds.

In the Japanese jungle crow, although the lectin binding patterns in the ONL and GL of the OB are reported by Yokosuka *et al.* [30], the lectin binding patterns in the present study are different from those previously reported. Since the results of the lectin histochemistry are sometimes influenced by the fixative solution and embedding agent [17], the differences between the lectin binding patterns in the present study and those reported by Yokosuka *et al.* [30] may be attributed to the differences in the fixative solution and embedding agent. Because 14 lectins among 21 lectins used in the present study stained the RCs of the OE and the ONL and GL of the OB, it is indicated that various glycoconjugates are expressed in the olfactory system of the Japanese jungle crow like that of many other species. Importantly, it was indicated that a small number of the RCs stained by SBA and VVA project their axons to the dorsocaudal region of the OB. In addition, it was indicated that several RCs stained by PHA-E project their axons to the ventral region of the OB. Therefore, in the olfactory system of the Japanese jungle crow, the RCs sensitive to the specific odoriferous substances may project their axons to the specific regions of the OB.

By the topographical, histological, and ultrastructural features of the NC and OB obtained in the present study, it is indicated that the Japanese jungle crow has less-developed olfactory system than other many birds and hardly depends on the olfaction. However, it is possible that the olfactory system in the Japanese jungle crow has the dedicated projection pathways for the specific odoriferous substances. To reveal how these pathways are related to the olfaction-medicated behaviors in birds, such as the reproductive, homing, and predatory behaviors [2, 13, 20, 28], further ethological and cytophysiological studies using several ligands are needed.

ACKNOWLEDGMENTS. The authors are very grateful to the Iwate Hunting Association, Japan, for providing animals used in the present study, and Messrs. Shuichiro Hayashi and Kuniaki Sasaki, Center of Regional Collaboration in Research and Education of Iwate University, Japan, for their cooperation in the studies using the scanning and transmission electron microscope.

REFERENCES

1. Adrian, E. D. 1951. Olfactory discrimination. *L'Annee Psychol.* **50**: 107–113.
2. Balthazart, J. and Taziaux, M. 2009. The underestimated role of olfaction in avian reproduction? *Behav. Brain Res.* **200**:

248–259.
3. Bang, B. G. 1971. Functional anatomy of the olfactory system in 23 orders of birds. *Acta Anat.* **79**: 1–76.
4. Bang, B. G. and Wenzel, B. M. 1985. Nasal cavity and olfactory system. pp. 195–225. *In*: Form and Function in Birds, Vol. 3 (Kings, A. S. and MacLelland, J. eds.), Academic Press, London.
5. Damjanov, I. 1987. Lectin cytochemistry and histochemistry. *Lab. Invest.* **57**: 5–20.
6. Day, I. N. and Thompson, R. J. 2010. UCHL1 (PGP 9.5): neuronal biomarker and ubiquitin system protein. *Prog. Neurobiol.* **90**: 327–362.
7. Eisthen, H. L. 1992. Phylogeny of the vomeronasal system and of receptor cell types in the olfactory and vomeronasal epithelia of vertebrates. *Microsc. Res. Tech.* **23**: 1–21.
8. Franceschini, V., Lazzari, M. and Ciani, F. 2000. Lectin cytochemical localisation of glycoconjugates in the olfactory system of the lizards *Lacerta viridis* and *Podarcis sicula*. *Anat. Embryol.* **202**: 49–54.
9. Franceschini, V., Lazzari, M. and Ciani, F. 2001. Lectin-binding patterns in the olfactory system of the lizard, *Physignathus lesueurii*. *J. Morphol.* **247**: 34–38.
10. Getchell, M. L. and Getchell, T. V. 1992. Fine structural aspects of secretion and extrinsic innervation in the olfactory mucosa. *Microsc. Res. Tech.* **23**: 111–127.
11. Graziadei, P. P. C. 1973. The ultrastructure of vertebrates olfactory mucosa. pp. 269–305. *In*: The Ultrastructure of Sensory Organs (Friedmann, I. ed.), North-Holland Publishing Co., New York.
12. Graziadei, P. and Bannister, L. H. 1967. Some observations on the fine structure of the olfactory epithelium in the domestic duck. *Z. Zellforsch.* **80**: 220–228.
13. Hagelin, J. C. and Jones, I. L. 2007. Bird odors and other chemical substances: a defense mechanism or overlooked mode of intraspecific communication? *Auk* **124**: 741–761.
14. Harriman, A. E. and Berger, R. H. 1986. Olfactory acuity in the common raven (*Corvus corax*). *Physiol. Behav.* **36**: 257–262.
15. Hirao, A. and Ookawara, S. 2008. Lectin binding patterns in the olfactory bulb of mallard ducks (*Anas platyrhynchos*). *Anim. Sci. J.* **79**: 686–692.
16. Kondoh, D., Yamamoto, Y., Nakamuta, N., Taniguchi, K. and Taniguchi, K. 2010. Lectin histochemical studies on the olfactory epithelium and vomeronasal organ in the Japanese striped snake, *Elaphe quadrivirgata*. *J. Morphol.* **271**: 1197–1203.
17. Leathem, A. and Atkins, N. 1983. Lectin binding to formalin-fixed paraffin sections. *J. Clin. Pathol.* **36**: 747–750.
18. Matsuzaki, O. 1995. Numbers of olfactory receptor cells and fine structure of olfactory nerves in various birds. *Zool. Sci.* **12**: 117–123.
19. Müller, H., Drenckhahn, D. and Haase, E. 1979. Vergleichend quantitative und ultrastrukturelle Untersuchungen am Geruchsorgan von vier Haustaubenrassen. *Z. Mikrosk.-anat. Forsch.* **93**: 888–900 (in German).
20. Nevitt, G. A., Losekoot, M. and Weimerskirch, H. 2008. Evidence for olfactory search in wandering albatross, *Diomedea exulans*. *Proc. Natl. Acad. Sci. U.S.A.* **105**: 4576–4581.
21. Okano, M. 1983. The olfactory organ in the fowl. pp. 123–131. *In*: The Brain and Behavior of the Fowl (Ookawa, T. ed.), Japan Sci. Soc. Press, Tokyo.
22. Plank, J. and Rychlo, A. 1952. A method for quick decalcification. *Zentralbl. Allg. Pathol.* **89**: 252–254 (in German).
23. Saito, S. and Taniguchi, K. 2000. Expression patterns of glycoconjugates in the three distinctive olfactory pathways of the clawed frog, *Xenopus laevis*. *J. Vet. Med. Sci.* **62**: 153–159.
24. Saito, S., Kobayashi, N. and Atoji, Y. 2006. Subdivision of the accessory olfactory bulb in the Japanese common toad, *Bufo japonicus*, revealed by lectin histochemical analysis. *Anat. Embryol.* **211**: 395–402.
25. Saito, S., Matsui, T., Kobayashi, N., Wakisaka, H., Mominoki, K., Matsuda, S. and Taniguchi, K. 2003. Lectin histochemical study on the olfactory organ of the newt, *Cynops pyrrhogaster*, revealed heterogeneous mucous environments in a single nasal cavity. *Anat. Embryol.* **206**: 349–356.
26. Steiger, S. S., Fidler, A. E., Valcu, M. and Kempenaers, B. 2008. Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? *Proc. R. Soc. B.* **275**: 2309–2317.
27. Taniguchi, K., Saito, S. and Taniguchi, K. 2011. Phylogenic outline of the olfactory system in vertebrates. *J. Vet. Med. Sci.* **73**: 139–147.
28. Wenzel, B. M. 1968. Olfactory prowess of the kiwi. *Nature* **220**: 1133–1134.
29. Yokosuka, M., Hagiwara, A., Saito, T. R., Aoyama, M., Ichikawa, M. and Sugita, S. 2009. Morphological and histochemical study of the nasal cavity and fused olfactory bulb of the brown-eared bulbul, *Hysipetes amaurotis*. *Zool. Sci.* **26**: 713–721.
30. Yokosuka, M., Hagiwara, A., Saito, T. R., Tsukahara, N., Aoyama, M., Wakabayashi, Y., Sugita, S. and Ichikawa, M. 2009. Histological properties of the nasal cavity and olfactory bulb of the Japanese jungle crow *Corvus macrorhynchos*. *Chem. Senses* **34**: 581–593.