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A descriptive and comparative lectin histochemical study of the vomeronasal system in pigs and sheep

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ABSTRACT

The accessory olfactory bulb (AOB) is the primary target of the sensory epithelium of the vomeronasal organ (VNO), and thus constitutes a fundamental component of the accessory olfactory system, which is involved in responses to behaviour-related olfactory stimuli. In this study we investigated the characteristics of the AOB, VNO, vomeronasal nerves (VNNs) and caudal nasal nerve (CdNN) in pigs and sheep, species in which olfaction plays a key behavioural role both in the neonatal period and in adulthood. The patterns of staining of the AOB by the *Bandeiraea simplicifolia* and *Lycopersicon esculentum* lectins were the same in the 2 species, whereas the *Ulex europeus* and *Dolichos biflorus* lectins gave different patterns. In both species, lectin staining of the AOB was consistent with that of the VNNs, while the CdNN did not label any of the structures studied. The entire sensory epithelium of the pig was labelled by *Ulex europeus* and *Lycopersicum esculentum* lectins, and all 4 lectins used labelled the mucomicrovillar surface of the sensory epithelium in sheep.

Key words: Accessory olfaction; ungulates.

INTRODUCTION

The size and morphology of the accessory olfactory bulb (AOB) shows marked variation between taxa (Crosby & Humphrey, 1939). In adult, young and embryonic mammals, the vomeronasal system (VNS) or parts of it generally exist, but with differing degrees of development, and even with differing configurations (Salazar et al. 1992, 1994). The literature contains extensive details with regard to such variability in the AOB (Hoffman, 1963). In bats, for example, structural characteristics of the AOB appear to correlate with feeding strategy (Frahm & Bhatnagar, 1980; Frahm, 1981). In other mammals, such as the mustelids, the AOB has been demonstrated in some cases (Jeserich, 1945) but not others (Jawlowski, 1956), although the particular morphological and topographic characteristics of this tissue in the mink may explain certain discrepancies (Salazar et al. 1998).

The chemical composition of the AOB similarly shows significant interspecies variation. This variation can be revealed by the use of various neurochemical techniques such as lectin histochemistry. However, the pattern of staining of the AOB obtained with a single lectin may differ even between species as closely related as the rat and the mouse (Key & Giorgi, 1986).

This variability in the morphological and histochemical characteristics of the AOB may shed light on the function of this organ within the VNS (Halpern, 1987), although the relationship between the VNS and reproduction is evident (Wysocki, 1979).

Interest in the study of the VNS has increased over recent years, especially since Johnson et al. (1985) reported the presence of the VNO in adult humans. Nowadays, the presence of the VNO in humans is generally accepted, not only during the prenatal period as has been known for some time (Humphrey, 1940) but also in adults (Smith et al. 1998). Recently, clear evidence of its functionality has been reported (Monti-Bloch et al. 1998; Stern & McClintock, 1998).

In the present descriptive and comparative study we

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investigated the VNS (excluding the vomeronasal amygdala) of 2 macrosmatic mammals, the sheep and the pig. These species, although they have been domesticated by man and live in captivity, continue to use the olfactory system in such critical moments of their habitual behaviour as reproduction and food selection.

MATERIALS AND METHODS

Tissue preparation

A total of 18 pigs and 16 sheep (in both species both males and females) were used. Of the 18 pigs, 4 were aged 2–12 wk ('young'), while the remaining 14 were more than 6 mo old ('adults'). Of the 16 sheep, 3 were aged 2–12 wk, while the remaining 13 were aged more than 6 mo. Nine of the adult pigs and 8 of the adult sheep were obtained directly from the slaughterhouse and studied by dissection, microdissection and conventional histology. After fixation, the tissue was embedded in paraffin and sectioned transversely, horizontally or parasagittally at 6–8 μm. Sections were alternately stained with haematoxylin/eosin, Tolivia stain (Tolivia et al. 1988) or Nissl methods. The remaining 5 adult pigs and 5 adult sheep were kindly donated by local farms and studied by histochemistry. The young animals were from our departmental animal facility and were used for histological examination. In all cases the ethical guidelines embodied in DHEW publication (NIH) 80–23 were observed.

Lectin histochemistry

The 10 animals studied by lectin histochemistry were killed with an overdose of sodium pentobarbitone administered intraperitoneally. The heads were perfused via the 2 common carotid arteries or transcardially, depending on size, with phosphate buffer (0.1 M, pH 7.3) and then with one of the 2 fixatives: 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3, or glacial acetic acid in absolute ethanol, 5% v/v. The olfactory bulbs, the vomeronasal organs (VNO) and sections of the nasal septum were removed. All samples, except 8 olfactory bulbs, were dehydrated in graded alcohols, infiltrated with xylene and embedded in paraffin. The AOB were sectioned horizontally and the VNO and nasal mucosa transversely at 6–8 μm. After cryoprotection in sucrose solution, the remaining olfactory bulbs were sectioned transversely or horizontally 30 μm on a cryostat. Four lectins, all biotin-conjugated (Sigma Chemical Company) were used: *Ulex europeus* agglutinin I (UEA-I), *Bandeiraea simplicifolia* lectin I isolectin B_4 (BSI-B_4), *Dolichos biflorus* agglutinin (DBA), and *Lycopersicon esculentum* agglutinin (LEA). The Vectastain ABC kit for detection of biotin was from Vector (Burlingame, USA).

Sections were dewaxed or processed free-floating, transferred to phosphate buffer (pH 7.3) and then incubated with (1) 2% bovine serum albumin in 0.1 M Tris buffer (pH 7.2) for 30 min, (2) lectin at various dilutions in 0.1 M Tris buffer containing 2% bovine serum albumin for 24 h at 4 °C, (3) phosphate buffer (2×10 min), (4) Vectastain ABC reagent (1:250 in phosphate buffer) for 90 min at room temperature, (5) phosphate buffer for 10 min, and (6) Tris-HCl (pH 7.6) for 5 min. Peroxidase activity was visualised by incubation in a solution containing 0.05% 3,3′-diaminobenzidine and 0.003% H_2O_2 in 0.2 M Tris-HCl buffer (pH 7.6), with monitoring of the reaction under a microscope; the reaction was stopped with 0.2 M Tris-HCl (pH 7.6).

Controls were performed by omitting the lectin from the labelling procedure, or by the preabsorption of lectins with excess amounts of respective sugar residues (coincubation with UEA-I and L-fucose, BSI-B_4 and D-galactose, DBA and N-acetyl-D-galactosamine or LEA and triacetylchitotriose). Some sections were lightly counterstained with cresyl violet.

RESULTS

The AOB occupied a practically identical position in the 2 species studied: ventral to the medial face of the main olfactory bulb (MOB), in association with the glomerular area of this structure (Fig. 1*a*). However, this general pattern diverged in young pigs with the AOB occupying a more dorsal position, as seen in transverse sections (Fig. 1*b*). By contrast, the AOB of young sheep was located in the same position as that of adults (Fig. 1*c*).

In both species, transverse and sagittal sections of the AOB revealed well defined stratification, with the vomeronasal nerve and the glomerular, external plexiform, mitral-tufted and granular layers clearly distinguishable (Fig. 1*d*).

Differences between the 2 species were observed with regard to binding of lectins to the AOB and other parts of the VNS. These differences are summarised in the Table and Figures 2, 3 and 4. In no case, was specific lectin binding observed in controls.

UEA-I did not bind either to the AOB or to

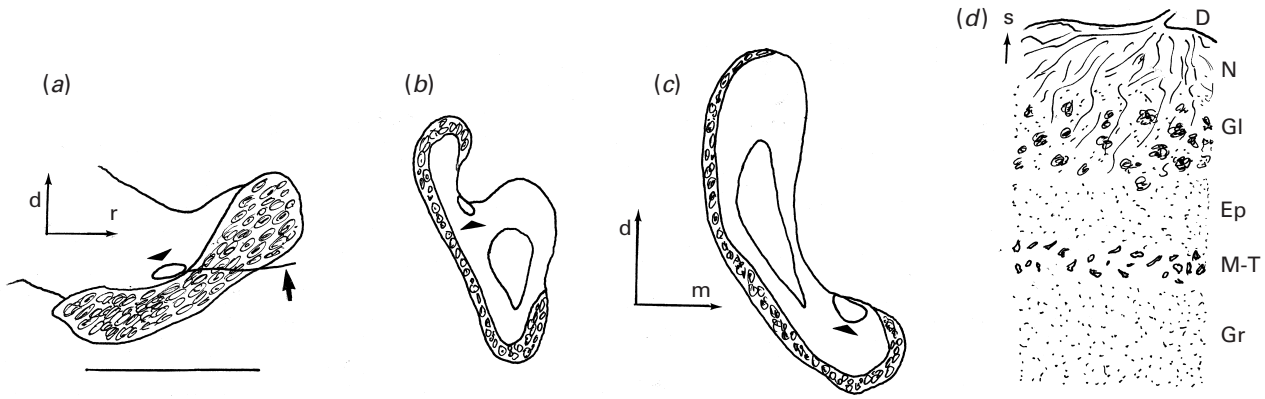

Fig. 1. Schematic drawings of (*A*) the topography of the AOB (arrowhead) on the medial face of the left main olfactory bulb of adult pig, showing the entrance of the VNNs (arrow); (*B*) a transverse section of the MOB of a young pig, showing the position of the AOB (arrowhead); (*C*) a transverse section of the MOB of an adult sheep, showing the position of the AOB (arrowhead); and (*D*) the AOB of an adult pig or sheep, showing the characteristic cell layers. The swirled areas within panels *B* and *C* are the glomeruli of the main olfactory bulb. d, dorsal; m, medial; r, rostral; s, superficial. N, olfactory nerve layer; Gl, glomerular layer; Ep, external plexiform layer; M-T, mitral-tufted cell layer; Gr, granular cell layer. A: bar, 1.63 cm.

surrounding tissues in sheep (Fig. 2*a*), whereas in the pig this lectin strongly labelled the AOB (Fig. 2*b*) and weakly labelled the glomerular layer of the MOB.

BSI-B_4 did not bind either to the sheep or pig AOB (Fig. 2*c*, *d*), although at high concentrations some labelling of glomeruli was observed in the sheep AOB. In both species, surrounding tissues showed generalised staining that even extended as far as the lateral olfactory tract; this staining was always more intense in sheep than in the pig.

DBA bound both to the AOB (Fig. 2*e*) and the glomerular layer of the MOB of sheep. In pig this lectin did not bind in the AOB, and produced no more than a diffuse background in surrounding tissues (Fig. 2*f*).

LEA labelled the AOB of both species in a similar way (Fig. 2*g*, *h*); the only difference was that staining, at equal dilutions, was more intense in pig than in sheep. LEA likewise stained the glomerular layer of the MOB in both species.

In all cases in which there was clear labelling of the AOB, binding was to the vomeronasal nerve and glomerular layers only.

The patterns of lectin binding to the epithelia of the VNO were also investigated. In sheep, all 4 lectins used mainly labelled, in different degree and extension, the mucomicrovillar surface of the sensory epithelium and the mucociliary surface of the respiratory epithelium (Fig. 3*a*, *d*, *g*, *j*). In the pig, UEA-I labelled the entire sensory and respiratory epithelia (Fig. 4*a*), BSI-B_4 labelled very weakly some cells of the respiratory epithelium (Fig. 4*d*), DBA labelled the respiratory epithelium (Fig. 4*g*), and LEA labelled the entire sensory epithelium and the mucociliary surface of the respiratory epithelium (Fig. 4*j*).

In sheep, the VNNs in the lamina propia of the sensory mucosa were stained by DBA (Fig. 3*h*) and LEA (Fig. 3*k*). There appeared to be some epineurial staining of the VNNs in the sheep (Fig. 3*e*, *h*). In the pig, these nerves were stained by UEA-I (Fig. 4*b*) and LEA (Fig. 4*k*). No lectin binding was observed in the remaining cases (Figs 3*b*, *e*, 4*e*, *h*). Finally, the caudal nasal nerve (CdNN) was not labelled by any of the lectins in either species (Figs 3*c*, *f*, *i*, *l*, 4*c*, *f*, *i*, *l*).

DISCUSSION

Apart from the shift in position observed in young pigs, the characteristics of the AOBs of the 2 species considered in the present study are similar to the 'classic' pattern observed in rodents (such as the rat and guinea pig) (Cajal, 1902) and in the rabbit (Cajal, 1902; McLean & Shipley, 1992). In our study it was not possible to distinguish mitral cells from tufted cells on morphological grounds, as has been noted in the rat (Takami & Graziadei, 1991).

The results of the present study indicate considerable differences in lectin binding between the AOB of pig and sheep. The patterns of staining of the AOB by LEA and BSI-B_4 lectins were the same in the 2 species, while labelling patterns obtained with the other 2 lectins (UEA-I and DBA) showed clear interspecies differences. The following aspects are of particular interest. First, UEA-I did not label the AOB of the sheep, and the sheep appears to be the only studied mammal in which this occurs. Second, BSI-B_4 did not label the AOB either of the sheep or the pig, despite the fact that this lectin is specific for the VNS in a wide group of animals (Ichikawa et al.

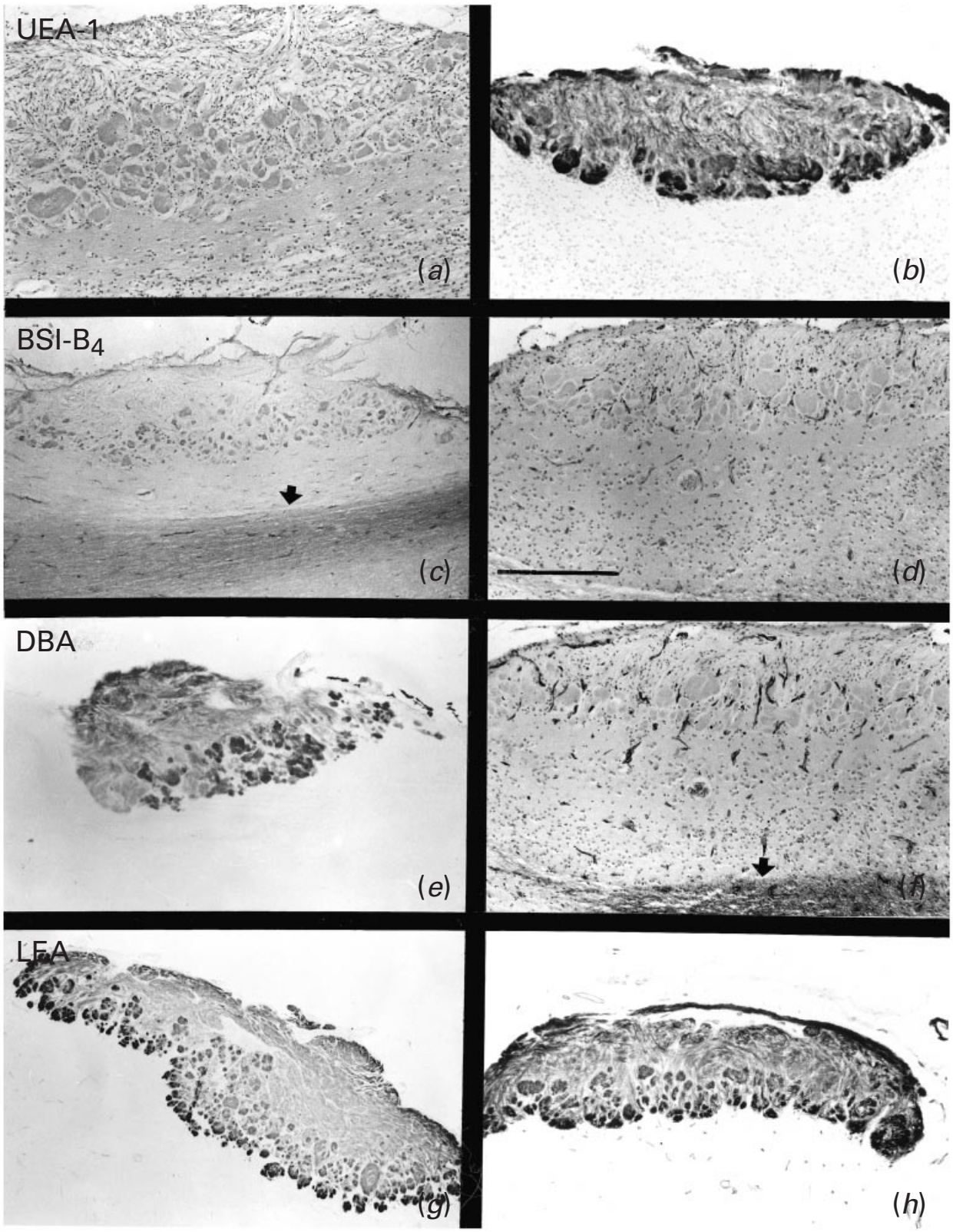

Fig. 2. Transverse sections showing staining of the AOB in the sheep (*a*, *c*, *e*, *g*) and pig (*b*, *d*, *f*, *h*) by UEA-I (*a*, *b*), BSI-B_4 (*c*, *d*), DBA (*e*, *f*) and LEA (*g*, *h*). Arrows, lateral olfactory tract. Bars: *a*, *b*, *d*, *f*, *h*, 400 μm; *c*, *e*, *g*, 800 μm.

1992). Third, DBA labelled the AOB of the sheep but did not do so in rodents (see Salazar & Sánchez Quinteiro, 1998 for revue and further discussion).

On the other hand, using lectins (Takami et al. 1992; Taniguchi et al. 1993) and other organic compounds (Imamura et al. 1985; Yoshida et al.

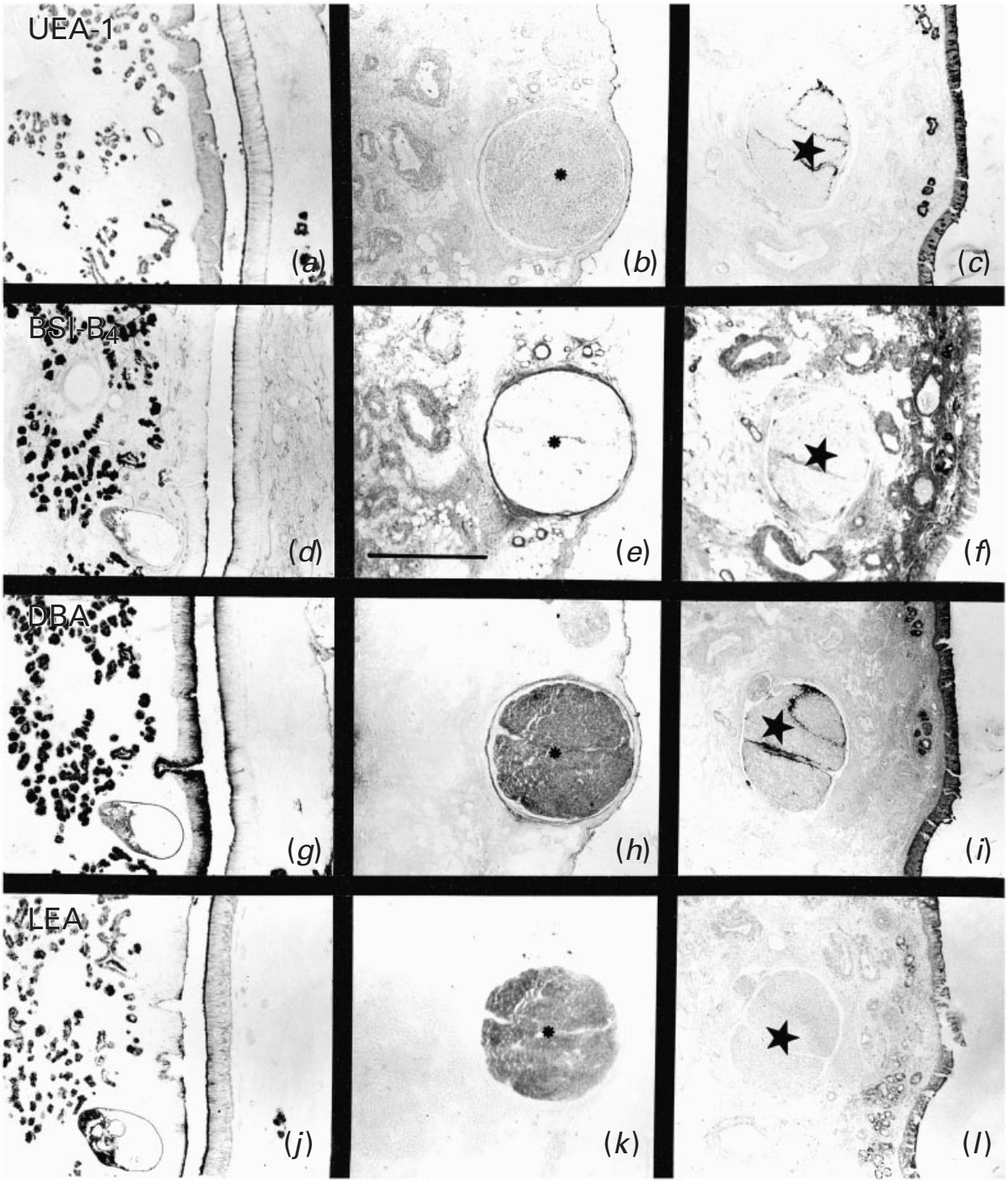

Fig. 3. Micrographs showing staining of the vomeronasal organ (*a*, *d*, *g*, *j*), vomeronasal nerves (asterisks) (*b*, *e*, *h*, *k*) and caudal nasal nerve (stars) (*c*, *f*, *i*, *l*) of the sheep by UEA-I (*a*, *b*, *c*), BSI-B_4 (*d*, *e*, *f*), DBA (*g*, *h*, *i*) and LEA (*j*, *k*, *l*). s, sensory or medial epithelium. Bars: *a*, *d*, *g*, *j*, 400 μm; and *b*, *c*, *e*, *f*, *h*, *i*, *k*, *l*, 800 μm.

1995) it has been demonstrated that the AOB shows histochemical zonation: the rostral and caudal parts of the AOB show clear labelling differences. In the present study, however, we did not observe such zonation of the AOB either in the sheep or the pig. One possible explanation for this result is that zonation is present in these species but is not revealed by the lectins used in the present study, despite the fact that these lectins may reveal AOB zonation in other animals.

The most evident explanation for the discrepancies quoted above is that there are differences in the sugars

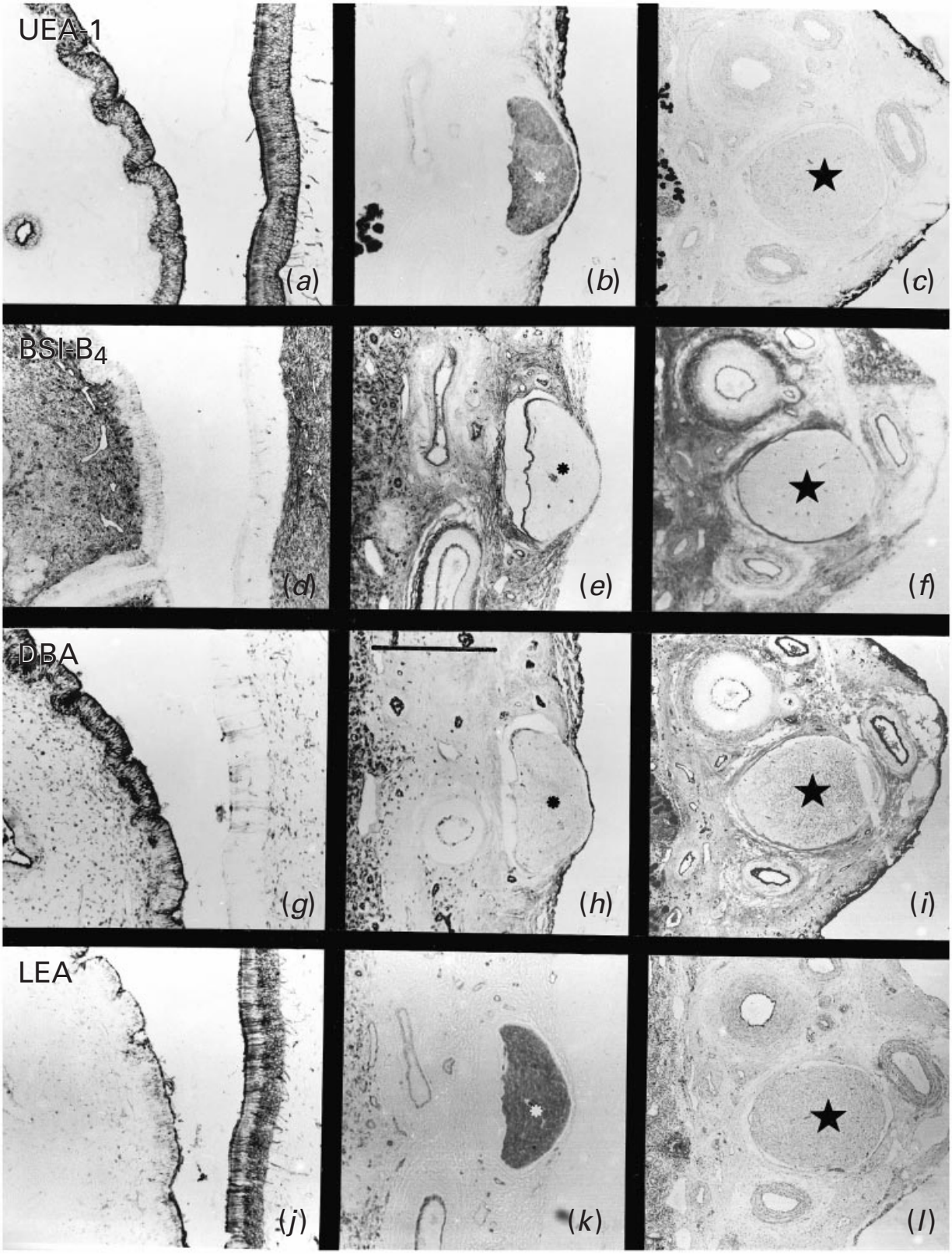

Fig. 4. Micrographs showing staining of the vomeronasal organ (*a*, *d*, *g*, *j*), vomeronasal nerves (asterisks) (*b*, *e*, *h*, *k*) and caudal nasal nerve (stars) (*c*, *f*, *i*, *l*) of the pig by UEA-I (*a*, *b*, *c*), BSI-B_4 (*d*, *e*, *f*), DBA (*g*, *h*, *i*) and LEA (*j*, *k*, *l*). S, sensory or medial epithelium. Bars: *a*, *b*, *d*, *e*, *g*, *h*, *j*, *k*, 400 μm; *c*, *f*, *i*, *l*, 800 μm.

Table. *Lectins binding to different parts of the vomeronasal system**

		Lectin (concentration)				
		UEA-I (10–30 μg/ml)	BSI-B_4 (10–50 μg/ml)	DBA (25–50 μg/ml)	LEA (10–30 μg/ml)	Structures
Sheep		−	−	+	+	AOB
Pig		+	−	−	+	
Sheep	Lateral epithelium	(+)	(+)	(+)	(+)	VNO
	Medial epithelium	(+)	(+)	(+)	(+)	
Pig	Lateral epithelium	+	(+)	+	(+)	
	Medial epithelium	+	−	−	+	
Sheep	Vomeronasal	−	−	+	+	Nerves
	Caudal nasal	−	−	−	−	
Pig	Vomeronasal	+	−	−	+	
	Caudal nasal	−	−	−	−	

* +, labelling of whole of structure; (+), part labelling; −, weak or absent labelling.

expressed in the AOBs of the pig and the sheep. Probably the range of affinities of each lectin for different sugar residues (exclusive affinity, major affinity or simple affinity) could be relevant in this regard (Etzler & Kabat, 1970; Allen & Johnson 1977; Wood et al. 1979; Nachbar et al. 1980).

With regards to the lectin binding in tissues associated directly with the VNS (the VNO, the VNNs, and the CdNN), we found that labelling of the VNNs was identical to that observed in the AOB, confirming the functional similarity of the 2 tissues. Furthermore, the CdNN were not labelled by any of the lectins used, in accordance with their origin (the maxillary nerve) and their target (the glandular tissue of the VNO) (Eccles, 1982). One tissue, the whole medial (sensory) epithelium of the VNO, did not show the expected staining responses (i.e. the same responses as the AOB and VNNs) in sheep; however, the mucomicrovillar surface of the sheep sensory epithelium was labelled by UEA-I and BSI-B_4, though neither of these 2 lectins stained the AOB and VNNs. This can probably be attributed to the presence of sialic acid and galactosamine residues in the mucus of the lumen of the VNO (Takami et al. 1994).

In conclusion, the coincidence between the lectin binding pattern in both the AOB and the VNNs suggests that some of the lectins employed in this study recognise the glycoconjugates present in some parts of the VNS: the surface of the vomeronasal axons along their course in the vomeronasal soft tissue and in the nasal septum, and their termination within glomeruli of the AOB, as summarised in the Table. Although the exact functional role of these glycoconjugates in the VNS remains to be determined, it is believed that they are involved in the mediation of cellular interactions (Jessell et al. 1990). The present study provides evidence that the expression of glycoconjugates in the primary vomeronasal projection is highly specific and more complex than is usually considered, with remarkable differences in the chemical composition of the AOB even among the closely related species considered in the present study.

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