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Lotus Lectin Labels Subpopulation of Olfactory Receptor Cells

William Bennett Stewart and Christopher Edward Touloukian

Section of Anatomy, Department of Surgery, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510, USA

Correspondence to be sent to: William Bennett Stewart, Section of Anatomy, Department of Surgery, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510, USA

Abstract

Experiments were performed to test the hypothesis that subsets of olfactory receptor cells could be recognized based on their lectin binding and that mapping of their projections onto the olfactory bulb would reveal details of anatomic organization of the olfactory nerve projection to the olfactory bulb. The results from one lectin, *Lotus*, were examined in detail. Olfactory receptor cells in the lateral part of the main epithelium were labeled, as well as scattered cells in the remainder of the epithelium. Glomeruli labeled by *Lotus* were concentrated primarily in the region of the olfactory bulb that receives its input from the lateral epithelium, although scattered glomeruli could be identified in other regions. Within the terminal field of these axons there was a mosaic pattern, with some glomeruli densely labeled, some lightly labeled and others unlabeled. These findings support the notion that there are biochemically distinct populations of olfactory receptor cells having localized distributions in the epithelium, with axons that coalesce to terminate in specific glomeruli, rather than diffusely over their projection field.
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Introduction

Several lines of evidence point to the existence of biochemically distinct subclasses of olfactory receptor cells. Some of these studies have used the monoclonal antibody method (Fujita *et al.*, 1985; Schwob and Gottlieb, 1986, 1988; Mori, 1987). Lectins have also been used to label olfactory receptor cells and their projections to the olfactory bulb (Barber, 1989; Gheri *et al.*, 1992; Riddle *et al.*, 1993). Molecular biology methods have provided evidence for a multigene family that may encode for odorant receptors (Buck and Axel, 1991; Buck, 1992). Anatomical studies using the *in situ* hybridization technique show that these receptors are organized in zones within the epithelium (Ressler, Sullivan *et al.*, 1993).

In this paper we report the results of a study using *Lotus* lectin to test the hypothesis that other subsets of olfactory receptor cells could be identified based on their expression of membrane sugars and that the spatial distribution of their projections to the olfactory bulb could be mapped. Prior studies have reported that *Ulex* and *Glycine* lectins show patchy staining of glomeruli of the main olfactory bulb (Key and Giorgi, 1986; Riggott and Scott, 1989) and that *Dolichos biflorus* (DBA) stained a subset of receptor cells in the epithelium and glomeruli scattered within a zone of the glomerular layer (Key and Akeson, 1993). These results suggested that staining with other lectins might allow mapping of the distribution of other populations of receptor cells and their projection to the olfactory bulb. A specific question to be addressed was whether olfactory cells that projected to scattered glomeruli in the olfactory bulb would come from scattered patches in the epithelium or would be

uniformly distributed. A related aim was to examine the distribution of the axons of these cells in light of what is known about the topographic organization of olfactory nerve projections. The results of this study show that a subclass of olfactory receptor cells can be identified using the lectin from *Tetragonolobus purpuraes* (*Lotus*) and that the axons of these cells converge onto glomeruli within defined regions of the olfactory bulb.

Materials and methods

Rats aged 14–25 days were anesthetized with sodium pentobarbital (70 mg/kg) and perfused transcardially with 2% paraformaldehyde in phosphate buffer or 10% buffered formaldehyde. The heads were cleaned and placed in 0.1 M EDTA in 0.1 M phosphate buffer. After 7–14 days decalcification, the heads were placed in 20% sucrose in 0.1 M phosphate buffer until sectioning. The heads were frozen and sectioned serially at 80 µm using a sliding microtome. The sections were collected in a cryoprotectant and stored at –20°C.

The sections were stained with a panel of lectins labeled with horseradish peroxidase (HRP). The lectins were dissolved in 3% bovine serum albumin in phosphate buffered saline with 0.1% Triton. The lectins were screened at concentrations between 0.001 and 0.1 mg/ml. For the screening phase of the study, staining took place overnight at room temperature. On the following day, the sections were reacted with the conventional diaminobenzidine procedure. The following lectins were screened: *Lotus*, *Helix*, *Vicia*, *Bandeiraea*, *Glycine*, *Maclura*, *Phytolacca* and *Ulex*. *Lotus* was chosen for further analysis because, of the lectins studied, it was the only one that produced a clear differential staining of both receptor cells and glomeruli. Every third section through the nose and olfactory bulb was stained to map the distribution of labeled receptor cells and glomeruli and to trace the labeled olfactory nerve fascicles.

Results

Olfactory receptor cells labeled by *Lotus* are located predominately in the lateral portion of the nasal cavity along the entire rostrocaudal axis (Figure 1A–D). The cells were evident along the lateral wall of the nasal cavity and along the base of the turbinates, where they arise from the lateral wall. The thicker epithelium on the turbinates further from their bases, in the dorsal recess and on the nasal septum had scattered cells labeled with *Lotus*. However, in one of the animals there was a small patch of epithelium on the ventral septum (see the asterisk in Figure 1C). Receptor cells in the vomeronasal organ were not labeled.

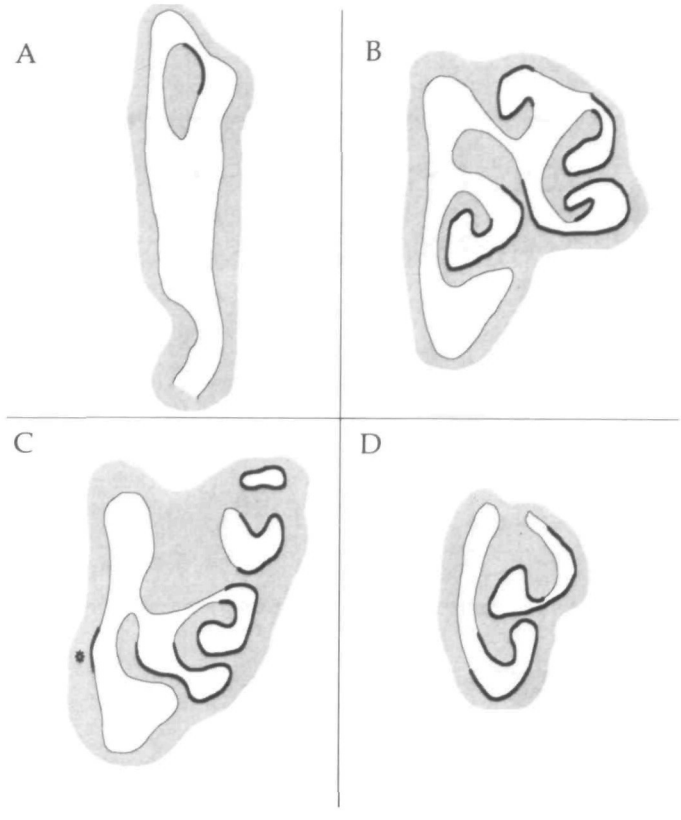

Figure 1 (A–D) Typical results from a study with *Lotus*. Representative sections from four regions the olfactory epithelium are illustrated, with (A) being the most rostral and (D) being the most caudal. Regions of the olfactory epithelium containing high concentrations of stained receptor cells are indicated by thick lines. Regions containing scattered labeled cells are indicated by light lines. The asterisk in (C) indicates a region of dense labelling in the ventral septum. Only one of the four animals had labelled cells in this region.

In the dorsolateral part of the epithelium (Figure 2A and B) receptor cell bodies as well as their dendrites' terminal knobs and axons were stained. Most, but not all, cells appeared to be labeled. In the olfactory bulb (Figure 2C) there was considerable variation among glomeruli in their degree of staining. Within the overall region of staining some glomeruli were stained densely (closed arrows) and some lightly, and some appeared to be unstained (star). Bundles of *Lotus*-positive axons could be identified in the nasal cavity and followed to the olfactory bulb (Figure 2C, open arrow). In the medial part of the nasal cavity, scattered olfactory receptor cells that were stained with *Lotus* could be identified (Figure 2D).

The stained glomeruli were located principally on the

lateral surface and on the ventral part of the medial surface of the olfactory bulb along its entire rostrocaudal extent. Previous studies of the topography of olfactory nerve connections show that these parts of the olfactory bulb receive their input from the regions of the epithelium where *Lotus* staining of receptor cells is concentrated (Astic and Saucier, 1986; Saucier and Astic, 1986; Astic *et al.*, 1987; Stewart and Pedersen, 1987). The results from four animals are summarized in Figure 3. For the olfactory bulb with the most glomeruli labeled (Figure 3D, filled circles), 13%

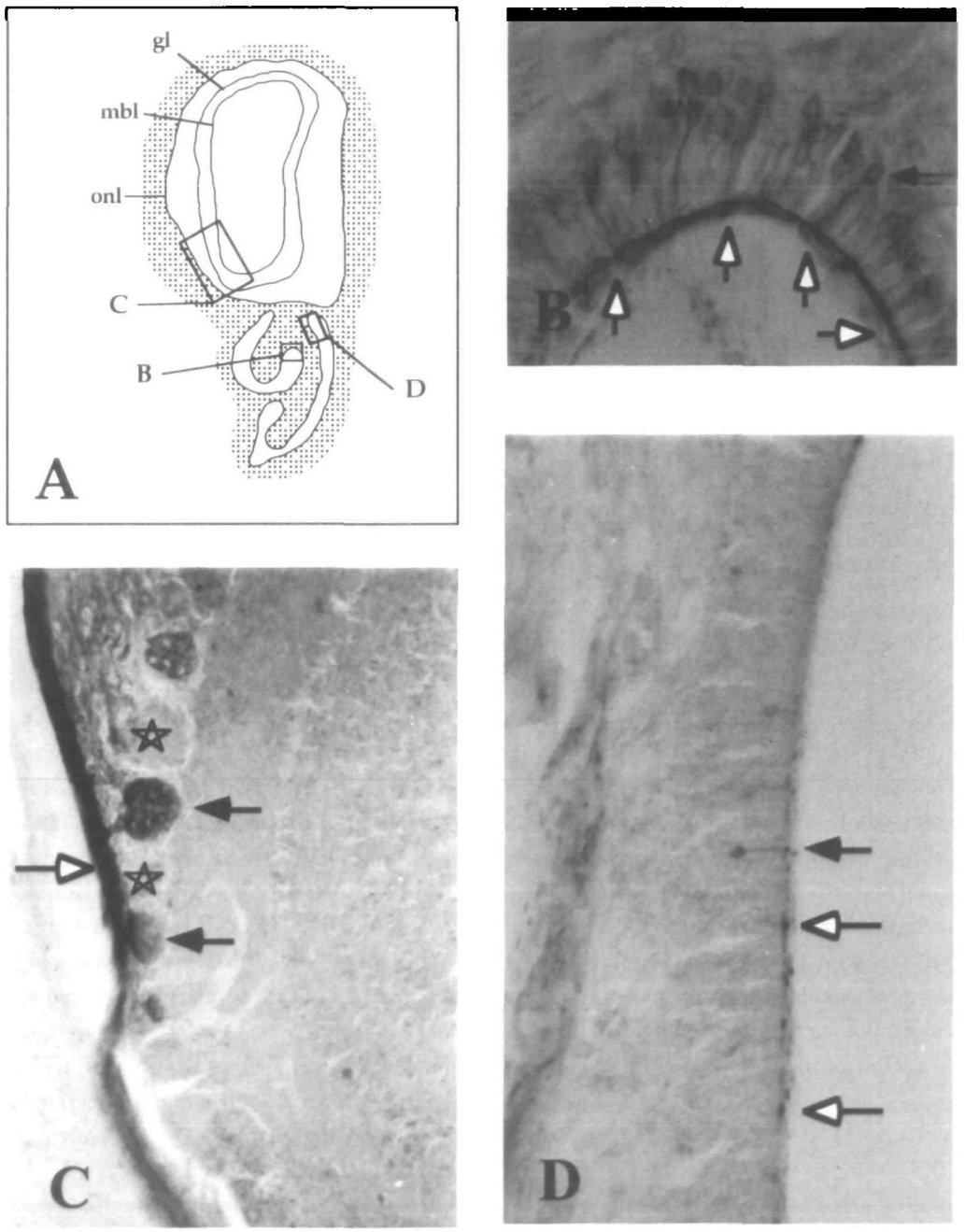

Figure 2 **(A)** A drawing of the nasal cavity and olfactory bulb. The regions shown in photomicrographs (B–D) are indicated. The lines indicate the mitral body layer (mbl), the inner border of the glomerular layer (gl) and the outer border of the olfactory nerve layer (onl). **(B)** Photomicrograph of a region of dense labeling of the olfactory epithelium. A labeled receptor cell (closed arrow) is indicated as well as labeling of terminal knobs (open arrows) (magnification: ×240). **(C)** Photomicrograph of a portion of the ventrolateral olfactory bulb. Within this field there is heterogeneous staining of glomeruli. There are both densely labeled glomeruli (closed arrows) and unlabeled glomeruli (stars). A labeled bundle of olfactory nerves can be seen on the edge of the olfactory nerve layer (open arrow) (magnification: ×80) **(D)** Photomicrograph of a region in the dorsomedial epithelium. A labeled receptor cell is indicated (closed arrows) as well as a region with labeled terminal knobs (between open arrows) (magnification: ×160).

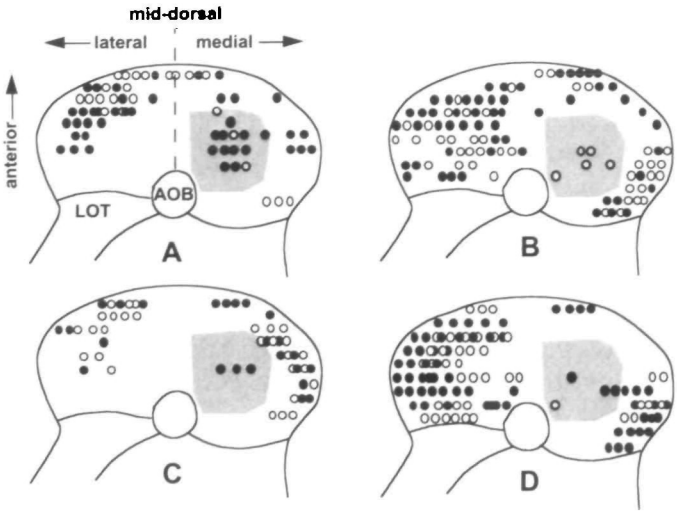

Figure 3 The position of glomeruli stained with *Lotus* in a two-dimensional representation of the glomerular layer Results from four animals (A–D) are displayed. The filled circles indicate stained glomeruli from the right olfactory bulb and the open circles indicate those from the left. The shaded zones indicate the region of the olfactory bulb that receives input primarily from the nasal septum

of glomeruli were stained. Within the zone of highest concentration of labeled glomeruli, 30% were labeled. In all of the rats there were some labeled glomeruli in dorsomedial part of the olfactory bulb (see the shaded area in Figure 3). This is the region of the olfactory bulb that receives input from the nasal septum and dorsal recess (Pedersen *et al.*, 1986; Stewart and Pedersen, 1987). The presence of labeled glomeruli in the dorsal portion of the olfactory bulb may due to the scattered receptor cells throughout the medial epithelium, including those regions that project to the dorsomedial part of the olfactory bulb. In one of the cases (Figure 3A), however, a strip of epithelium with a cluster of labeled cells is present on the ventral part of the nasal septum on the right (see also Figure 1C). The presence of this patch of cells in this location is consistent with the appearance of a larger number of *Lotus*-positive glomeruli in the dorsomedial region of the right bulb in this animal.

Since the primary sugar specificity of *Lotus* is for fucose, sections were incubated as described above with 1.0 M fucose added to the solution. Under these conditions, the *Lotus* staining in both the epithelium and the olfactory bulb could be blocked.

Discussion

The spatial distribution of *Lotus* labeled cells is markedly different than that reported in the mouse using DBA lectin (Key and Akeson, 1993). The DBA lectin stains receptor cell throughout the nasal cavity, but the highest concentration of stained cells is in the anterior and medial epithelia. The ventral and lateral epithelia have only widely scattered cells. Furthermore, the axons of these cells terminate in DBA-positive glomeruli concentrated in the dorsomedial region of olfactory bulb, where *Lotus* staining is sparse. Unlike *Lotus*, DBA also stains some of the cells of the vomeronasal organ.

There are, however, important similarities in the staining pattern. The cells are concentrated in specific regions of the olfactory epithelium. Within the region of highest density of stained cells, not all cells are labeled. Scattered labeled cells are also evident in other regions of the epithelium. As far as density of staining of the glomeruli of the olfactory bulb is concerned, both lectins stained the glomeruli heterogeneously. Within a region of the olfactory bulb, some of the glomeruli stained intensely, some weakly and some not at all. The distribution of labeled glomeruli is consistent with what is known about the topographic organization of the olfactory nerve projections.

Barber (1989) has also reported staining, using *Lotus* and *Ulex* lectins, in the olfactory bulb of the rat. *Ulex* and *Lotus* both have primary sugar specificity for α-L-fucose. The results from his study indicated that both lectins stained the olfactory nerve and all glomeruli; and in addition, *Lotus* stained widely scattered nuclei and tracts. This difference between *Ulex* and *Lotus* may be due to the fact that these two lectins have different affinities for branched oligosaccharides (Wu *et al.*, 1987). Barber's results were obtained mostly in tissue treated by alcohol fixation. When aldehyde fixation was used, staining was not as robust. In addition, other studies using *Ulex* lectin with aldehdye-fixed tissue show a pattern of staining similar but not identical to that reported here for *Lotus* (Riggott and Scott, 1989). These studies make it clear that the staining patterns with lectins are highly influenced by the fixation methods. It should be noted, however, that the differences in staining across regions of the olfactory bulb and epithelium reported here are consistent among animals and are unlikely to be due to spatial gradients of fixation.

A variety of anatomical methods have also highlighted the convergent nature of the olfactory nerve projections to the olfactory bulb. Studies in the rabbit have shown that degeneration within the terminal field of an olfactory nerve fascicle is not uniform (Land, 1973). Within the terminal field, glomeruli with very dense degeneration staining are intermingled with glomeruli that have light or no staining.

In addition, experiments studying the transport of HRP or amino acids from the nose to the olfactory bulb have found that densely labeled glomeruli occur adjacent to unlabeled ones (Land and Shepherd, 1974; Stewart, 1985). Astic and co-workers (Astic *et al.*, 1987) have also demonstrated with very small injections of HRP into the olfactory bulb that labeled cells in the epithelium occur in large patches. Within these patches, the cells are not located in dense clumps but are distributed throughout the epithelial zone that projects to that general region of the bulb. They suggest, therefore, that there is considerable redundancy in the epithelium to olfactory bulb projection. Topographic studies performed by Kauer (1987) in salamander show that small fascicles of olfactory nerve project densely to a portion of the olfactory bulb and lightly to the remainder of the bulb.

The lectin findings may also be related to the results of the electro-olfactogram studies performed in the salamander that show there are regions of maximal response for a given odor, but that responses of a smaller size may be obtained from the entire epithelial sheet (MacKay-Sim *et al.*, 1982). This physiological finding suggests that there are localized regions where receptor cells with the same properties are concentrated, but that these cells are also present in lower concentrations in the rest of the epithelium. This is the kind of pattern that is obtained with both *Lotus* and DBA.

The lectin results are also of interest in light of what is known about spatial patterns of functional activation of glomeruli in the olfactory bulb. Recordings made with large electrodes in the rabbit olfactory bulb indicate that glomeruli have different spectra of sensitivities to odors (Leveteau and MacLeod, 1966). This was true of glomeruli that were close to each other. Another line of evidence supporting variation in the sensitivity of glomeruli to odors has come from the 2-deoxyglucose (2DG) method (Stewart *et al.*, 1979). With this method, odor stimulation produces punctate foci of 2DG uptake. These foci tend to overly small groups of glomeruli. There are unlabeled or lightly labeled glomeruli scattered within the overall region of increased functional activity.

Studies using the *in situ* hybridization method to look for the expression of presumed odorant receptor genes show that there are bands of expression running from anterior to posterior in the nose. This general distribution is similar to that reported here for *Lotus* and elsewhere for *Dolichos* (Key and Akeson, 1993). Detailed examination of labeling within a zone reveals that all cells do not express the receptor mRNA. Labeled cells appear to be scattered throughout the zone but $<1\%$ of cells appear to be involved (Ressler *et al.*, 1993). The fraction of cells labeled by DBA and *Lotus* lectins appears to be much larger. In addition, both of these lectins label widely scattered cells throughout the epithelium, which is not the case for the gene expression studies. These discrepancies suggest that the subpopulations of receptor cells identified by these two lectins are different from those identified as expressing receptor mRNAs of the same subfamily.

The monoclonal antibody methods yield a third kind of pattern. All receptor cells within a broad region of the olfactory epithelium appear to labeled by this method (Fujita, Mori *et al.*, 1985; Schwob and Gottlieb, 1986). The axons of these cells can be traced to the olfactory bulb, where they appear to terminate with uniform density across their terminal field, although a mosaic pattern of staining may be observed at the periphery of the fields.

In summary, previous studies, using tract tracing methods, have shown that small regions of the olfactory bulb receive input from a large proportion of the olfactory epithelium (Stewart *et al.*, 1979; Clancy *et al.*, 1985; Astic and Saucier, 1986; Pedersen *et al.*, 1986; Astic *et al.*, 1987; Stewart and Pedersen, 1987). The present study provides evidence that biochemically identified subpopulations of receptor cells, scattered over a large region of the epithelium, do not project diffusely onto the olfactory bulb. They project to glomeruli within a broad region of the olfactory bulb, which are interspersed with glomeruli that do not appear to be targets.

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