Chem. Senses 23: 477–481, 1998

Heterogeneity in the Accessory Olfactory System

Mimi Halpern, Lena Shnayder Shapiro and Changping Jia

Department of Anatomy and Cell Biology and Program in Neural and Behavioral Science, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203, USA

Correspondence to be sent to: Dr Mimi Halpern, Department of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, NY 11203, USA. e-mail: mhalpern@netmail.hscbklyn.edu

Abstract

The mammalian accessory olfactory bulb (AOB) is chemoarchitecturally heterogeneous in that it stains differentially with a number of markers; the receptor cells that project to the AOB are similarly heterogeneous. What is the significance of this heterogeneity? We have found that the AOB of the gray, short-tailed opossum, *Monodelphis domestica*, stains differentially with a number of 'markers': antibodies to olfactory marker protein (OMP) and the α subunit of the G protein G_{i2}, the lectin of *Vicia villosa* and NADPH-diaphorase. These markers stain the rostral AOB more strongly than the caudal AOB whereas, the G protein subunit $G_{o\alpha}$ is located predominantly in the posterior subdivision of the AOB. This heterogeneity in the chemoarchitecture of the AOB may reflect a fundamental organizational dichotomy within the vomeronasal system that corresponds to a functional dichotomy. The vomeronasal sensory epithelium also exhibits a chemoarchitectural heterogeneity: receptor cells in the basal third are $G_{o\alpha}$-immunoreactive whereas the cells in the middle third are $G_{i2\alpha}$-immunoreactive. Tracing studies using WGA-HRP demonstrate that the neurons in the middle third of the vomeronasal sensory epithelium project their axons to the anterior AOB whereas those in the basal third appear to project to the posterior AOB.

The main olfactory and accessory olfactory systems are represented in the periphery by sensory epithelia containing bipolar neurons that terminate in glomeruli of the main (MOB) and accessory (AOB) olfactory bulbs respectively. One of the prominent current hypotheses concerning chemosensory coding in the main olfactory system proposes that sensory receptor cells with different complements of receptor proteins on their distal processes send specific projections to glomeruli localized in particular sectors of the MOB. In the vomeronasal system, where the glomeruli of the AOB are less distinct, no mechanism of coding similar to that in the main olfactory system has been proposed.

It is generally believed that stimulus coding in the main olfactory system is a reflection of the topographical relationship between the receptor cells of the olfactory epithelium and the glomeruli of the MOB where their axons terminate (Figure 1). However, it has remained a contentious issue whether the glomeruli of the MOB represent a locus for the segregation of information derived from primary sensory neurons responding to distinct odorants or classes of odorants (Kauer, 1980, 1987; Kauer *et al.*, 1994; Schoenfield and Buck, 1994). One approach to this issue is to determine if there are clear differences in the olfactory bulb glomeruli that are reflective of differences in groups of receptor cells.

Characterization of biochemically defined subsets of olfactory neurons may result in identification of neuronal subsets with similar sensitivity spectra to odorants (Schwob, 1992). In rat and mouse olfactory systems bipolar neurons expressing distinct putative receptors are segregated topographically, to a certain degree, in the epithelium (Buck, 1993; Ressler *et al.*, 1993; Vassar *et al.*, 1993). However, within each topographic zone several different types of putative receptors may be expressed and the neurons expressing these different receptors are distributed, apparently at random, within the circumscribed zone. Thus, in the olfactory epithelium a partial pattern of spatial segregation based on receptor type is present (partial topographic correspondence; Figure 1). However, the role of this spatial pattern for the encoding of particular odor stimuli has yet to be determined.

In recent years a chemoarchitectural heterogeneity has been observed in main and accessory olfactory systems in both receptor cells and the nerve/glomerular layers of their respective bulbs. The heterogeneity in the accessory olfactory bulbs of rat, mouse, rabbit, hamster and opossum has been demonstrated with monoclonal antibodies raised to specific carbohydrate moities (Mori *et al.*, 1987; Schwarting and Crandall, 1991; Schwarting *et al.*, 1992 a,b, 1994), lectin staining (Takami *et al.*, 1992; Taniguchi *et al.*, 1993; Shapiro *et al.*, 1995a), antibodies to G proteins

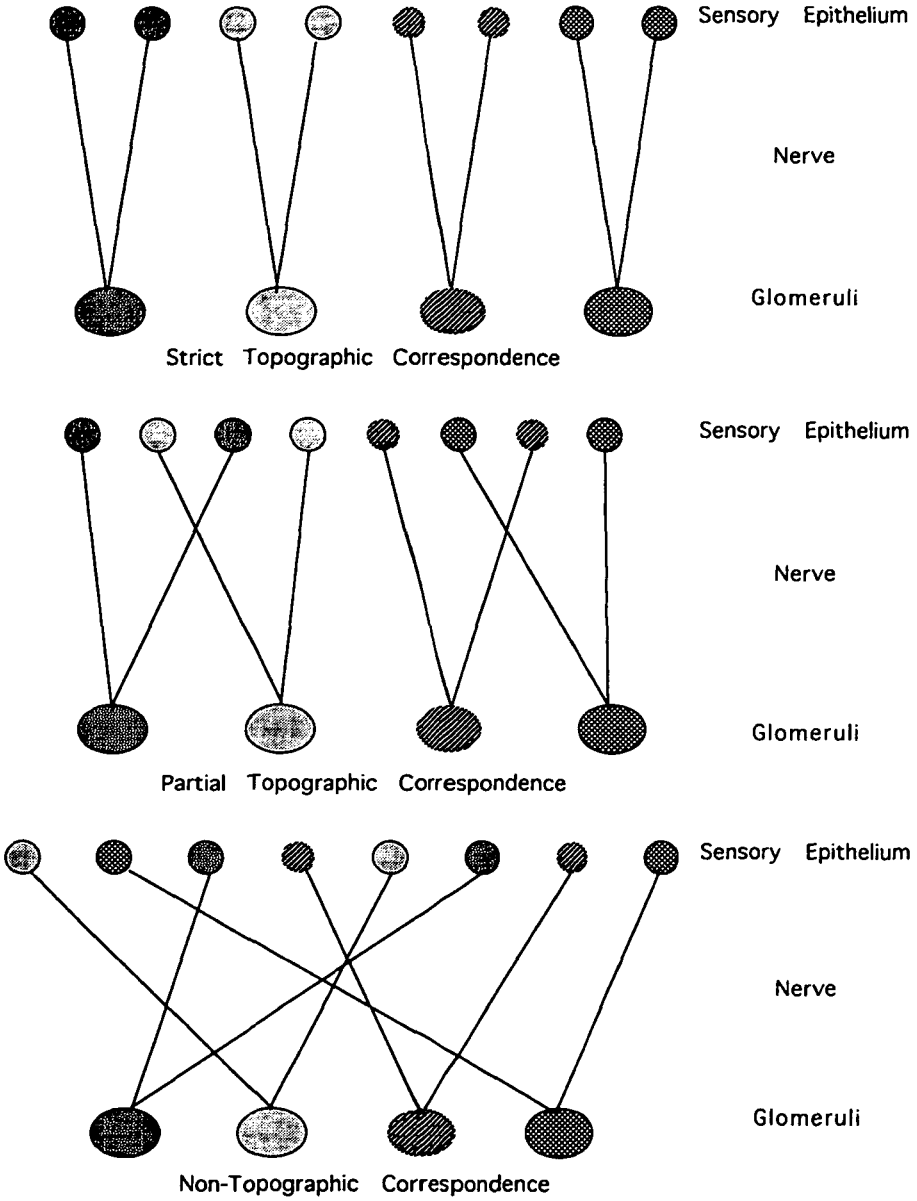

Figure 1 Three models of topographic correspondence between chemically identified cells in the sensory epithelium of chemoreceptive organs and their termination sites in glomeruli of the main or accessory olfactory bulb.

(Shinohara *et al.*, 1992; Halpern *et al.*, 1995), antibodies to olfactory marker protein (Shnayder *et al.*, 1993) and NADPH diaphorase histochemistry (Shnayder *et al.*, 1994; Halpern and Jia, 1995). Most recently, in rat, mouse and opossum two populations of vomeronasal receptor cells have been identified using antibodies to G proteins (Halpern *et al.*, 1995; Jia and Halpern, 1995a,b, 1996; Berghard and Buck, 1996) and each of these two populations appear to project to one of the two chemoarchitecturally defined subdivisions of the accessory olfactory bulb (Shapiro *et al.*, 1995b; Jia and Halpern, 1995a,b, 1996). In addition, an *in situ* hybridization study using probes to $G_{\alpha i2}$ and $G_{\alpha o}$ confirmed the immunohistochemical findings of differential localization of receptor neurons expressing these proteins in the vomeronasal sensory epithelium (Berghard and Buck, 1996).

This chemoarchitectural heterogeneity in the opossum AOB and VNO is illustrated for the G proteins $G_{\alpha i2}$ and $G_{\alpha o}$ in Figure 2. We conclude from these observations, as well as those of Jia and Halpern (1996) and Berhard and Buck (1996) in the mouse, that the vomeronasal organ contains at least two types of receptor neurons located in different sublayers of the receptor cell layer. The axons of these cells intermingle in the vomeronasal nerve but become

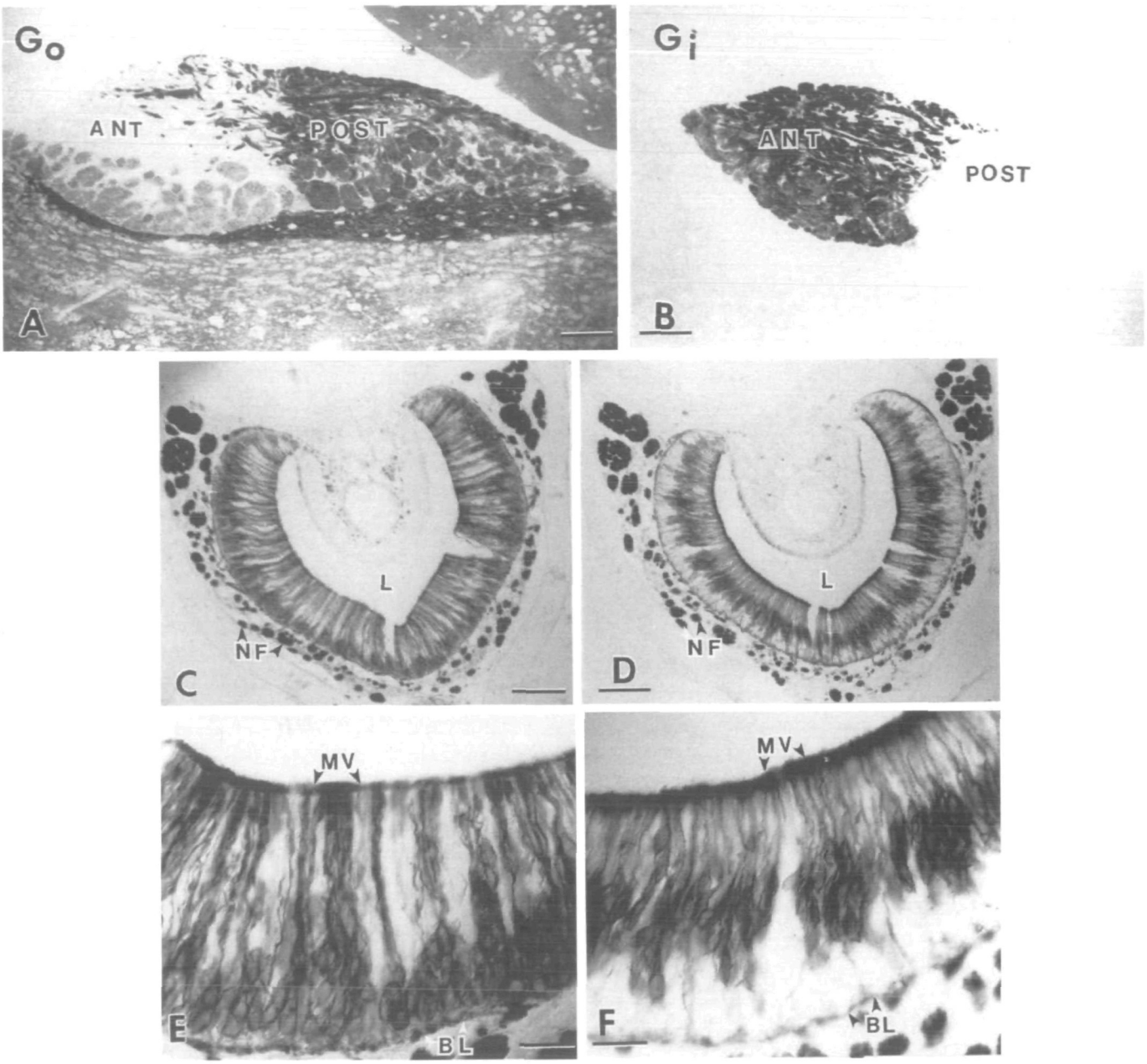

Figure 2 Photomicrographs of sections through the accessory olfactory bulb **(A** and **B)** and vomeronasal organ **(C, D, E** and **F)** of grey short-tailed opossums stained with antibodies to G_o (A, C and E) and G_i (B, D and F) proteins. Ant = anterior AOB; Post = posterior AOB; L= lumen of vomero- nasal organ; NF = vomeronasal nerve fiber bundles; MV = microvillar border of sensory epithelium; BL = basal lamina of sensory epithelium. Bar = 80 μm in A, B, C and D; 20 μm in E and F.

segregated at the level of the accessory olfactory bulb, where they terminate in distinct subdivisions of that structure (Figure 3).

What is the potential significance of this duality in the accessory olfactory system? It is clearly an anatomical substrate for maintaining separate channels of information from the periphery to more central structures. However, if it is the anatomical basis for sensory coding then we are in need of a demonstration that different chemical signals activate the separate channels. The heterogeneity may represent use of different signal transduction mechanisms within the system. Certainly the universal demonstration, among mammals so far studied, that G proteins are differentially expressed in the two parts of the vomeronasal system supports this notion, as does the demonstration that βNADPH diaphorase reactivity is differentially present in the vomeronasal systems of some mammals. Another possibility is that the differential expression may be related to axon–substrate interactions that are involved in axonal guidance and matching of receptor cells with their

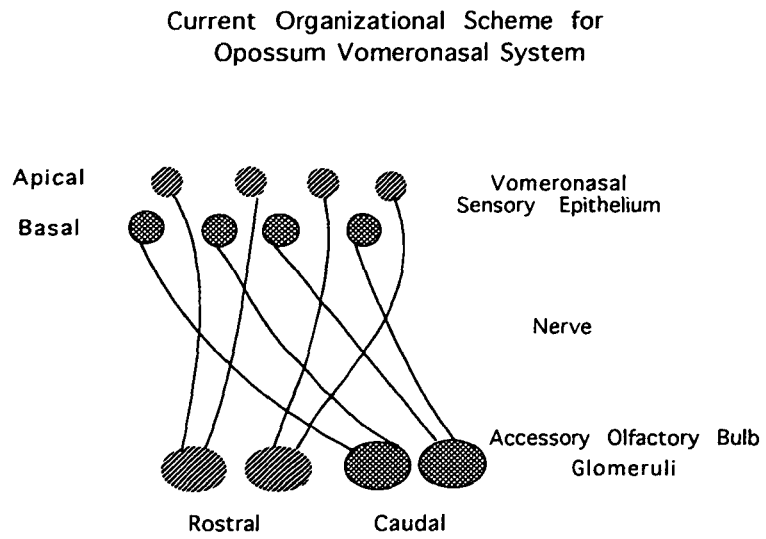

Figure 3 Current model of topographic correspondence between the chemically identified cells in the vomeronasal epithelium and the glomeruli of the accessory olfactory bulb.

appropriate targets in the AOB. For example, the two G proteins may be involved in pathway finding and target selection. G_i and G_o, both sensitive to PTX, have been implicated in neurite-promoting functions of cell adhesion molecules (Doherty *et al.*, 1991; Strittmatter *et al.*, 1991).

Comparisons among mammals of the chemoarchitectonic heterogeneity within the accessory olfactory system reveal some striking similarities and differences (Table 1). For example, whereas G_o is localized in the posterior AOB and G_i is localized in the anterior AOB in the rat, mouse and opossum (Shinohara *et al.*, 1992; Halpern *et al.*, 1995; Jia and Halpern, 1995a,b, 1996), the pattern of lectin staining (Takami *et al.*, 1992; Taniguchi *et al.*, 1993; Shapiro *et al.*, 1995a), olfactory marker protein localization and the pattern of NADPH diaphorase reactivity differs in these three groups of mammals (Davis, 1991; Porteros *et al.*, 1994; Shnayder *et al.*, 1994; Halpern and Jia, 1995). Do these differences between species represent fundamental differences in the organization of the accessory olfactory system or do they represent different methods for solving similar problems of sensory information transduction, coding and segregation? These issues are at present unresolved.

Acknowledgements

This work was supported by a grant from the NIDCD #DC02745 awarded to M.H.

References

Berghard, A. and **Buck, L.B.** (1996) *Sensory transduction in vomeronasal neurons: evidence for G_{ao}, G_{ai2} and adenylyl cyclase II as major components of a pheromone signal cascade.* J. Neurosci., 16, 909–918.

Buck, L.B. (1993) *Receptor diversity and spatial patterning in the mammalian olfactory system.* In Chadwick, E., Marsh, J. and Goode, J. (eds), The Molecular Basis of Smell and Taste Transduction. Wiley, Chichester, CIBA Foundation Symposium 179, pp. 51–67.

Davis, B.J. (1991) *NADPH-diaphorase activity in the olfactory system of the hamster and rat.* J. Comp. Neurol., 314, 493–511.

Doherty, P., Ashton, S.V., Moore, S.E. and **Walsh, F.S.** (1991) *Morpho-regulatory activities of NCAM and N-dadherin can be accounted for by G protein-dependent activation of L- and N-type neuronal Ca^{2+} channels.* Cell, 67, 21–33.

Halpern, M. and **Jia, C.** (1995) *Differential localization of NADPH-diaphorase activity in the mouse vomeronasal system.* Abstr. Soc. Neurosci., 21, 1181.

Halpern, M., Shapiro, L.S. and **Jia, C.** (1995) *Differential localization of G proteins in the opossum vomeronasal system.* Brain Res., 677, 157–161.

Jia, C.-P. and **Halpern, M.** (1995a) *Differential expression of G proteins in the vomeronasal system of the opossum (*Monodelphis domestica*).* Chem. Senses, 20, 714.

Jia, C. and **Halpern, M.** (1995b) *Differential expression of G proteins in subclasses of vomeronasal receptor neurons and their separate terminations in the accessory olfactory bulb of the rat and mouse.* Abstr. Soc. Neurosci., 21, 1181.

Jia, C. and **Halpern, M.** (1996) *Subclasses of vomeronasal receptor neurons: differential expression of G proteins (G_{ia2} and G_{oa}) and segregated projections to the accessory olfactory bulb.* Brain Res., 719, 117–128.

Kauer, J.S. (1980) *Some spatial characteristics of central information processing in the vertebrate olfactory pathway.* In van der Tarre, H. (ed.), Olfaction and Taste VII. IRL Press, London, pp. 227–236.

Kauer, J.S. (1987) *Coding in the olfactory system.* In Finger, T.E. and Silver, W.L. (eds), Neurobiology of Taste and Smell. John Wiley & Sons, New York, pp. 205–231.

Kauer, J.S., White, J., Wellis, D.P. and **Cinelli, A.R.** (1994) *Observations and modeling of distributed processing in the olfactory system.* In Kurihara, K., Suzuki, N. and Ogawa, H. (eds), International Symposium on Olfaction and Taste XI. Springer-Verlag, Tokyo, pp. 433–439.

Kream, R.M., Davis, B.J., Kawano, T., Margolis, F.L. and **Macrides, F.** (1984) *Substance P and catecholaminergic expression in neurons of the hamster main olfactory bulb*. J. Comp. Neurol., 222, 140–154.

Monti Graziadei, G.A., Stanley, R.S. and **Graziadei, P.P.C.** (1980) *The olfactory marker protein in the olfactory system of the mouse during development*. Neuroscience, 5, 1239–1252.

Mori, K., Imamura, K., Fujita, S.C. and **Obata, K.** (1987) *Projections of two subclasses of vomeronasal nerve fibers to the accessory olfactory bulb in the rabbit*. Neuroscience, 20, 259–278.

Porteros, A., Alonso, J.R., Arevalo, R., Garcia-Ojeda, E., Crespo, C. and **Aijon, J.** (1994) *Histochemical localization of NADPH-diaphorase in the rat accessory olfactory bulb*. Chem. Senses, 19, 413–424.

Ressler, K.J., Sullivan, S.L. and **Buck, L.B.** (1993) *A zonal organization of odorant receptor gene expression in the olfactory epithelium*. Cell, 73, 597–609.

Schoenfield, T. and **Buck, L.** (1994) Symposium on Spatial Coding: Molecules to Behavior. AChems XVI, Sarasota, FL.

Schwob, J.E. (1992) *The biochemistry of olfactory neurons: stages of differeentiation and neuronal subsets*. In Serby, M.J. and Choba, K.L. (eds), Science of Olfaction. Springer-Verlag, New York, pp. 80–125.

Schwarting, G.A. and **Crandall, J.E.** (1991) *Subsets of olfactory and vomeronasal sensory epithelial cells and axons revealed by monoclonal antibodies to carbohydrate antigens*. Brain Res., 547, 239–248.

Schwarting, G.A., Deutsch, G., Gattey, D.M. and **Crandall, J.E.** (1992a) *Glycoconjugates are stage- and position-specific cell surface molecules in the developing olfactory system. 1. The CC1 immunoreactive glycolipid defines a rostrocaudal gradient in the rat vomeronasal system*. J. Neurobiol., 23, 120–129.

Schwarting, G.A., Deutsch, G., Gattey, D.M. and **Crandall, J.E.** (1992b) *Glycoconjugates are stage- and position-specific cell surface molecules in the developing olfactory system. 2. Unique carbohydrate antigens are topographic markers for selective projection patterns of olfactory axons*. J. Neurobiol., 23, 130–142.

Schwarting, G.A., Drinkwater, D. and **Crandall, J.E.** (1994) *A unique neuronal glycolipid defines rostrocaudal compartmentalization in the accessory olfactory system of rats*. Dev. Brain Res., 78, 191–200.

Shapiro, L.S., Ee, P.-L. and **Halpern, M.** (1995a) *Lectin histochemical identification of carbohydrate moieties in opossum chemosensory systems during development, with special emphasis on VVA-identified subdivisions in the accessory olfactory bulb*. J. Morphol., 224, 331–349.

Shapiro, L.S., Li, C-S., Jia, C.-P. and **Halpern, M.** (1995b) *Histochemical, immunocytochemical and tract tracing studies investigating the heterogeneity of the primary vomeronasal pathway in the Brazilian gray short-tailed opossum,* Monodelphis domestica. Chem. Senses, 20, 777–778.

Shinohara, H., Asano, T. and **Kato, K.** (1992) *Differential localization of G-proteins G_i and G_o in the accessory olfactory bulb of the rat*. J. Neurosci., 12, 1275–1279.

Shnayder, L., Schwanzel-Fukuda, M. and **Halpern, M.** (1993) *Differential OMP expression in opossum accessory olfactory bulb*. NeuroReport, 5, 193–196.

Shnayder, L., Rook, M. and **Halpern, M.** (1994) *NADPH-diaphorase histochemistry in the nasal chemosensory systems of immature and adult opossum*. Chem. Senses, 19, 544–545.

Strittmatter, S.M., Vanenzuela, D., Vartanian, T., Sudo, Y., Zuber, M.X. and **Fishman, M.C.** (1991) *Growth cone transduction: G_o and GAP-43*. J. Cell Sci. Suppl., 15, 27–33.

Takami, S., Graziadei, P.P.C. and **Ichikawa, M.** (1992) *The differential staining patterns of two lectins in the accessory olfactory bulb of the rat*. Brain Res., 598, 337–342.

Taniguchi, K., Nii, Y. and **Ogawa, K.** (1993) *Subdivisions of the accessory olfactory bulb, as demonstrated by lectin-histochemistry in the golden hamster*. Neurosci. Lett., 158, 185–188.

Vassar, R., Ngai, J. and **Axel, R.** (1993) *Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium*. Cell, 74, 309–318.

Accepted March 18, 1997