Chem. Senses 25: 199–205, 2000

Rewiring the Olfactory Bulb: Changes in Odor Maps following Recovery from Nerve Transection

Richard M. Costanzo

Department of Physiology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA 23298-0551, USA

Correspondence to be sent to: Richard M. Costanzo, Department of Physiology, PO Box 980551, Virginia Commonwealth University, Richmond, VA 23298-0551, USA. e-mail: rcostanz@hsc.vcu.edu

Abstract

Recent studies have shown that axons from olfactory receptor subtypes converge onto glomeruli in fixed positions within the olfactory bulb. Different receptor subtypes project to different glomeruli, forming a spatial distribution of odor information or 'odor maps'. Olfactory receptor neurons are continuously replaced throughout the life span of an animal, yet they preserve this highly localized mapping of receptor subtypes. In this study we used a transgenic mouse (P2-*IRES*-tau–lacZ) to map axons from a single receptor subtype (P2 receptors) in order to determine if regenerating axons were able to re-establish the P2 receptor map following nerve transection. Results confirm that P2 receptor axons retain their capacity to grow back to the olfactory bulb and converge onto glomeruli following nerve transection. However, the location and number of convergence sites was significantly altered compared to the control map. This change in the spatial distribution of axons alters the topography of odor mapping and has important implications for the processing of olfactory information. Findings from this study may explain why animals recovering from nerve injury require odor training before odor discrimination is restored. Future studies of olfactory receptor mapping could prove helpful in planning strategies to rewire connections in the brain and to restore function following injury or neurological disease.

Introduction

The processing of olfactory information begins with the detection of odorants by olfactory receptor neurons (ORNs). There are ~1000 different receptor subtypes distributed in four zones within the nasal epithelium (Buck and Axel, 1991; Ressler *et al.*, 1993; Strotmann *et al.*, 1994). ORNs send axons to the olfactory bulb (OB) where they establish synaptic connections with second-order neurons in structures called glomeruli (Shepherd, 1972; Kosaka *et al.*, 1998). ORN subtypes expressing the same receptor protein are found within the same epithelial zone and converge onto two out of ~1800 glomeruli in the mouse bulb. One of these two glomeruli is located on the medial side of the bulb, receiving input from the medial surface of the nasal cavity; the other is located laterally, receiving its input from the lateral surface. (Royet *et al.*, 1988; Ressler *et al.*, 1994; Vassar *et al.*, 1994; Mombaerts, 1996). This arrangement of converging axons from the 1000 different ORN subtypes results in the formation of 'odor maps' in the OB. These odor maps are thought to play an essential role in odor quality discrimination (Mombaerts, 1996; Bozza and Kauer, 1998; Wang *et al.*, 1998).

Olfactory receptor neurons are capable of continuous neurogenesis and replacement through out the life span of an animal (Graziadei and Monti Graziadei, 1977). They are also replaced in response to chemical lesions or nerve injury. During recovery from injury, replacement neurons grow new axons and re-establish connections with the OB (Graziadei and Monti Graziadei, 1980). Physiological studies have shown that the replacement neurons restore function in both the sensory epithelium (Simmons and Getchell, 1981a) and the OB (Simmons and Getchell, 1981b; Costanzo, 1985). Behavioral experiments have shown that the ability to make odor discriminations is restored within 40 days of recovery, although additional training or relearning is required to bring performance back to control levels (Yee and Costanzo, 1995, 1998).

What are the mechanisms underlying recovery of olfactory function after nerve injury? It has been shown that regenerated axons have the capacity to grow back to the OB and re-establish functional synapses with second-order mitral cells (Costanzo, 1985). But are the normal projection patterns (odor maps) restored during recovery? And do axons from receptor subtypes project back to the same glomeruli? To address these questions we conducted nerve regeneration experiments using a genetically altered strain of mice (P2-*IRES*-tau–lac-Z) (Mombaerts, 1996). With this strain of mice, it is possible to label axons from a single receptor subtype (P2 receptors) and to trace the P2 axons to

their projection sites in the OB. Following recovery from nerve transection, P2 axons projected to and converged within individual OB glomeruli. However, the number and location of these glomeruli in the rewired bulb differed from those observed in controls. These findings demonstrate that the axon projections, or odor maps, are altered during recovery from nerve transection. This may explain why additional training is required to restore olfactory discrimination after recovery from nerve injury.

Materials and methods

Animals

Experiments were performed using a transgenic strain of mice (P2 *IRES*-tau–lacZ) obtained from the laboratory of Dr Richard Axel (Howard Hughes Medical Institute, Columbia University, NY). These mice have been genetically altered so that olfactory receptor neurons expressing the P2 receptor subtype also express a tau–lacZ fusion protein. This makes it possible to visualize individual receptor neurons and their axon projections to the OB (Mombaerts *et al.*, 1996; Wang *et al.*, 1998). By tagging the P2 receptor cells we can visualize the normal convergence of axons mapping onto a single glomerulus in a fixed position in control bulbs, and detect changes in the map that occur following recovery from nerve transection. The P2 mouse provides an excellent animal model for investigating axon replacement and mechanisms of sensory mapping in the brain.

Nerve transection

Adult mice (>4 weeks of age) were anesthetized with 70 mg/kg of sodium pentobarbitol and then the dorsal surface of the left OB was exposed. A small Teflon blade was used in a surgical procedure (left olfactory nerve transection, LNTX) to cut all nerve fibers emerging from the left nasal cavity that project to the left OB. Nerve transection was accomplished by inserting the blade down along the medial and ventral surface of the left bulb, across the anterior and ventral surface and then along the lateral surface of the bulb. Manipulation of the bulb with a small jet stream of saline solution improved visualization and access for blade insertion. The right OB and associated nerve fibers were left intact and served as an internal control for all LNTX animals. Upon completion of the LNTX procedure, the skin incision was sutured closed. Animals were returned to their home cage after they had fully recovered from the anesthesia. LNTX animals (n = 8) were examined after recovery periods of 2.5 (n = 2) and 4.5 (n = 6) months. Control animals (n = 5) and surgical shams (n = 2) were also included in the study.

Whole mount staining

Animals were killed by exposure to CO_2 followed by rapid decapitation. Skin and soft tissue were removed from the skull. The heads were carefully dissected down the midline producing separate tissue samples for the right (control) and left (LNTX) sides. Tissue was fixed for 30 min on ice with 4% paraformaldehyde fix, washed at room temperature with buffer A [100 mM phosphate buffer (pH 7.4), 2 mM $MgCl_2$ and 5 mM EGTA] once for 5 min and then a second time for 25 min. This was followed by two 5 min washes with buffer B [100 mM phosphate buffer (pH 7.4), 2 mM $MgCl_2$, 0.01% sodium desoxycholate, and 0.02% Nonidet P40]. The blue XGal reaction was generated by overnight exposure in the dark to buffer C (buffer B, with 5 mM potassium ferricyanide, 5 mM potassium-ferrocyanide and 1 mg/ml of Xgal). This reaction was stopped by two 5 min washes in phosphate buffer. The tissue was then transferred to cold fix solution and stored in the refrigerator. Whole mount tissue was examined using a Wild M400 photomicroscope. Photographic images of the medial surface of the bulb and nasal cavity were obtained using Kodak 160T Elite chrome slide film. The slides were digitized using a slide scanner and composite images were then arranged using Adobe Photoshop v. 5.0.

Results

P2 convergence in control animals

On the medial surface of both the right and left OB of control animals we consistently observed the convergence of P2 receptor axons onto a single fixed locus. This was also seen on the control side (right OB) of LNTX animals. Figure 1A shows a whole mount preparation from a control P2 mouse. The dark blue stain reveals the projections of P2 axon fibers. Axons are seen coursing along the septal wall of the nasal cavity and then converging onto a fixed locus on the medial surface of the bulb. Figure 1B shows the same preparation after removal of the septal wall and exposure of the turbinates located on the lateral wall of the nasal cavity. P2 axons from the lateral surface of the nasal cavity (turbinates) converge onto a fixed locus on the lateral surface of the bulb (not shown in Figure 1). At high magnification (Figure 1C) discrete bundles of P2 axons can be seen passing through openings in the cribriform plate. The cribriform plate defines the anterior wall of the mouse skull and separates the OB from the nasal cavity. After making contact with the OB, P2 axons begin to converge onto a single fixed position in the bulb.

P2 connections after recovery from LNTX

After 2 months of recovery, substantial reconnection of P2 axons was observed. Figure 2 shows examples of projection patterns for replacement axons following recovery at 2.5 and 4.5 months. After recovery from nerve transection, P2 axons projected to multiple loci and were widely distributed across the surface of the OB. In two experimental animals examined at 2.5 months recovery the termination of axons appeared to be diffuse (see Figure 2A). However, at

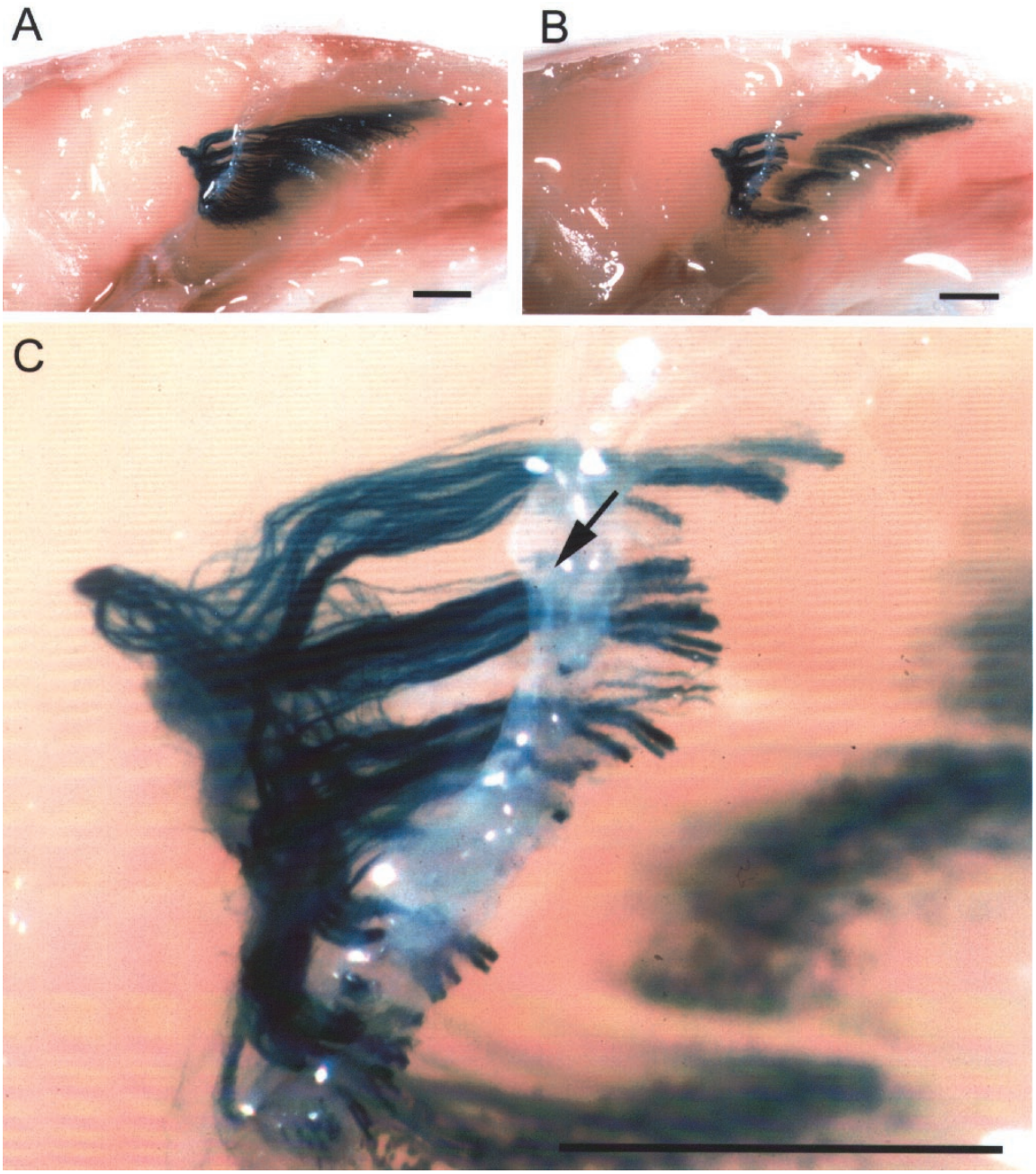

Figure 1 Mapping of the P2 receptor neurons in a control animal. Photographs of whole mount preparations showing the medial surface of the left OB and nasal cavity. The axons from P2 odorant receptor subtypes all converge onto a single fixed position in the OB. **(A)** P2 axons are seen coursing along the septal wall of the intact nasal cavity. **(B)** After removal of the septal wall the zonal distribution of P2 receptors can be seen on the lateral wall (turbinates) of the nasal cavity. **(C)** At high magnification P2 axon bundles can be seen passing through openings in the cribriform plate (arrow). After making contact with the OB, P2 axons converge onto a single loci (glomerulus). Calibration bars (A–C), 1 mm.

the longer recovery times (4.5 months) axons frequently converged and terminated in discrete loci, often forming a small spherical knob (Figure 2B–D). On the control side of experimental animals (not shown in Figure 2), P2 axons routinely converged onto a single fixed loci on the medial surface of the right bulb. The control side showed normal P2 axon mapping and confirmed that the surgical lesion was confined to the left OB. Although regenerated axons on the lesion side (left bulb) also showed convergence onto discrete loci, they did not converge to the normal P2 glomeruli. Instead they projected to multiple loci distributed in different regions of the bulb. These data demonstrate that the new axon connections established following recovery from LNTX do not restore the original P2 map.

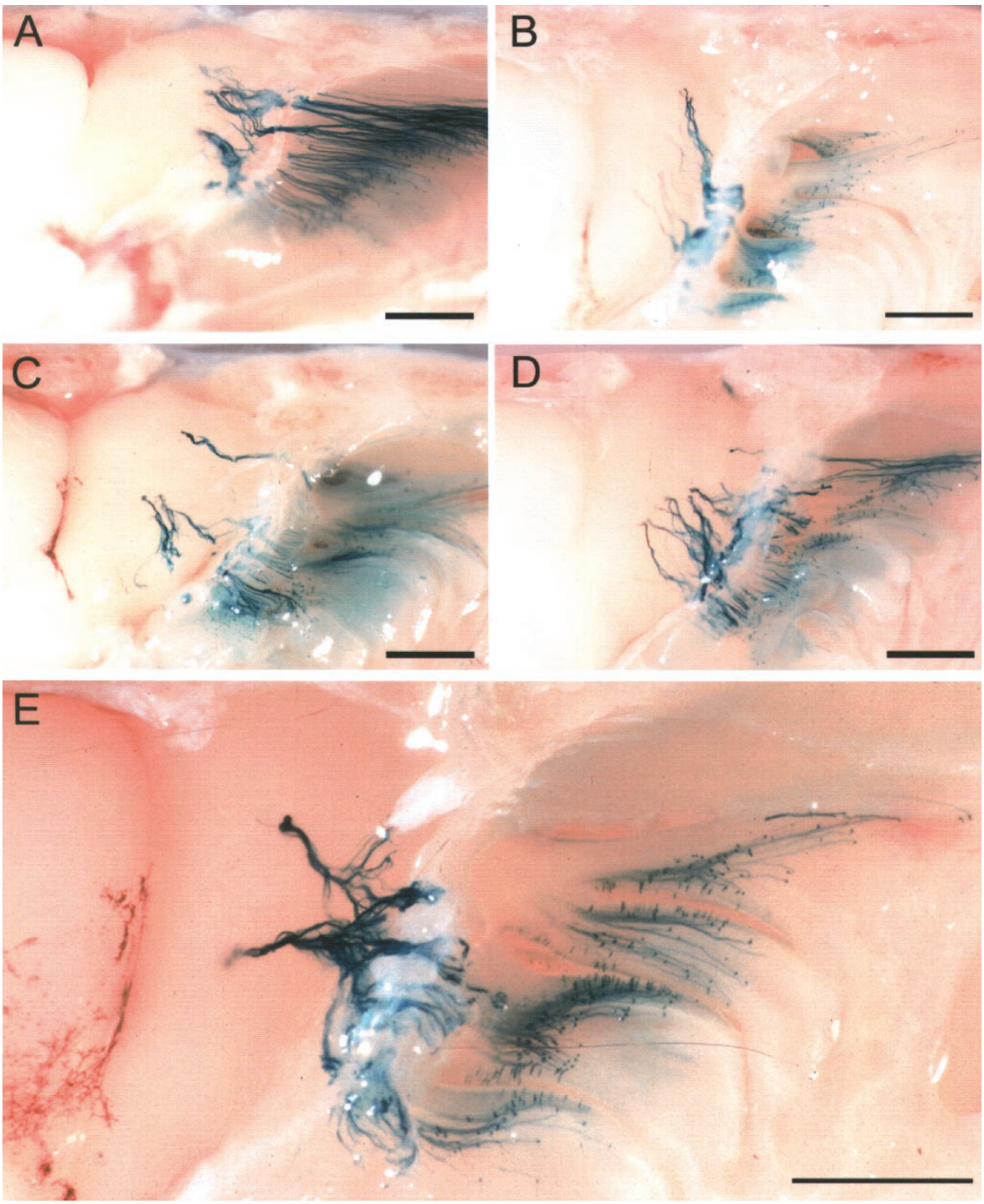

Figure 2 Changes in P2 mapping after recovery from LNTX surgery. **(A)** After 2.5 months (76 days) of recovery, axons projected to multiple locations in the bulb and their termination appeared to be diffuse. **(B–D)** P2 axon projections at 4.5 months (131–134 days) recovery. In these animals replacement axons converge onto multiple sites covering a wide range of bulb locations and the axon terminations were more discrete, often forming small ball-like structures. **(E)** In this animal the normal P2 target tissue (anterior third of the bulb) was removed at the time of LNTX surgery. After 4.5 months of recovery P2 axons were able to grow back to posterior regions of the bulb, where they converged onto two discrete loci. These data demonstrate that P2 receptor convergence does not require a specific glomerulus located in a fixed position in the bulb.

In two animals, the target area of the bulb containing the P2 convergence site was removed at the time of the LNTX procedure. The result from one of these animals is illustrated in Figure 3E. Removal of the anterior third of the bulb in this animal assured complete removal of the P2 convergence site (target glomerulus) and associated axon bundles located within the nerve layer of the bulb. After a recovery period of 4.5 months, P2 axons exhibited a remarkable amount of convergence, even in the absence of the normal target tissue. Surprisingly, the removal of a significant amount of bulb tissue did not prevent new axons from reaching the OB. P2 axons retained their ability to converge onto small discrete loci, but convergence was now in a new (more posterior) region of the bulb. This high degree of convergence in the absence of the target P2 glomerulus demonstrates that a specific target site or fixed location is not required for axon convergence. In addition, P2 axon fibers exhibited the capacity to travel considerable distances in the remaining bulb before they terminated onto small localized regions (glomeruli). These data confirm that the presence of a specific P2 target glomerulus is not needed for the convergence of P2 axons, and that olfactory axons demonstrate a remarkable capacity to re-innervate the OB even when there is a significant loss of bulb tissue.

Discussion

P2 convergence is maintained after injury

Experiments were designed to address the question of whether or not regenerating P2 receptors have the capacity to restore the original P2 projection map after recovery from nerve injury. The results demonstrate that after recovery from LNTX, axons from the P2 receptors do re-establish connections with the OB. However, these connections do not map to a single P2 locus (Figure 2). Significant changes in the P2 mapping were observed. Regenerated axons produced a much wider distribution of projections across multiple regions of the OB. Nevertheless, the convergence of P2 axons onto individual glomeruli was observed. Thus, P2 axon convergence was preserved during recovery, even though the location of the target sites was significantly altered. These data suggest that convergence of ORN subtypes is an intrinsic property of P2 receptors, but that the termination sites of converging axons can change. Such findings are consistent with other studies that attribute axon convergence and guidance in part to the receptor subtypes (Bozza and Kauer, 1998; Bulfone *et al.*, 1998; Wang *et al.*, 1998). When an odorant receptor is deleted from a neuron using gene targeting strategies, convergence is not observed and axons appear to wander rather than converge onto a single glomerulus (Wang *et al.*, 1998). In receptor substitution experiments ORN axons continue to converge onto glomeruli but the location of convergence is shifted, suggesting that while odorant receptors play an instructive role in target selection they are not the sole determinants in the guidance of axons to their target (Wang *et al.*, 1998). It is likely that the mechanisms of axonal guidance responsible for the convergence of olfactory receptor neurons involve multiple factors.

What are the functional consequences of nerve transection and the subsequent changes in the mapping of olfactory receptor neurons? Why isn't the map restored? Injury to the olfactory nerves, such as that produced by the LNTX procedure, is a traumatic event. The surgical transection of nerve fibers results in a disruption of supporting structures and blood vessels. Schwann cells and glia cells provide the underlying scaffolding along which axons grow as they project from the epithelium to their targets in the OB. These structures are established during development along with the initial growth of receptor cell axons. It is likely that the alignment and integrity of axonal supporting structures is significantly disrupted by the LNTX procedure. During recovery, replacement axons may not have access to many of these path-finding or guidance structures. This could explain why axons end up projecting into new regions of the OB. In contrast to nerve transection, chemical lesions such as exposure to ZN_2SO_4 or methyl bromide gas are likely to lesion receptor neurons and leave the underlying supporting structures intact (Stewart *et al.*, 1983; Burd, 1993; Schwob *et al.*, 1995). It is conceivable that with chemical lesions many of the supporting matrixes would remain intact and provide a substrate for axons to restore the P2 map. Future studies comparing recovery after chemical lesion may provide some answers concerning the importance of supporting cells.

At 2.5 months recovery (Figure 2A) the termination of P2 axons did not appear to be as focused as that observed at 4.5 months (Figure 2B–E). It is conceivable that odor exposure and functional activity within the bulb are prerequisites for axon convergence. Axons may initially attempt to establish connections with many different glomeruli during recovery. However, restoration of functional connections and permanent synapse formation may require a critical period of odor exposure. Thus, projections would appear more diffuse when they first reach the bulb, but would become focused as functional synapses are formed. Axons that do not experience functional activity would be pruned back, as is thought to occur during normal olfactory development.

Significance of the altered map

This study demonstrates that odor receptor mapping is significantly altered following nerve injury. What are the functional consequences of changing the odor maps? Is it necessary for receptor neurons to project to specific, fixed locations in the OB? Or can the olfactory system encode information using a new map?

Behavioral studies of animals recovering from olfactory nerve transection have shown that it is possible to restore odor discrimination even if receptor mapping onto the OB is

significantly altered (Koster and Costanzo, 1996; Yee and Costanzo, 1998). This restoration of function, however, requires additional odor training. Animals must first relearn the identification of odors before they can again perform odor discrimination tasks. These findings suggest that if the odor maps are altered following recovery, animals can learn to adapt to new patterns of neural activity. A similar observation has been made in patients recovering from traumatic head injury, who as a result of their injury have impaired olfactory function (Costanzo *et al.*, 1995). These patients often report that odors produce unique and different sensations, but they cannot identify these odors. After feedback and training, the patient is often able to identify the odor as being different from other odors and in many cases can identify the odor by associating it with its unique sensation. However, patients insist that the odor 'does not smell the way it used to' and typically it has an unpleasant association. These clinical findings are consistent with the hypothesis that odor receptor projection mapping is significantly altered following olfactory nerve injury. Even though it is possible to learn to adapt to the changes in the way odors are mapped onto the brain, it is unlikely that the odor sensations will be restored to normal. What can be restored is the ability to discriminate between different odor sensations and the ability to perform simple odor dependent tasks. This may require odor training or conditioning, but restoration of some odor discrimination function is possible. Complete recovery or the return of normal odor sensations may require restoration of the original odor maps. Future research using the olfactory system as a model for neural regeneration may lead to the development of strategies to restore sensory maps and to rewire the brain following injury or neurological disease.

Acknowledgements

We thank Dr Frank Margolis for his generous gift of antibodies to OMP, Richard Axel and his colleagues for providing the P2 mice, and Steven Chao and Fan Wang for help with the P2 whole mount methods and their participation in preliminary experiments. This work was supported by National Institute on Deafness and Other Communication Disorders, NIH Grant DC00165 to R.M.C.

References

Bozza, T.C. and **Kauer, J.S.** (1998) *Odorant response properties of convergent olfactory receptor neurons*. J. Neurosci., 18, 4560–4569.

Buck, L. and **Axel, R.** (1991) *A novel multigene family may encode odorant receptors: a molecular basis for odor recognition*. Cell, 65, 175–187.

Bulfone, A., Wang, F., Hevner, R., Anderson, S., Cutforth, T., Chen, S., Meneses, J., Pedersen, R., Axel, R. and **Rubenstein, J.L.** (1998) *An olfactory sensory map develops in the absence of normal projection neurons or GABAergic interneurons*. Neuron, 21, 1273–1282.

Burd, G.D. (1993) *Morphological study of the effects of intranasal zinc sulfate irrigation on the mouse olfactory epithelium and olfactory bulb*. Microsci. Res. Tech., 24, 195–213.

Costanzo, R.M. (1985) *Neural regeneration and functional reconnection following olfactory nerve transection in hamster*. Brain Res., 361, 258–266.

Costanzo, R.M., DiNardo, L.J. and **Zasler, N.D.** (1995) *Head injury and olfaction.* In Doty, R.L. (ed.), Handbook of Olfaction and Gustation. Marcel Dekker, New York, pp. 493–502.

Graziadei, P.P.C. and **Monti Graziadei, G.A.** (1977) *Continuous nerve cell renewal in the olfactory system.* In Jacobson, M. (ed.), Handbook of Sensory Physiology, Vol. IX: Development of Sensory Systems. Springer, Berlin, pp. 55–83.

Graziadei, P.P.C. and **Monti Graziadei, G.A.** (1980) *Neurogenesis and neuron regeneration in the olfactory system of mammals. III. Deafferentation and reinnervation of the olfactory bulb following section of the fila olfactoria in rat*. J. Neurocytol., 9, 145–162.

Kosaka, K., Toida, K., Aika, Y. and **Kosaka, T.** (1998) *How simple is the organization of the olfactory glomerulus? The heterogeneity of so-called periglomerular cells*. Neurosci. Res., 30, 101–110.

Koster, N.L. and **Costanzo, R.M.** (1996) *Electrophysiological characterization of the olfactory bulb during recovery from sensory deafferentation*. Brain Res., 724, 117–120.

Mombaerts, P. (1996) *Targeting olfaction*. Curr. Opin. Neurobiol., 6, 481–486.

Mombaerts, P., Wang, F., Dulac, C., Chao, S.K., Nemes, A., Mendelsohn, M., Edmondson, J. and **Axel, R.** (1996) *Visualizing an olfactory sensory map*. Cell, 87, 675–686.

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.

Ressler, K.J., Sullivan, S.L. and **Buck, L.B.** (1994) *A molecular dissection of spatial patterning in the olfactory system*. Curr. Opin. Neurobiol., 4, 588–596.

Royet, J.P., Souchier, C., Jourdan, F. and **Ploye, H.** (1988) *Morphometric study of the glomerular population in the mouse olfactory bulb: numerical density and size distribution along the rostrocaudal axis*. J. Comp. Neurol., 270, 559–568.

Schwob, J.E., Youngentob, S.L. and **Mezza, R.C.** (1995) *Reconstitution of the rat olfactory epithelium after methyl bromide-induced lesion*. J. Comp. Neurol., 359, 15–37.

Shepherd, G.M. (1972) *Synaptic organization of the mammalian olfactory bulb*. Physiol. Rev., 52, 864–917.

Simmons, P.A. and **Getchell, T.V.** (1981a) *Physiological activity of newly differentiated olfactory receptor neurons correlated with morphological recovery from olfactory nerve section in the salamander*. J. Neurophysiol., 45, 529–549.

Simmons, P.A. and **Getchell, T.V.** (1981b) *Neurogenesis in olfactory epithelium: loss and recovery of transepithelial voltage transients following olfactory nerve section*. J. Neurophysiol., 45, 516–528.

Stewart, W.B., Greer, C.A. and **Teicher, M.H.** (1983) *The effect of intranasal zinc sulfate treatment on odor-mediated behavior and on odor-induced metabolic activity in the olfactory bulbs of neonatal rats*. Brain. Res., 284, 247–259.

Strotmann, J., Wanner, I., Helfrich, T., Beck, A., Meinken, C., Kubick, S. and **Breer, H.** (1994) *Olfactory neurons expressing distinct odorant receptor subtypes are spatially segregated in the nasal neuroepithelium*. Cell Tissue Res., 276, 429–438.

Vassar, R., Chao, S.K., Sitcheran, R., Nuñez, J.M., Vosshall, L.B. and

Axel, R. (1994) *Topographic organization of sensory projections to the olfactory bulb*. Cell, 79, 981–991.

Wang, F., Nemes, A., Mendelsohn, M. and **Axel, R.** (1998) *Odorant receptors govern the formation of a precise topographic map*. Cell, 93, 47–60.

Yee, K.K. and **Costanzo, R.M.** (1995) *Restoration of olfactory mediated behavior after olfactory bulb deafferentation*. Physiol. Behav., 58, 959–968.

Yee, K.K. and **Costanzo, R.M.** (1998) *Changes in odor quality discrimination following recovery from olfactory nerve transection*. Chem. Senses, 23, 513–519.

Accepted November 29, 1999