A Model for Axon Navigation Based on Glycocodes in the Primary Olfactory System

James St John[1] and Brian Key[1,2]

[1]Department of Anatomy & Developmental Biology, School of Biomedical Sciences, University of Queensland, Brisbane 4072, Australia and [2]Centre for Functional and Applied Genomics, Institute of Molecular Biosciences, University of Queensland, Brisbane 4072, Australia

Correspondence should be sent to: Brian Key, e-mail: brian.key@uq.edu.au

Introduction

In rodents, primary olfactory sensory neurons reside in the psuedostratified olfactory neuroepithelium lining the caudal nasal cavity. Each neuron expresses a single odorant receptor and all neurons expressing the same receptor are typically located in one of four bands of neuroepithelium lining the nasal cavity (Buck and Axel, 1991; Vassar *et al.*, 1993, 1994). While there are ~1000 different odorant receptor genes, there are probably many more distinct subpopulations of sensory neurons due to regional differences in the nasal cavity of expression of cell surface carbohydrates (Key and Akeson, 1993; Puche and Key, 1996; Dowsing *et al.*, 1997; St John and Key, 2001; Storan *et al.*, 2004) and putative cell adhesion molecules such as OCAM (Yoshihara *et al.*, 1997). The differential expression of odorant receptors does more than just provide a large repertoire of sensory receptors for the detection of odors. These molecules have been shown to play a major axon guidance role (Mombaerts *et al.*, 1996). All primary olfactory neurons expressing the same odorant receptor protein project axons to the olfactory bulb where they converge and typically form two synaptic glomeruli with the dendrites of second-order olfactory neurons and interneurons. While this projection pattern in itself is not unique it is the fact that these glomeruli are positioned in topographically fixed positions in the bulb that creates a complex navigational problem for growth cones.

In the retinotecal pathway, retinal neurons project topographically from the retina on to the tectum in a point-to-point map. The maintenance of near-neighbor relations between the sense organ and the target allows the use of complementary gradients of chemorepulsive receptors and ligands to simply determine the topography. This is not the case in the primary olfactory system since the mosaic and stochastic expression of odorant receptors in the olfactory neuroepithelium negates the use of any simple matching gradient of ligand in the bulb. Moreover, it requires that the axons of highly dispersed neurons must sort out and converge on to defined points in space in the bulb at positions which have little spatial correlation to the position of parent neurons in the nasal cavity. Mechanisms are needed to sort about 1000 different subpopulations of axons to enable them to converge and form glomeruli.

Role of sorting and selective fasciculation in axon guidance

Examination of axons expressing the P2 odorant receptor during the earliest stages of glomerular formation revealed that these axons do not selectively fasciculate (Royal and Key, 1999). Thus, the initial sorting out of axons expressing one particular receptor from those axons expressing other receptors must not be via selective adhesion mediated by odorant receptors. A clue to the putative basis of this sorting came from observations of the behavior of axons expressing specific cell surface carbohydrates recognized by the plant lectin *Dolichos biflorus* agglutinin (DBA) (Key and Akeson, 1993). These

axons arise from neurons widely dispersed through the dorsal lateral nasal cavity of the mouse and they course within mixed nerve fiber fascicles as they project to the olfactory bulb. There is no evidence of selective fasciculation of these axons into bundles exclusively containing this subpopulation of axons within the peripheral nerve fascicles. However, at the level of the olfactory bulb there is a radical change in the trajectory of these axons. As they enter the outer nerve fiber layer these axons begin to sort out from other axons and self fasciculate into discrete bundles. These bundles of DBA stained axons then innervate numerous mosaically-distributed glomeruli particularly in the dorsocaudal olfactory bulb.

This sorting behavior is not peculiar to these axons since it has also been shown that axons expressing different levels of lactosamine containing carbohydrates also sort out as they enter the bulb (Puche and Key, 1996). Large fascicles of axons are present that contain low, medium and high levels of these sugars and these sorted axon bundles innervate glomeruli exhibiting similar levels of these lactosamine sugars. Lactosamine has recently been shown to be selectively present in unique glycoforms of the neural cell adhesion molecule NCAM which mediate olfactory axon fasciculation *in vitro* (Storan *et al.*, 2004). It appears that sugars such as lactosamine may be responsible for the sorting of axons into large bundles while DBA ligands may further subdivide these fascicles into smaller discrete fascicles. Thus, as axons course through the nerve fiber layer they begin to become progressively sorted into smaller and smaller fascicles on the basis of the expression of cell surface carbohydrates. These sugars can be considered as providing a cell surface signature or 'glycocode' that allows axons to be sorted prior to their convergence on glomerular targets. Most likely this self-fasciculation, mediated by carbohydrates, is a necessary prerequisite for the targeting of axons to specific glomerular sites in the olfactory bulb, based on expression of odorant receptor.

It is envisaged that axons expressing lactosamine consist of subpopulations of axons expressing many different odorant receptors. For instance, some of these axons may express the P2 odorant receptor and thus lactosamine may provide one mechanism for sorting these axons within a hierarchy of different glycocodes. Axons within these lactosamine bundles may also express DBA ligands which in turn express a smaller repertoire of odorant receptors. There may be several reiterations of this sorting into fascicles of smaller and smaller size coded by the expression of discrete cell surface carbohydrates (St John and Key, 2001). However, it is unlikely that axons expressing a single odorant receptor express an entirely unique glycode not shared by other receptor subpopulations since, as noted above, axons expressing the same odorant receptor never self-fasciculate en route to their target (Royal and Key, 1999). It appears that axons expressing P2 odorant receptors fasciculate with other axons expressing different receptors but sharing the same cell surface carbohydrates as they approach their prospective glomerular targets. P2 axons probably defasciculate from these small

fascicles in response to other guidance cues in the target zone. With subsequent development, as more and more P2 axons grow into the bulb, some of these dispersed P2 axons form small distinct fascicles that enter the glomerulus in random approach patterns, as is observed in mature glomeruli (Royal and Key, 1999).

Conclusion

Glycocodes are not the only mechanism sorting axons (Key and St John, 2002; St John *et al.*, 2002). Most likely molecules such as NCAM-180 (Treloar *et al.*, 1997), OCAM (Yoshihara *et al.*, 1997) and Neuropilin-1 (Crandall *et al.*, 2000; Taniguchi *et al.*, 2003), which are expressed by large subpopulations of axons that course in like bundles to the olfactory bulb, are also mediating sorting and selective fasciculation. There is probably a hierarchy of multiple cues that are responsible for guiding axons to the vicinity of their target where local guidance cues then become dominant. The role of timing of axon growth into the bulb should also be considered in models of axon navigation in the olfactory system. The behavior of axons such as selective sorting is clearly dependent on the environmental context in which the axons are growing, which varies with time of outgrowth of axons to the bulb. We have not dealt here with the possible mechanisms responsible for the fasciculation of axons expressing the same cell surface glycocodes; however, it is likely that receptors such as the galectin family of carbohydrate binding proteins are involved in this behavior (Storan *et al.*, 2004).

Summary

A major question in developmental neurobiology concerns understanding the molecular and cellular mechanisms underlying axon growth and guidance in the developing nervous system. Deciphering the complex interplay of molecular signals responsible for establishing axon and nerve pathways is essential for any effective therapeutic approach to regeneration and repair of injured nervous systems. While many molecules have been shown both *in vitro* and *in vivo* to participate in growth cone navigation there is no pathway in the mammalian nervous system for which we understand the principal mechanisms driving the establishment of axon pathways from neuron of origin to target cell. The primary olfactory system is one region that demands particular attention due to it peculiar regenerative capacity. It is the only region in the mammalian nervous system that exhibits continual neuronal turnover and axon growth and guidance throughout embryonic development as well as adult life. Understanding the unique characteristics of axon navigation in this system should provide insight into why the rest of the nervous system is so refractory to regeneration and repair. We present here a model for axon navigation based on the use of cell surface glycocodes for the sorting and selective fasciculation of primary olfactory axons in the nerve fiber layer of the olfactory bulb.

References

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

Crandall, J.E., Dibble, C., Butler, D., Pays, L., Ahmad, N., Kostek, C., Puschel, A.W. and Schwarting, G.A. (2000) *Patterning of olfactory sensory connections is mediated by extracellular matrix proteins in the nerve fiber layer of the olfactory bulb.* J. Neurobiol., 45, 195–206.

Dowsing, B., Puche, A.C., Hearn, C. and Key, B. (1997) *The presence of novel N-CAM glycoforms in the rat olfactory system.* J. Comp. Neurol. 285, 514–527.

Key, B. and **Akeson, R.A.** (1993) *Distinct subsets of sensory olfactory neurons: possible role in the formation of the mosaic olfactory projection.* J. Comp. Neurol., 335, 355–368.

Key, B. and **St John, J.** (2002) *Axon navigation in the mammalian nervous system. Where to next?* Chem. Senses, 27, 245–260.

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

Puche, A.C. and **Key, B.** (1996) N-*Acetyl-lactosamine in the rat olfactory system: expression and potential role in neurite growth.* J. Comp. Neurol., 364, 267–287.

Royal, S.J. and **Key, B.** (1999) *Development of P2 olfactory glomeruli in P2-IRES-tau-LacZ transgenic mice.* J. Neurosci., 19, 9856–9864.

St John, J. and **Key, B.** (2001) *Chemically and morphologically identifiable glomeruli in the rat olfactory bulb.* J. Comp. Neurol., 436, 497–507.

St John, J., Clarris, H.J. and Key, B. (2002) *Multiple axon guidance cues establish the olfactory topographical map: how do these cues interact?* Int. J. Dev. Biol., 46, 639–647.

Storan, M.J., Magnaldo, T., Biol-N'Garagra, M-C., Zick, Y. and Key, B. (2004) *Expression and putative role of lactoseries carbohydrates present on NCAM in the rat primary olfactory pathway.* J. Comp. Neurol., 475, 289–302.

Treloar, H.B., Tomasiewicz, H., Magnuson, T. and Key, B. (1997) *The central pathway of primary olfactory axons is abnormal in mice lacking the N-CAM-180 isoform.* J. Neurobiol., 32, 643–658.

Taniguchi, M., Nagao, H., Takahashi, Y.K., Yamaguchi, M., Mitsui, S., Yagi, T., Mori, K. and Shimizu, T. (2003) *Distorted odor maps in the olfactory bulb of semaphoring 3A-deficient mice.* J. Neurosci., 23, 1390–1397.

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

Vassar, R., Chao, S.K., Sitcheran, R., Nunez, J.M., Vosshall, L.B. and Axel, R. (1994) *Topogrpahic organization of sensory projections in the olfactory bulb.* Cell, 79, 981–991.

Yoshihara, Y., Kawasaki, M., Tamada, A., Fujita, H., Hayashi, H., Kagamiyama, H. and Mori, K. (1997) *OCAM: A new member of the neural cell adhesion molecule family related to zone-to-zone projection of olfactory and vomeronasal axons.* J. Neurosci., 17, 5830–5842.