

● PERSPECTIVE

Radial glia interact with primary olfactory axons to regulate development of the olfactory bulb

The developing olfactory system – merging of the peripheral and central nervous systems: The olfactory system is responsible for the sense of smell and is comprised of a complex topographic map that regenerates throughout life. In rodents each olfactory sensory neuron expresses one of ~1,300 odorant receptors with the neurons being distributed mosaically within the epithelium. The axons of the sensory neurons do not maintain near-neighbour relationships and instead project to disparate topographic targets in the olfactory bulb within the central nervous system. The development of the targets relies on the intermingling of the sensory axons with the interneurons, glia and second order neurons of the olfactory bulb. Thus the formation of the olfactory system involves the coordinated integration of the axons of the peripheral olfactory sensory neurons with the cells of the olfactory bulb.

While the final topographic map exhibits high precision of axon targeting, this is not the case during its development. In the embryonic and postnatal olfactory system many olfactory sensory axons make errors and mis-project into incorrect targets or over-project past the target layer and penetrate the deeper layers of the olfactory bulb (**Figure 1**; Graziadei et al., 1980; Amaya et al., 2015). These mis-targeted axon errors need to be corrected and the mis-targeted axons removed. The glia of the olfactory system, olfactory ensheathing cells, have been shown to remove the debris arising from degenerated olfactory axons (**Figure 1**; Su et al., 2013; Nazareth et al., 2015) along the nerve fascicles. More recently it has been shown that radial glia within the deeper layers of the olfactory bulb are the principal cells that phagocytose the debris arising from axons that over-project past their target layers (Amaya et al., 2015).

Mis-targeting of axons occurs from the very first attempts of axons to reach the olfactory bulb: The olfactory sensory neurons arise from the olfactory placode that lines the future olfactory epithelium within the nasal cavity. The axons of the sensory neurons project in mixed fascicles to the olfactory bulb. Upon penetrating the cribriform plate and entering the nerve fibre layer of the olfactory bulb the axons defasciculate and sort out so that axons arising from neurons that express the same odorant receptor converge together and project to their target glomeruli with each axon ultimately projecting to a single glomerulus. Despite the numerous axon guidance cues that are present, the axons often make targeting errors with over-projecting axons being observed from as early as embryonic day 11.5 in mouse (Graziadei et al., 1980; Amaya et al., 2015). With later development, when glomeruli are

being formed, the axons often inappropriately project into several glomeruli, branch prematurely, or over-project past the target layer (Tenne-Brown and Key, 1999). Axons that do over-project into the deeper layers can continue growing for considerable distances (Tenne-Brown and Key, 1999). The mis-targeting of sensory axons is a normal occurrence, but increased evidence of mis-targeting has been shown when specific guidance molecules or molecules of important cellular function are perturbed (Baker et al., 1999; St John et al., 2006). Eventually the mis-targeted axons degenerate and while the trigger for their degradation is unknown, it is likely to be a consequence of the inability of the axons to connect with appropriate second order neurons. However, it is possible that direct contact with the radial glia leads to the degradation of the axons and this can be explored in future work.

Olfactory sensory neurons continually regenerate: The olfactory sensory neurons are the only neurons in the nervous system that are directly exposed to the environment. The dendrites of the sensory neurons extend to the surface of the olfactory epithelium where they interact with inhaled odours. As a consequence of this exposure the sensory neurons are subject to attack by pathogens and destruction by toxic chemicals with an estimated 1–3% of neurons being turned over each day. The neurons are replaced from a population of neural precursor cells that line the basal layer of the epithelium and they project the newly generated axons into the olfactory bulb. Thus there is continual turnover of olfactory sensory neurons and the debris from the degenerated axons needs to be removed in order to maintain a healthy environment for the remaining axons, as well as for the new axons. In the adult olfactory system, axon targeting exhibits a high degree of precision unless there is widespread regeneration of sensory neurons such as can occur during injury or infection. In particular, when the olfactory system undergoes widespread degeneration followed by extensive regeneration as can occur during exposure to toxic chemicals the olfactory axons show considerable mis-targeting with the majority of axons initially being unable to project to their correct glomeruli (St John and Key, 2003).

Olfactory ensheathing cells remove axon debris: The removal of the debris arising from mis-targeted or degraded olfactory axons has been attributed to the glia of the olfactory system, olfactory ensheathing cells (OECs), that wrap around the axon fascicles. Rather than relying on infiltrating macrophages to remove debris, it has been shown that the resident OECs are the predominant phagocytic cells in the embryonic olfactory system from as early as E14.5 in mouse (Nazareth et al., 2015). The OECs continue to be the major phagocytic cells in the postnatal and adult (Su et al., 2013) olfactory system as well as during widespread degeneration (Su et al., 2013; Nazareth et al., 2015). Macrophages are able to phagocytose the olfactory axon debris, but as they are largely excluded from the axon fascicles they therefore play a minor role (Nazareth et al., 2015). Thus OECs are not only

crucial to the growth and guidance of olfactory axons, but also for removing debris from degraded olfactory axons. However, the OECs are only able to remove the debris within the axon fascicles (**Figure 1**). As olfactory sensory axons also over-project into the deeper layers of the olfactory bulb, cells that reside within the olfactory bulb must be responsible for the removal of debris arising from the over-projected axons.

Radial glia regulate olfactory bulb development: Radial glia are one of the principal cell types in the embryonic olfactory bulb and are crucial to its development. The cell bodies of the radial glia are initially located close to the ventricle in the central bulb and they project their processes radially to the outer surface of the developing olfactory bulb (**Figure 1**). It is here at the junction between the central nervous system and the peripheral nervous system that the interactions between the olfactory sensory axons and the olfactory bulb cells occur. Olfactory sensory axons that project to the correct target zone intermingle with the processes of the radial glia and astrocytes leading to the formation of proto-glomeruli (Bailey et al., 1999). However, olfactory sensory axons that over-project into the deeper layers of the olfactory bulb migrate along the processes of the radial glia (Amaya et al., 2015). Radial glia have been shown to aid cell migration and are precursors to other glia cells. Unlike in the neocortex, the radial glia in the olfactory bulb have convoluted projections that differ in their final location within the olfactory bulb's outer layers, with some ramifying in the glomerular layer and others in the developing external plexiform layer (Bailey et al., 1999). Thus radial glia play numerous roles in regulating development of the early brain.

Radial glia phagocytose debris from over-projecting olfactory axons: To determine which cells phagocytose the debris from over-projected olfactory sensory axons during early development of olfactory system, we utilized the OMP-ZsGreen transgenic reporter line of mice in which olfactory sensory axons express the bright and stable green fluorescent protein ZsGreen (Amaya et al., 2015). In these reporter mice, the projections of individual axons could be easily traced and debris from degraded axons could be detected after they were phagocytosed by other cells. By examining the trajectory of the over-projecting axons it was shown that the over-projecting olfactory sensory axons travelled along the processes of the radial glia that extended from the ventricle out towards the developing nerve fibre layer of the olfactory bulb (Amaya et al., 2015). It has previously been suggested that radial glia repel olfactory axons (Gonzalez et al., 1993) thus it is interesting that the over-projecting axons maintain close contact with the processes of the radial glia. Perhaps this is a consequence of the general inability of the over-projecting axons to detect repulsive signals that would normally indicate the olfactory sensory axons to terminate in the outer layer of the olfactory bulb.

As the over-projecting axons proceeded deeper into the olfactory bulb, it was apparent that they became degraded

(Amaya et al., 2015). Fortunately, the strong and stable fluorescence of the OMP-ZsGreen reporter molecule enabled the degraded debris to be easily detected. The axon debris accumulated principally around the ventral surface of the ventricle in the region of the cell bodies of the radial glia. Immunostaining and three-dimensional reconstruction of the radial glia confirmed that the ZsGreen axon debris was internalized by the radial glia (Amaya et al., 2015). Several markers were used to identify if other cells portrayed similar phagocytic properties however no other cell type was observed to be involved in the removal of axonal debris. Thus it was concluded that the axon debris was phagocytosed by the processes of the radial glia and then transported internally to the soma of the radial glia (**Figure 1**).

Olfactory ensheathing cells influence the development of the radial glia: The crucial interactions between the cells of the peripheral and central nervous system that regulate the development of the olfactory bulb are highlighted by the influence of OECs on the development of radial glia. At around E16 of normal development, the processes of the radial glia are concentrated to the central region of the olfactory bulb, with few processes extending to the nerve fibre layer. Thus a distinct region is formed between the processes of the radial glia and the OECs which represents the presumptive external plexiform layer (Amaya et al., 2015). However, in mice which lack the transcription factor Sox10 which is associated with central nervous system development, the OECs fail to proliferate and migrate properly with the result that the nerve fibre layer of the olfactory bulb is poorly populated by OECs. In these Sox10 knockout mice the distribution of the radial glia was also clearly perturbed and the processes of the radial glia extended all the way out to the nerve fibre layer. In addition, the orientation of the cell bodies was disorganized in the Sox10 knockout mice and rather than being radially aligned, they lacked a definite uniform orientation (Amaya et al., 2015). Coincident with the altered morphology of the radial glia was an increase in the amount of olfactory sensory axon debris that was present in the deeper layers of the olfactory bulb. Importantly the debris did not accumulate within the cell bodies of the radial glia indicating that the phagocytic ability of the radial glia was reduced and hence the increased accumulation of debris within the deeper layer of the olfactory bulb (Amaya et al., 2015). As radial glia do not express Sox10 the effect on the distribution and morphology of radial glia was indirect and likely to be influenced by the OECs.

Conclusion: Numerous errors occur during the development of the olfactory system and olfactory sensory axons often fail to terminate in the target zone of the olfactory bulb. The mis-targeted axons often over-project into the deeper layers of the olfactory bulb where they interact with the processes of radial glia. The excess axons are subsequently degraded and phagocytosed by OECs along the axon fascicles and by radial glia within the deeper layers of the olfactory bulb. It is apparent that the growth and distribution of OECs influences the growth of radial glia and thus there is

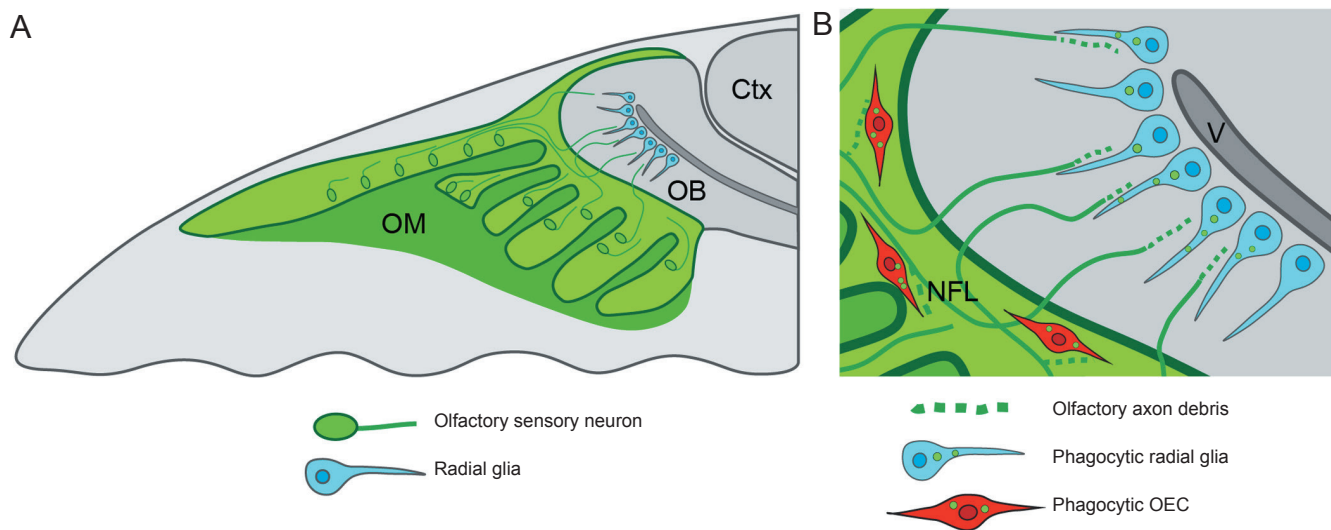


Figure 1 Schematic sagittal view of the mouse olfactory system.

(A) Olfactory sensory neurons (green) line the olfactory mucosa (OM) within the nasal cavity. The neurons project axons to the olfactory bulb (OB) within the central nervous system. Some axons over-project into the deeper layers of the olfactory bulb. Radial glia (blue) are present within the deeper layer of the olfactory bulb. (B) Close up schematic of the olfactory bulb. Olfactory sensory axons branch and over-project inappropriately. Axons that over-project often follow the processes of the radial glia. The inappropriate branches degrade (dashed lines) and the axon debris is phagocytosed by radial glia within the deeper layer of the olfactory bulb, and by olfactory ensheathing cells (OECs) within the nerve fibre layer which lines the outer region of the olfactory bulb. Ctx: Cortex; NFL: nerve fiber layer; V: ventricle.

an important interplay between the axons and OECs of the peripheral nervous system and the cells of the central nervous system as they integrate to form the olfactory system.

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