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Correction of aberrant axon growth in the developing mouse olfactory bulb

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ABSTRACT

During development of the primary olfactory system, sensory axons project from the nasal cavity to the glomerular layer of the olfactory bulb. In the process axons can branch inappropriately into several glomeruli and sometimes over-shoot the glomerular layer, entering the deeper external plexiform layer. However in the adult, axons are rarely observed within the external plexiform layer. While chemorepulsive cues are proposed to restrict axons to the glomerular layer in the embryonic animal, these cues are clearly insufficient for all axons in the postnatal animal. We hypothesised that the external plexiform layer is initially an environment in which axons are able to grow but becomes increasingly inhibitory to axon growth in later postnatal development. We have determined that rather than having short localised trajectories as previously assumed, many axons that enter the external plexiform layer had considerable trajectories and projected preferentially along the ventro-dorsal and rostro-caudal axes for up to 950 μm. With increasing age, fewer axons were detected within the external plexiform layer but axons continued to be present until P17. Thus the external plexiform layer is initially an environment in which axons can extensively grow. We next tested whether the external plexiform layer became increasingly inhibitory to axon growth by microdissecting various layers of the olfactory bulb and preparing protein extracts. When assayed using olfactory epithelium explants of the same embryonic age, primary olfactory axons became increasingly inhibited by extract prepared from the external plexiform layer of increasingly older animals. These results demonstrate that primary olfactory axons can initially grow extensively in the external plexiform layer, but that during postnatal development inhibitory cues are upregulated that reduce axon growth within the external plexiform layer.

Introduction

Primary olfactory neurons that express the same odorant receptor are mosaically distributed within the olfactory neuroepithelium and appear to have no particular spatial relation to each other (Ressler et al., 1993; Vassar et al., 1994). Despite this distribution, primary olfactory axons arising from these neurons are able to sort out, fasciculate together and target glomeruli to form the highly specific and topographically fixed olfactory map (Mombaerts, 1996; Ressler et al., 1994; Royal and Key, 1999; Treloar et al., 2002; Vassar et al., 1994). The development of this complex projection pattern involves the migration of numerous cell types and the extension of their processes into different layers of the olfactory bulb. While the timing of many events is coincident with the appearance of glomeruli, the cellular interactions driving the formation of this unique synaptic neuropil remain to be determined. We have reported that numerous sensory axons exhibit exuberant growth and project aberrantly into deeper layers of the olfactory bulb during development (Royal and Key, 1999; Tenne-Brown and Key, 1999). Others have instead noted the specificity with which primary olfactory axons are able to home to their target and form glomeruli without the need for error correction (Dynes and Ngai, 1998; Klenoff and Greer, 1998; Potter et al., 2001; Treloar et al., 2002; Wang et al., 1998).

There is no generic mechanism that governs the development of topographic connections in different neural pathways. In the visual system, retinal axons initially form a diffuse map on the tectum which is subsequently refined into a strict topography as a result of pruning and lateral branching (Hindges et al., 2002; Simon and O'Leary, 1992; Yates et al., 2001). In contrast, dorsal root sensory axons establish a correct topographic map in the dorsal horn of the spinal cord as the pathway forms (Silos-Santiago et al., 1995). Dorsal root axons always grow specifically to their target lamina without branching or over-shooting into inappropriate regions in the dorsal horn (Ozaki and Snider, 1997). The extent of error formation by sensory axon projections in the olfactory bulb and the cues that lead to their removal still remains to be resolved.

During development in the olfactory nerve pathway, while many axons project directly to a glomerulus (Dynes and Ngai, 1998; Klenoff and Greer, 1998; Potter et al., 2001; Treloar et al., 2002; Treloar et al., 1999; Wang et al., 1998) it is clear that many axons make errors in

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targeting, branch into numerous glomeruli, and can over-project past the target layer into the deeper layers of the olfactory bulb (Royal and Key, 1999; Santacana et al., 1992; Tenne-Brown and Key, 1999). The development of P2-odorant glomeruli in mice commences around embryonic day 14.5 (E14.5) when P2 axons coalesce in a broad locus underlying the presumptive glomerular layer (Royal and Key, 1999). Over the next 5 days the axons develop into discrete glomerular structures. During this period the P2-odorant receptor axons are intermingled with other odorant receptor axons, branch into neighbouring glomeruli and project into deeper layers of the olfactory bulb. It is not until postnatal day 7.5 that P2 glomeruli become distinct and separate, with all axons terminating within the target glomeruli (Royal and Key, 1999). This initial inaccuracy of targeting is not restricted to P2 axons, but is typical of the majority of axons. Tenne-Brown and Key (1999) reported that at P1.5, 54% of axons did not terminate directly in a glomerulus, and that 22% of axons projected aberrantly into the external plexiform layer. However, axons were reported to project directly to their glomerular target by P5.5. Similarly in rat, aberrant projections into the external plexiform layer have been observed up to P9 (Royal and Key, 1999). Thus the mechanisms that regulate axonal targeting are not precise and many axons are not initially successful in recognising their target glomeruli.

We hypothesised that the external plexiform layer is initially an environment in which axons are able to grow, but becomes increasingly inhibitory with later development. In the present study we addressed this issue and found that in early postnatal animals, many sensory axons are able to enter the external plexiform layer and are capable of growing tangentially for long distances where they form a diffuse plexus. We then examined axon outgrowth from olfactory epithelial explants obtained from the same embryonic age animals and found that with increasing developmental age, the external plexiform layer becomes increasingly repulsive to primary olfactory axons. These results show that the external plexiform layer contributes to restricting primary olfactory axons to the superficial layers of the olfactory bulb.

Results

Primary olfactory axons have extensive trajectories in the external plexiform layer

Previous studies have revealed that during development many primary olfactory axons aberrantly project past their target, the glomerular layer, and continue into the deeper external plexiform layer (EPL) of the olfactory bulb (Bailey et al., 1999; Graziadei et al., 1980; Key and Akeson, 1993; Santacana et al., 1992; Tenne-Brown and Key, 1999; Treloar et al., 1996). This exuberant radial growth may be a consequence of the inability of some growth cones to recognize appropriate stop signals (Poeck et al., 2001) in the glomerular layer. If this was the case then once axons have entered the EPL they may grow unchecked. However, over-projecting axons have been noted to loop in the EPL and then turn back towards the glomerular layer, suggesting that the EPL is non-conducive for primary olfactory axon growth (Treloar et al., 1997, 2002). We were interested in determining the extent of primary olfactory axon growth within the EPL. Do primary olfactory axons only project a short distance into the EPL and then return to the glomerular layer or do they find the EPL a region in which they can continue to extend?

Our initial analysis of coronal sections from early neonatal bulbs confirmed that primary olfactory axons do project deep past the glomerular layer and into the underlying EPL, with some even projecting into the internal plexiform layer (Fig. 1A, B). Immunostaining for the olfactory marker protein (OMP) revealed numerous axons coursing radially within the EPL. While many axons were observed looping back as if they were repelled by the mitral cell layer, there was considerable granular or punctate staining of the EPL which suggested the presence of transversely-sectioned axons (Fig. 1B). In coronal sections, it is difficult

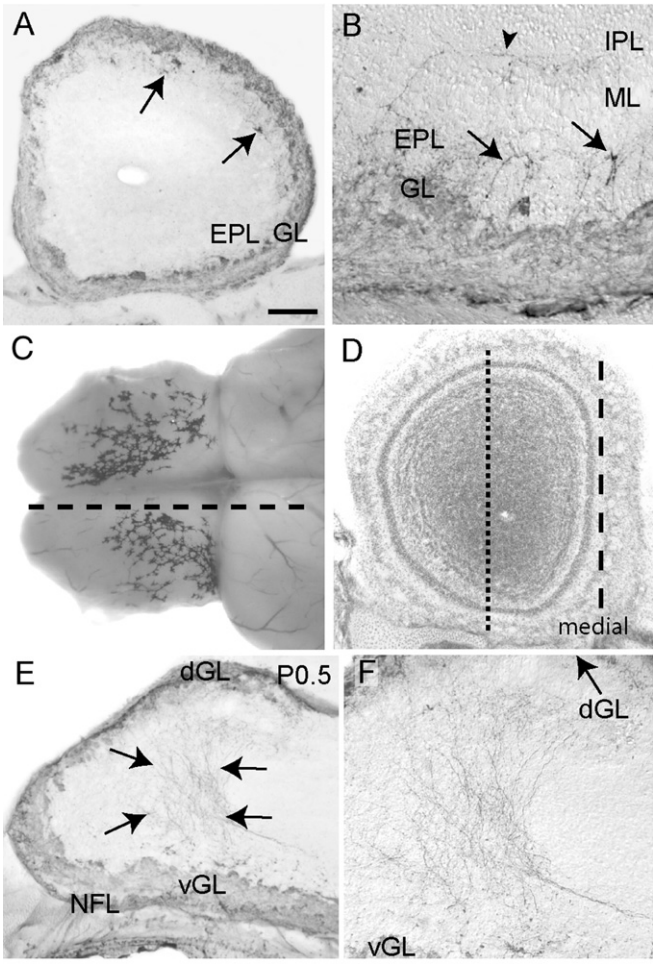

Fig. 1. Parasagittal sections reveal extent of axon trajectories within the external plexiform layer. (A) Coronal section of P0.5 mouse olfactory bulb immunoreacted with antibodies against OMP. Numerous axons penetrated the external plexiform layer (EPL) with some axons forming aggregrates (arrows) within the EPL. (B) Higher power view of axons within the EPL. These axons appeared to project short distances and often looped back (arrows) towards the glomerular layer (GL). Occasionally axons projected past the mitral cell layer (ML) into the internal plexiform layer (arrowhead). (C) Parasagittal sections (100 μm) of mouse olfactory bulbs and cortex were cut (dashed line) to provide flat-mount sections of the EPL. (D) Medial parasagittal sections (dashed line) were cut through extensive areas of the EPL, whereas other parasagittal sections (dotted line) passed through central regions of the bulb. (E) Immunohistochemistry using antibodies against OMP in a glancing section through the EPL of P0.5 olfactory bulb revealed extensive projections (arrows) of primary olfactory axons within the EPL; vGL = ventral glomerular layer, dGL = dorsal glomerular layer. (F) Higher power view of E; vGL is slightly in view at the bottom left, dGL is out of view but indicated by the arrow. E–F are parasagittal sections with rostral to the left and dorsal to the top. Scale bar is 125 μm in A, D; 50 μm in B; 250 μm in C,E; 100 μm in F.

to determine the trajectory of axons that have turned and grown tangentially within this layer. We addressed this question by examining thick (100 μm) parasagittal sections of the olfactory bulb. Flat-mounts of the EPL on the medial and lateral surfaces of the bulb were present in some of these sections (Fig. 1C, D), enabling us to examine the extent of the trajectory of axons as they coursed tangentially within this layer. These thick parasaggital sections clearly revealed the extent to which the primary olfactory axons projected into the EPL and formed a loose and extensive network throughout this layer (Fig.s 1E–F, 2). OMP stained axons appeared to extend throughout the ventral-dorsal breadth of the bulb at postnatal day (PD) 0.5 (Fig. 1E). However, because of the extent of this exuberant growth it was not possible to trace the trajectory of axons past the midpoint in the dorsoventral axis (Fig. 1F). This is probably why they were previously not recognized in coronal sections of the bulb and explains the granular staining in the EPL (see Fig. 1B). While in parasagittal sections these deeper growing axons