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Research Report

The shape of the olfactory bulb influences axon targeting

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ABSTRACT

Each primary olfactory neuron in the mouse expresses a single type of odorant receptor. All neurons expressing the same odorant receptor gene typically project to two topographically fixed glomeruli, one each on the medial and lateral surfaces of the olfactory bulb. While topographic gradients of guidance receptors and their ligands help to establish the retinotectal projection, similar orthogonal distributions of cues have not yet been detected within the olfactory system. While odorant receptors are crucial for the final targeting of axons to glomeruli, it is unclear whether the olfactory bulb itself provides instructive cues for the establishment of the topographic map. To begin to understand the role of the olfactory bulb in the formation of the olfactory nerve pathway, we developed a model whereby the gross shape of the bulb in the P2-IRES-tau-LacZ line of mice was radically altered during postnatal development. We have shown here that the topography of axons expressing the P2 odorant receptor is dependent on the shape of the olfactory bulb. When the dorsoventral axis of the olfactory bulb was compressed during the early postnatal period, newly developing P2 axons projected to multiple inappropriate glomeruli surrounding their normal target site. These results suggest that the distribution of local guidance cues within the olfactory bulb is influenced by the shape of the olfactory bulb and that these cues contribute to the topographic positioning of glomeruli.

1. Introduction

Primary olfactory neurons in the mouse express one of ~1000 odorant receptors. Those neurons expressing the same receptor type are mosaically distributed throughout zones in the nasal cavity (Ressler et al., 1993; Strotmann et al., 1994; Vassar et al., 1993) and typically project axons to two topographically fixed glomeruli, one each on the medial and lateral surfaces of the olfactory bulb (Ressler et al., 1993; Vassar et al., 1994). What are the mechanisms that enable these axons to navigate to their target sites and form glomeruli in the appropriate topographic location in the olfactory bulb during development? The odorant receptors themselves are known to play a role in topographic targeting (Mombaerts et al., 1996) and several adhesion molecules and guidance receptors such as galectin-1 (Puche et al., 1996), N-CAM (Treloar et al., 1997), neuropilin-1 (Pasterkamp et al., 1998) and its ligand Semaphorin 3A (Crandall et al., 2000) as well as neuropilin-2 (Cloutier et al., 2002; Walz et al., 2002) are involved in olfactory axon guidance. However, the specific cues responsible for generating the topographic map remain elusive.

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While the expression of spatially restricted guidance cues in the olfactory bulb has been proposed to determine the location of glomeruli (Key and St John, 2002; St John et al., 2002), others have suggested that intrinsic self-organizational properties of olfactory axons are sufficient for emergence of the map (Feinstein and Mombaerts, 2004). In this latter model, the olfactory bulb is believed to merely provide a permissive substrate for axon growth. Rather than olfactory axons recognizing specific topographic cues, as in the retinotectal system, they are instead hypothesized to sort out autonomously in a highly stereotyped spatiotemporal order.

Following genetic or physical ablation of the olfactory bulb, axons arising from neurons expressing the P2 odorant receptor are able to form glomerular-like loci which suggests that these axons have a cell autonomous ability to, at least, converge (Bulfone et al., 1998; St John et al., 2003). Furthermore, we have previously shown that regenerating olfactory axons are able to form the olfactory nerve and glomerular layers in the presence of an artificial biological scaffold, similar in size and shape to the olfactory bulb, although they are not able to form glomeruli in appropriate positions (Chehrehasa et al., 2006). However, it remains to be determined what role the olfactory bulb itself plays in the establishment of the topographic map.

One means of assessing the role of the olfactory bulb in the formation of the olfactory nerve pathway is to experimentally alter the normal shape of this tissue during development. We developed a model whereby the gross shape of the olfactory bulb in P2-IRES-tau-LacZ mice was disrupted without perturbing existing olfactory nerve connections. We have demonstrated here that this altered shape caused growing P2 axons to project to multiple inappropriate glomeruli rather than to a single glomerulus. These results demonstrate for the first time that the shape of the olfactory bulb is an important determinant in the targeting of olfactory axons to specific glomeruli.

2. Results

2.1. *Unilateral bulbectomy alters the gross morphology of the remaining olfactory bulb*

We hypothesized that the gross shape of the bulb contributes to the topographic organization of glomeruli. It is envisaged that the shape either influences the spatial and temporal ordering of axons as they converge to form glomeruli or that it determines the positioning of intrinsic axon guidance cues. We developed a model whereby the gross shape of the olfactory bulb was disrupted during development, without perturbing the existing projections of olfactory axons to their glomeruli. This was achieved by unilaterally removing an olfactory bulb and the accompanying rostral forebrain from

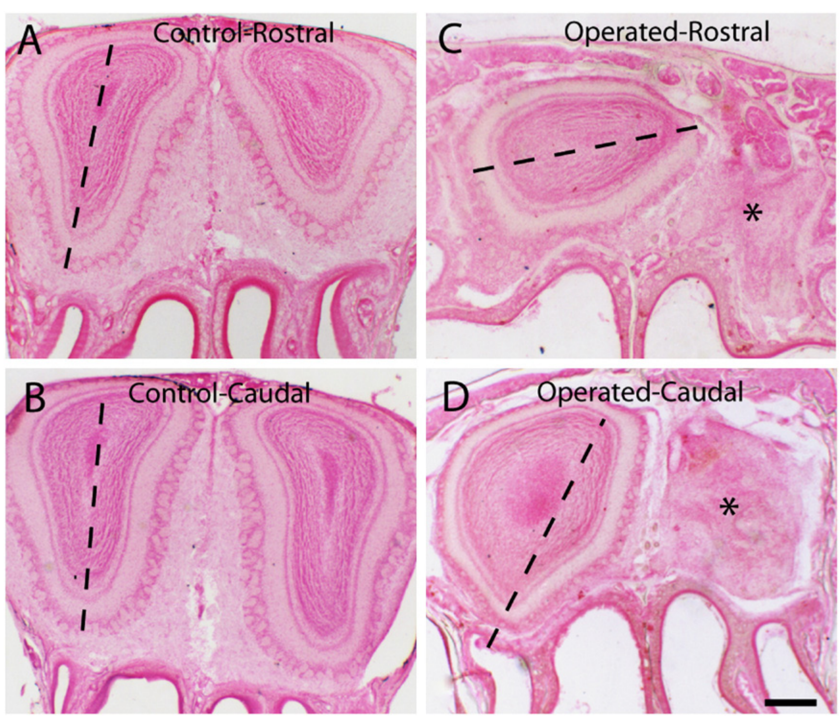

Fig. 1 – Unilateral bulbectomy perturbs the gross morphology of the remaining olfactory bulb. Panels are coronal sections through the olfactory bulb, with dorsal to the top. (A, B) In control animals at 4 weeks, the width of the olfactory bulb was approximately one-half of its height with a longer vertical than horizontal axis (dashed line). (C) However, at 4 weeks post-surgery in animals which underwent unilateral bulbectomy at P3, the rostral portion of the remaining olfactory bulb appeared flattened with a horizontal axis almost twice the width of its height (dashed line). This collapsed morphology was most prominent in the rostral half of the bulb. Asterisk indicates the position of tissue that has filled the bulbectomized cavity. (D) In the caudal region, the collapsed morphology was less prominent and the olfactory bulb had a longer vertical than horizontal axis (dashed line). Asterisk indicates tissue in the bulbectomized cavity. Scale bar = 400 μm in panel A; 600 μm in panel B; 550 μm in panels C, D.