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Research report

Neonatal focal denervation of the rat olfactory bulb alters cell structure and survival: a Golgi, Nissl and confocal study

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Abstract

Contact between sensory axons and their targets is critical for the development and maintenance of normal neural circuits. Previous work indicates that the removal of afferent contact to the olfactory bulb affects bulb organization, neurophenotypic expression, and cell survival. The studies also suggested changes to the structure of individual cell types. The current work examines the effects of denervation on the morphology of mitral/tufted, periglomerular, and granule cells. Focal denervation drastically changed mitral/tufted cell structure but had only subtle effects on periglomerular and granule cells. Denervated mitral/tufted cells lacked apical tufts and, in most cases, a primary dendrite. In addition, the denervated cells had more secondary processes whose orientation with respect to the bulb surface was altered. Our results suggest that contact between olfactory axons and the bulb is necessary for cell maintenance and may be critical for the ability of mitral/tufted cells to achieve adult morphology

Theme: Development and regeneration

Topic: Sensory systems

Keywords: Denervation; Olfactory system; Olfactory nerve; Development

1. Introduction

Denervation causes a wide range of both cellular and cytoarchitectural alterations. Many investigators have employed sensory systems for studying the consequences of the loss of afferent input, perhaps due to their well-understood wiring diagrams and ease of study. For example, it has long been known that enucleation causes rapid atrophy and cell death in the visual system during early development [27]. Similarly, neonatal cochlea removal results in reductions in the number and size of specific populations of target neurons [3,4,21,29,57,58]. In addition to determining cell size and number, innervation also plays a role in maintaining cell structure. Deafferentation of the chick nucleus laminaris and the rodent cochlear nucleus causes reductions in field area, length and fine structure of target neuron dendrites [12,47,54,58,59]. The severity of these effects relies heavily on the age at which the manipulation is performed. A possible mechanism underlying such changes is afferent regulation of cytoskeletal proteins [13,25].

Several classic experiments indicate that the olfactory system is also affected by deafferentation. Removal of the rabbit peripheral olfactory organ causes reductions in the size of the olfactory bulb and several of its constituent cell types, including mitral/tufted cells, the primary targets of olfactory sensory neurons [37]. Ablation of the olfactory epithelium in young animals causes reductions in mitral/tufted cell soma size and dendritic length and alterations in intracellular constituent organization (causing an 'atrophic' appearance [37,48–50]). The observed changes in mitral/tufted cell structure may be attributable to a reduction in

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olfactory neuron activity and/or coincident release of trophic factors.

Our previous work using neonatal focal denervation [8] showed that contact between the olfactory nerve and bulb is crucial to the establishment of normal bulb architecture, the survival of mitral/tufted cells and the integrity of cellular processes. In particular, MAP2 immunocytochemistry indicated a disruption in cellular processes in denervated zones, perhaps reflecting a change in mitral/tufted cell dendritic structure. A developmental analysis showed that the changes incurred by focal denervation result from a cascade of events that begins soon after the surgery is performed [9]. One such change was a significant decrease in the number of mitral/tufted cell profiles by postnatal day (P) 20. The retention of some profiles in denervated zones suggested that extensive reorganization must occur to ensure the survival of remaining cells.

Focal denervation (accomplished by placing a small Teflon chip between the cribriform plate of the ethmoid bone and the olfactory bulb) provides a number of advantages over other techniques used to study the role of afferent contact in target cell structure: (i) the surgery can be easily performed shortly after birth; (ii) unlike mechanical or chemical deafferentation, the method ensures that specific regions will be completely denervated and others will be spared; and (iii) unlike other methods in which reinnervation occurs [11,59], this method is permanent, allowing an examination of long-term denervation on cell structure. In the current study, we used a combination of Golgi impregnation, Nissl staining and confocal microscopy to analyze the effects of neonatal focal denervation on mitral/tufted cell structure. Focally denervated bulbs contained regions in which large segments of the olfactory nerve layer and glomerular layer were absent, juxtaposed to regions receiving apparently normal innervation. The following studies examine the relationship between the extent of innervation and cell size and morphology.

2. Materials and methods

2.1. Animals

Offspring of Long-Evans-hooded rats purchased from Charles River Laboratories (Wilmington, MD) served as subjects in these experiments. Rats were housed in polypropylene cages and given food and water ad libitum. The colony room was maintained on a 16/8-h light/dark cycle. All experiments were endorsed by the Animal Care and Use Committee of the University of Virginia and followed guidelines set by the National Institutes of Health. Every attempt was made to minimize the number of animals required.

2.2. Surgery

On P1 (the day after the day of birth), pups were anesthetized via hypothermia and underwent Teflon implant insertion or sham surgery. Teflon implants were constructed by shaving thin strips from a large sheet and cutting them into small (1–2×2 mm) rectangles. For both experimental and control animals, incisions were made through the skin and bone overlying the rostral pole of the right olfactory bulb. For experimental animals the Teflon implant was inserted between the cribriform plate and the rostral olfactory bulb with a pair of fine forceps. Sham surgery consisted of the entire procedure without Teflon insertion. The insertion severed existing olfactory connections and prevented in-growth of new axons in particular regions of the bulb [8,9]. Following surgery pups were warmed and returned to their mothers or colony.

2.3. Tissue preparation

Rat pups were killed by lethal injection of sodium pentobarbital (0.39 mg drug/g body weight) on P10, 20 or 30. One group, used to examine mitral/tufted cell soma size, was perfused with phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde and processed for paraffin embedding (four experimental and control pups at each age). Horizontal paraffin sections (8-μm) were stained with cresyl violet, and sections representing the midpoint of the dorso-ventral extent of the bulb and containing a large portion of the subependymal layer (SEL) were selected. At least three, non-adjacent sections were measured for each animal. Large cells, in or adjacent to the mitral cell layer showing abundant cytoplasm and a clear nucleus were included in measures of mitral/tufted cell size. The entire cell body (nucleus and cytoplasm) was outlined at 40× and an area measurement obtained using a microcomputer-based image analysis program (MCID, Imaging Research, St. Catharine's, Ont.). The average mitral/tufted cell profile size for anterior, lateral and medial regions was then determined for each animal. The method resulted in ~1400 cells being measured at each age. Comparisons of profile areas were then made between denervated and intact zones in the contralateral control bulbs across ages. As it is difficult to assess the degree of innervation of areas outside the shadow of the Teflon implants, contralateral bulbs were used for standard comparisons to ensure that all comparisons were made with fully innervated regions. Results were expressed as percentage differences between operated and control bulbs (right bulb relative to left bulb). Measures of glomerular layer area, an index of the amount of first nerve fibers in the region [8,9], were made for each bulb region and then compared to cell area measurements to examine the relationship between innervation and mitral/tufted cell profile size.

Pups in a second group (25 experimental rats at P30) were treated with the same anesthesia and then decapitated. Brains were placed in Golgi-Cox solution and processed according to techniques described previously [7]. Briefly, following 5 weeks incubation in staining solution, the

tissue was embedded in celloidin, mounted on blocks and cut on a sliding microtome at 120 μm. Sections were developed, dehydrated and cleared before mounting in DPX (Aldrich Chemical). Mitral/tufted, periglomerular and granule cells from control and denervated bulbs were reconstructed at 5000× with a camera lucida (~10/group) and analyzed via standard techniques [5,16] to assess total dendritic length and number of branches. Cells were chosen for analysis by standard techniques: the first cells encountered that were fully impregnated with stain and located so that nearly all processes were contained within the section were selected. Cell types were identified on the basis of detailed descriptions of cell morphology [15,18,31,48,51,56]. While enough of the cell morphology was retained to allow positive identification of neurons in denervated regions as relay cells, due to the disruption of normal lamination patterns, we could not determine whether the cells were mitral, displaced mitral or deep tufted cells, and the data are reported from the combined category 'mitral/tufted' cells. Because (a) there is substantial variability in the effects of the experimental procedure from subject to subject, (b) the Teflon implants only denervate a relatively small region and (c) denervation results in a severe reduction in the number of cells, it was difficult to collect a large sample from age, sex, and littermate-matched control and experimental subjects. However, the changes seen were large and consistent. Differences noted between experimental and control bulbs were likely due to focal denervation because (i) right and left bulbs were processed together, (ii) no differences in staining density or cell numbers were noted in right versus left neocortices, (iii) innervated regions of manipulated bulbs were indistinguishable from controls at this level of analysis, and (iv) denervated regions from numerous animals showed similar cell structural features. Photomicrographs were obtained with a digital camera (Spot, Diagnostic Instruments, Sterling Heights, MI) compiled and labeled with Adobe Photoshop and Illustrator.

A third group of pups underwent Teflon insertion on P1 and were reared until P30. After vascular perfusion as outlined above, the lateral olfactory tract was severed with a 27-gauge needle just caudal to the olfactory bulb and a small crystal of DiI (1,1′-dihexadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; $DiIC_{16}$(3); Molecular Probes, Eugene, OR) was inserted into the cavity. The site was sealed with 5% gelatin or agar solution and the tissue left in the dark for 16–18 months. Vibratome sections were cut at 70 μm, counterstained with a fluorescent Nissl-stain (Sytox, Molecular Probes, Eugene, OR) and viewed with an Olympus Fluoview 300 confocal microscope.

3. Results

3.1. Nissl study: mitral/tufted cell soma size

While no specific marker has been reported for mitral cells, they are easily discerned in Nissl-stained tissue. Mitral cell somata are restricted to the 'mitral cell layer', an obvious region that it is bounded by two relatively cell free zones (the external and internal plexiform layers; Fig. 1a). The layer itself is comprised of three cell types: mitral cells (found almost entirely in the superficial side of the thin layer except in very young animals), granule cells (small profiles found predominately in the deep regions), and glia. Mitral cells are distinguishable by their large somas, conspicuous, pale spherical nuclei, and the emergence of large dendritic processes from their apical aspects (see below). In P10 experimental animals, mitral cells are easily detectable in the outer portion of the mitral cell layer as their morphology is still similar to that seen in controls (Fig. 2; see also Fig. 1 in Ref. [9]). By P30, in denervated zones the mitral cell layer has lost it regular appearance, though it is still easily apparent sandwiched between two plexiform zones (Figs. 1 and 2) and divisible into a deep zone with small cells and a superficial area with larger cells. The fact that these cells can be filled by placing DiI into the lateral olfactory tract (Fig. 1b), along with their size and location, indicates that they are indeed mitral or tufted cells.

Quantitative evaluations indicated that, as expected, there was no laterality in mitral/tufted cell profiles of control animals. By P10, mitral/tufted cell soma profiles in denervated regions were an average of 11% smaller than profiles in the contralateral bulb. By P20, profile size showed a significant reduction of 27%, and by P30, profiles were almost 50% smaller (Figs. 2 and 3a). Soma size was positively correlated (P10: $r^2=0.47$; P20: $r^2=0.56$; P30: $r^2=0.55$) with the area of the glomerular region (defined as the cell rich zone lying superficial to the external plexiform layer) at each age (Fig. 3b).

3.2. Golgi study

3.2.1. Normal morphology

In control animals and unmanipulated bulbs of experimental animals, Golgi-impregnated mitral/tufted cells were readily identifiable due to the size, shape and location of their cell bodies. Primary dendrites extending from the soma and ending in an apical tuft were commonly found (Fig. 4, bottom panel; Fig. 5b). These arborizations were large, tended to fill the entire glomerulus, contained few spines but numerous varicosities and never extended beyond the glomerulus. Branch terminations within the glomerulus were discernible due to round, ovoid or tear-shaped terminal varicosities. Unlike the fan-shaped branching pattern of external tufted cells, mitral and deep tufted cells exhibited a wider angle of branching and spherical apical tufts within the glomerulus. Secondary dendrites filled the EPL in a bilaminar or trilaminar [32,41,44] pattern (Fig. 4, bottom panel; Fig. 5b). Each mitral cell had several secondary dendrites, and these could often be followed for long distances within the EPL.

Periglomerular cell somata, found throughout the

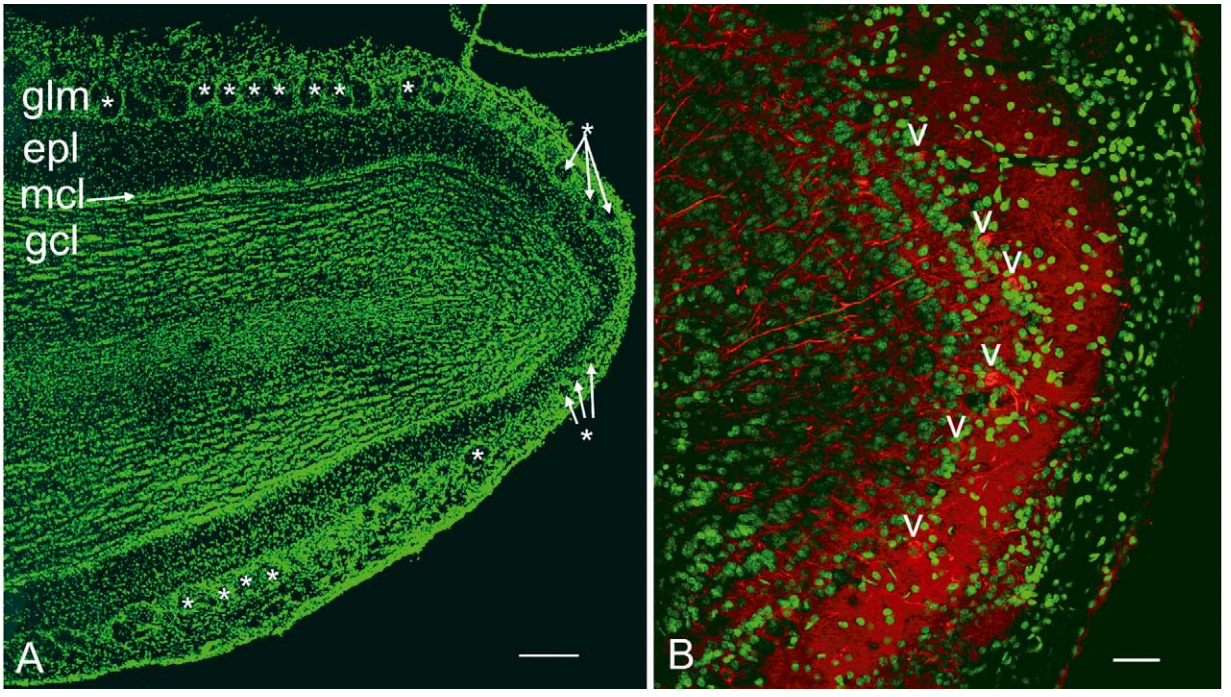

Fig. 1. (a) Low power confocal micrograph of a Nissl-stained horizontal section through a focally-denervated olfactory bulb. The Teflon chip was located anterior and slightly lateral to the rostral pole of the bulb, resulting in a denervated zone lying between the two sets of arrows. Note that glomeruli (examples marked by asterisks) are evident on the medial (top) and lateral (bottom) sides, but not in the denervated zone. Abbreviations: EPL, external plexiform layer; GCL, granule cell layer; GLM, glomerular layer; MCL, mitral cell layer (the line of cells indicated by the arrow. Note that this zone becomes disorganized in the denervated region). Scale bar represents 200 μm. (b) Higher power image of the denervated region depicted in left panel. Nissl-staining resulted in the green image, while the red represents DiI labeling. DiI was placed in the lateral olfactory tract to fill axons and cell bodies that contribute to the bundle. Note the red axons approaching the region from the deep granule cell layer (left side of figure), and the six examples of labeled somata in the cellular region corresponding the mitral cell zone (arrows). Scale bar represents 60 μm.

glomerular layer, were much smaller and had a spherical or oblong shape (Fig. 6b). Spines or short dendrites were often observed on cell bodies. In all cases, a single dendrite extended from the soma and arborized within one glomerulus. These extensions had no uniform orientation relative to bulb laminae. Arbors began a short distance from the cell body and appeared to contribute to a circumscribed portion of a glomerulus. The elaborate dendritic arbors had numerous, sometimes very thin, spines and contained varicosities both along their branches and at their termination points. Periglomerular cells were distinguishable from superficial short axon cells since the latter variety branched more rarely. They were also unlikely to be external tufted cells since tufted cells have a

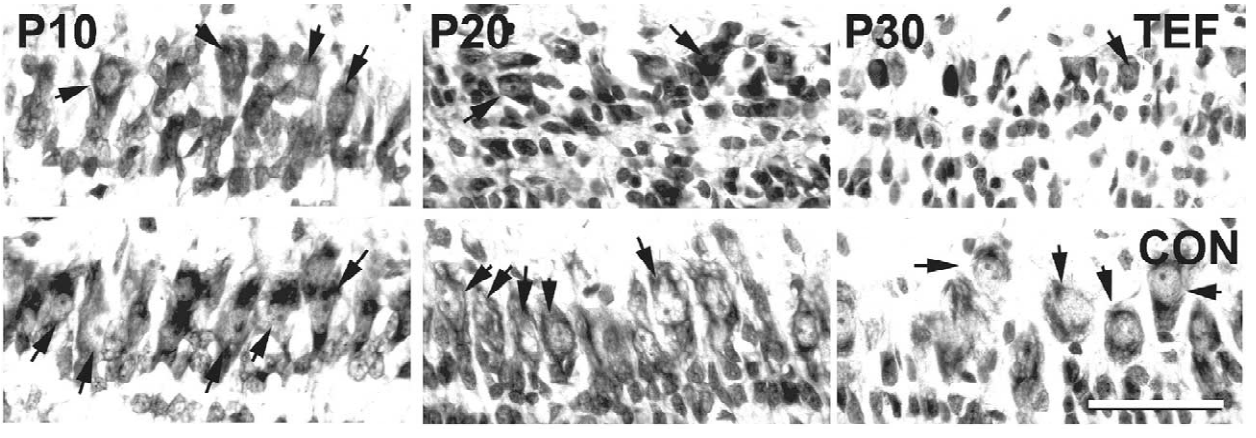

Fig. 2. Nissl-stained paraffin sections through the mitral cell layer in experimental (TEF) and control (CON) bulbs of P10, 20 and 30 rats. Profile size appears reduced by P20. Scale bar represents 100 μm.

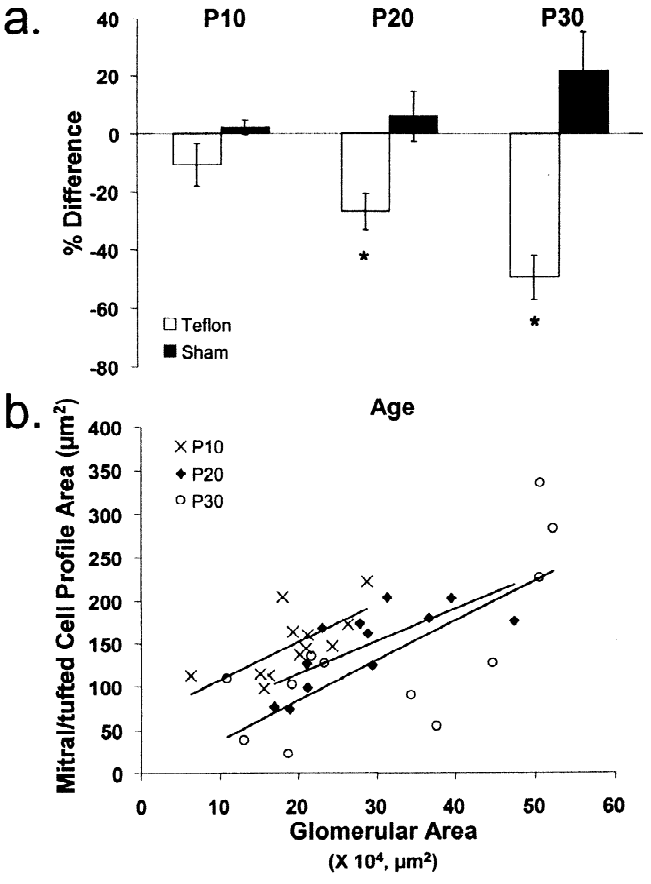

Fig. 3. (a) Comparison of mitral/tufted cell profile size in right and left bulbs from P10, 20 and 30 rats. Profile size was significantly reduced by P20 (*$P<0.05$). (b) Correlation between mitral/tufted cell profile area and glomerular layer area, an index of the degree of innervation (P10: $r^2=0.47$; P20: $r^2=0.56$; P30: $r^2=0.55$).

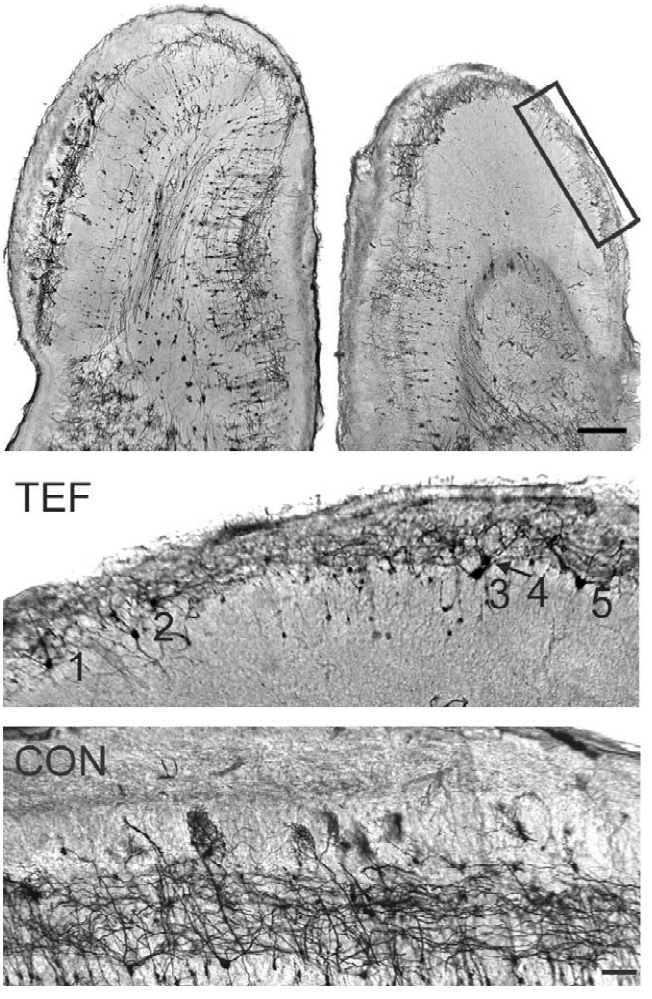

Fig. 4. Upper panel: Golgi-Cox stained horizontal section through the bulbs of a P30 rat showing experimental (right) and control bulbs (left). Scale bar represents 500 μm. Middle panel: High magnification photomicrograph of the boxed, lateral aspect of the experimental bulb shown in the upper panel. Mitral/tufted cells (1–5) were reconstructed in Fig. 5. Lower panel: High magnification photomicrograph of the lateral aspect of a control bulb showing numerous impregnated mitral/tufted cells with primary dendrites and apical tufts. Scale bar for middle and lower panels represents 100 μm.

larger soma, few dendritic spines and very extensive arbors.

Granule cell bodies were located in the mitral cell layer and throughout the granule cell layer to the subependymal zone. The small, ovoid somata usually gave rise to a number of spine-like appendages as well as basal and apical dendrites (Fig. 7b). The most identifiable feature was a lengthy apical dendrite extending radially as a relatively straight process that, after a variable distance, branched repeatedly. The branches, which occurred between the mitral cell layer and the superficial EPL, occurred in clusters at the apical extent as well as occasionally along the principal shaft and followed a more tortuous path than the parent process. They contained numerous spines, or 'gemmules', especially after the initial branch point. Variably sized varicosities along the initial dendritic segment as well as along the branches were also found. The spines themselves varied in size and type; both sessile and pedunculated varieties were found. According to the classification of Mori et al. [41], at least three types of granule cells were identified in control bulbs based on the location of their somata, branches and gemmules (Type I (Fig. 7b, cells 1, 2), Type II (Fig. 7b, cell 3), Type III (Fig. 7b, cells 4, 5)). Basal dendrites were much shorter than apical processes, though they could be quite complex. Like apical dendrites, they had a perpendicular orientation relative to the mitral cell layer, and often had a number of branches and spines. However, unlike apical dendrites, basal dendrites often originated from several locations on the cell body.

3.2.2. Experimental bulbs

A striking feature of Teflon-treated bulbs, and one noted in detail in the previous two papers [8,9], was the reduction in size of superficial bulb layers, and the apparent compression of cells and their processes (Fig. 1a). Of the three cell types examined, the morphology of the

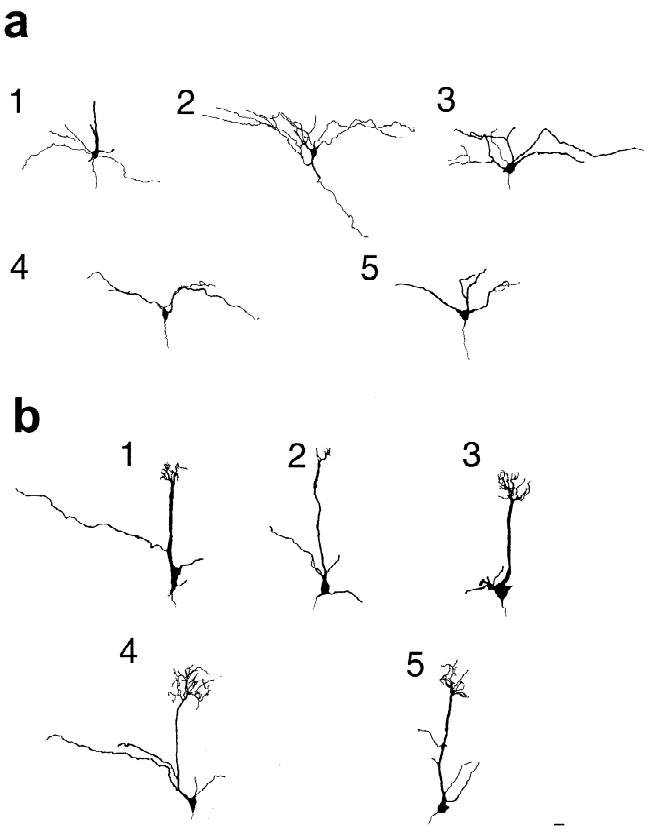

Fig. 5. Camera lucida reconstructions of mitral/tufted cells in the experimental bulb shown in Fig. 4 (a, 1–5) and in control bulbs (b) Scale bar represents 25 μm.

mitral/tufted cells appeared to be most affected by denervation. Although their structure differed considerably from mitral/tufted cells in normal bulbs, cells were still identifiable due to their large and characteristically shaped somata and position within the mitral cell layer (Figs. 1a and 2). In many cases, a primary dendrite was not discernible and apical tufts were never found (Fig. 5). Dendrites extended tangentially from the cell body coming within a few microns of the bulb surface or drooped towards the granule cell layer. Some cells contained many more processes than typically seen in normal tissue (Fig. 5a, cells 2, 3), primarily due to extra dendrites leaving the cell body, and/or from additional branches. Axonal branches (Fig. 5a, cell 2) were also encountered, a finding similar to that noted in HRP-injected tufted and Type II mitral cells (Ref. [44], although in normal bulbs these branches never re-entered the EPL).

Periglomerular cells in denervated bulbs were much more similar to those found in control tissue. In most cases, soma shape and size was normal, and no gross differences in dendritic morphology were observed (Fig. 6a), although an occasional cell with altered soma shape or size, or stunted dendritic extensions was found (Fig. 6a, cells 2, 3). Quantitative analyses indicated that the number of dendritic branches was nearly identical (means 7.6 vs. 6.6 in control vs. experimental pups), but the total length of branches in experimental pups was ~55% of that seen in controls. Mean total number of spines/dendritic length was very similar between the two groups (control: 15.4/145.2: experimental: 8.4/79.4; both values 0.106). Periglomerular cell position relative to other layers was less apparent due to the widespread loss of olfactory axons and superficial bulb layers. As was noted in control bulbs, they had no specific orientation relative to the bulb surface.

Golgi-filled granule cells were common in denervated bulbs. Although their structure was more variable than those found in innervated regions, they were still identifiable due to soma size and the characteristic extension of apical and basal dendrites (Fig. 7a). Nonetheless, their somata contained many more spine-like appendages and the dendrites appeared less organized than in control

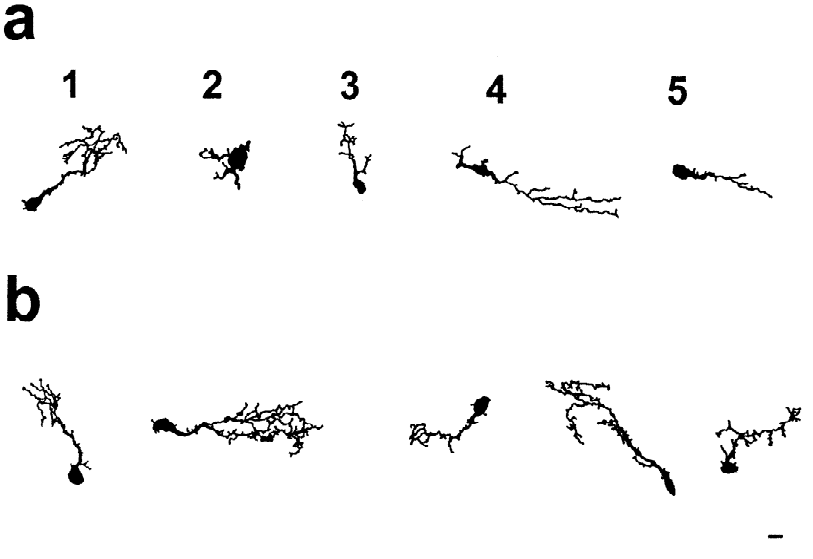

Fig. 6. Camera lucida reconstructions of periglomerular cells found in experimental (a; 1–5) and control (b) bulbs. Scale bar represents 25 μm.

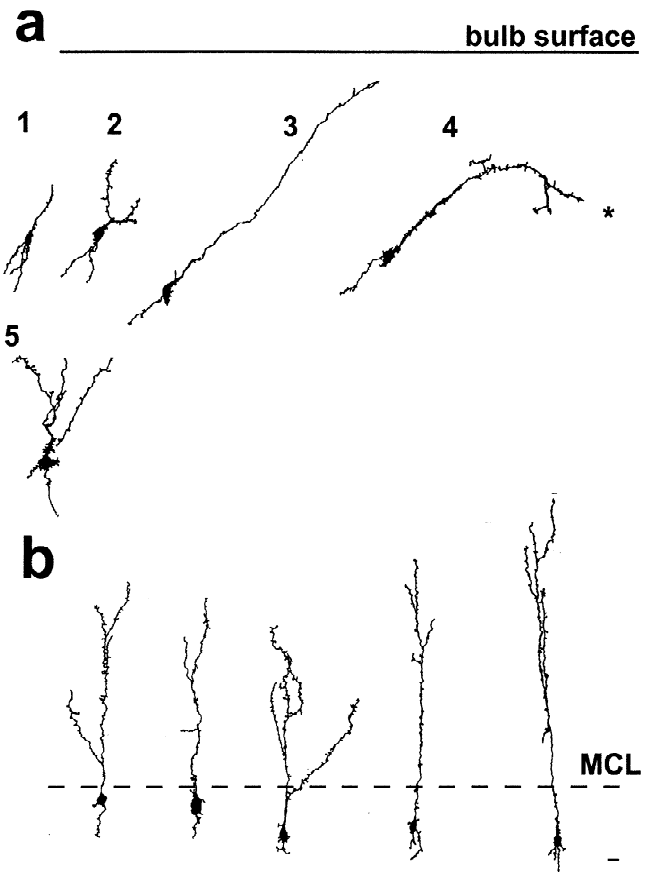

Fig. 7. Camera lucida reconstructions of granule cells found in experimental (a; 1–5, asterisk indicates mitral/tufted cell secondary dendrites) and control bulbs (b). Scale bar represents 25 μm.

regions. In denervated zones there were more branches that occurred closer to the cell body and dendrites covered a wider but shorter field, making the dendrites appear compressed within the shrunken EPL (Fig. 7a, cells 1, 2). In most cases, the initial segment and the branches of the apical dendrites extended at an oblique, rather than perpendicular, orientation to the bulb laminae/surface (Fig. 7a, cells 1–4). No obvious differences in the number or structure of dendritic spines were observed. Several cases of granule cells whose somata were centered in denervated zones but whose apical dendrites extended towards a pocket of innervation were found (Fig. 7a, cells 3, 4). In one such case, the apical dendrite was as long as those found in control bulbs, but it extended at an oblique angle relative to the mitral cell layer possibly due to a nearby region of innervation (Fig. 7a, cell 3). This dendrite had very few spines and only two, very short, branches. The apical extent of the dendrite was only a few microns away from the bulb surface. Another case in which the primary dendrite followed a similar oblique path, a granule cell's apical branches were seen in close proximity to the secondary dendrites of a mitral/tufted cell that lay deep to a few remaining glomeruli neighboring a region of denervation (Fig. 7a, cell 4). After extending diagonally away from the mitral cell layer the dendrite made an abrupt turn away from the bulb surface and towards the mitral/tufted cell's dendrites. The terminal branches of this cell appeared thicker than usual.

Quantitative analyses were similar to that for the periglomerular cells. While the total number of dendritic branches was nearly identical in the two groups, total dendritic length in the experimental group was only 68% of that in controls. Mean total number of spines/dendritic length were very similar between the two groups (control: 23.6/202.4 μm or 0.117; experimental: 19.4/139.6 μm or 0.139).

4. Discussion

The results outlined above, along with those from previous papers [8,9], indicate that focal denervation has varied effects depending on the cell class examined. For example, mitral/tufted cells undergo severe reductions in number, with remaining cells exhibiting shrunken or distorted morphologies. Surviving granule and periglomerular cells, however, maintain relatively normal morphology. Each of these groups will be described in more detail below.

4.1. Mitral/tufted cells

Our previous work showed that afferent denervation of the developing olfactory bulb causes a reduction in the number of mitral/tufted cells by P30, and MAP2 immunostaining indicated that there was a reorientation of neuronal processes in superficial bulb layers [8]. A second study indicated that these changes begin shortly after the manipulation is performed [9]. Although there is an impressive reduction in mitral/tufted cell number, perhaps even more intriguing is the fact that cells survive the manipulation given that their primary synaptic partners are no longer available. Results reported above (Fig. 3) indicate that average mitral/tufted cell soma size begins to decrease shortly after denervation, and steadily declines to 50% of the normal size by P30. The correlation between cell profile area and glomerular layer area suggests that contact with sensory afferents is directly responsible for the maintenance of normal somatic structure.

Mitral cell soma size is affected by a number of manipulations. For example, reductions occur after ablation of the olfactory epithelium early in development [49,50]. Rearing rats in an environment containing a preponderance of a single odorant, or in air that has been largely deodorized, results in regions of severe soma shrinkage [28,45,46,52]. Unilateral closure of an external naris, a procedure that reduces airflow over the ipsilateral sensory epithelium, also reduces mitral/tufted cell size [2,39] without reducing cell number [2,16] suggesting that remaining spontaneous activity may be sufficient for the

survival of sensory neuron targets. Soma size, therefore, appears to be a reasonable assay of alterations in the type and/or quantity of afferent input in the cell.

Analyses of Golgi-impregnated tissue revealed consistent, dramatic changes in mitral/tufted cell morphology. Several lines of evidence support the striking finding that mitral cells in the shadow of the Teflon implants rarely contain characteristic apical tufts. For example, MAP2 immunostained tissue revealed that glomerulus-like nets of fibers are rare in the denervated zone and that most processes are tangential to the bulb surface [8]. Furthermore, apical tufts were never observed in either the Golgi-stained or confocal studies reported above. Denervation-induced atrophy was not limited to the primary dendrite even though it is the only region directly exposed to olfactory sensory neuron contact. Degeneration of secondary dendrites, which receive contacts from other sources of stimulation, including granule cells, other mitral cells, and centrifugal inputs (reviewed in Ref. [14]), indicated that afferent activity is essential to the structural integrity of the whole cell. The correlation between mitral/tufted cell size and the area of the overlying glomerular layer suggests that sensory neuron contact and/or activity is directly responsible for maintaining cell structure. Our results also support the finding that the extent of denervation corresponds with changes throughout the bulb [37].

Denervated mitral/tufted cells in partially bulbectomized bulbs are capable of reorienting their primary dendrites towards novel glomeruli [17], but such plasticity was not observed following focal denervation. At least three reasons for such apparent immutability of connections exist: (i) unlike partial bulbectomy, focal denervation prohibits ingrowth of new axons into distinct regions of the bulb, implying permanent denervation for a specific population of mitral cells; (ii) existing glomeruli that are unaffected by the procedure retain their normal mitral/tufted cell targets, thereby restricting denervated cells from moving to occupy spared glomeruli; and (iii) maintenance of a precise topography between axons and their targets may prevent denervated cells from contacting spared or regenerated afferents. This hypothesis is supported by theories suggesting that the bulb functions through a number of relatively independent columnar units [6,20,24].

While mitral/tufted cells are the most mature cell type in the bulb at birth [22,39,40,42], some are relatively less mature and structural and functional development continues into the postnatal period [1,22,33–35]. Neonatal denervation may deprive cells of the stimulation required to achieve their mature form [36,55,57,58]. Indeed, many denervated mitral/tufted cells resemble those seen during early development [35,36,55]. Perhaps the cells were arrested in an immature state by denervation. The range of morphologies seen at birth (and perhaps after denervation) may permit sampling of a wider range of afferents [23,35,55]. However, several studies suggest that immature cells are less likely to survive denervation [10,38], implying that the only cells likely to be found in denervated regions must have been relatively mature (synaptically secure) at birth.

As expected, the effects of surgically deafferenting the bulb are more severe than those seen after functional denervation (reducing activity while leaving physical connections intact). For example, naris closure results in slowed dendritic development as does genetic knockout of the cyclic nucleotide gated channel thought to be essential in the stimulus transduction process [30,36]. Therefore, physical contact with afferent fibers is an important feature that regulates patterns of early maturation.

4.2. Interneurons

While the analyses of Golgi-impregnated tissue revealed consistent and dramatic changes in mitral/tufted cell morphology, only moderate alterations were observed in granule cell dendrites and few obvious differences noted in periglomerular cells. The relatively normal structure of granule cells was perhaps not surprising given their adaptability at the structural and ultrastructural levels [7,16,19] (but see Ref. [53]). Along the same lines, the similarity between periglomerular cells in denervated and control bulbs implies a degree of malleability in the face of extreme environmental changes. However, all three cell types examined here are comprised of heterogeneous groups [26,41,44,43] whose members may be differentially affected by denervation [8]. For example, our results suggest that tyrosine hydroxylase-, GABA- and calbindin-, but not calretinin-immunoreactive populations are lost following neonatal denervation [9]. Thus, our protocol may have selected for analysis those populations that are resistant to denervation.

A specific aim of this work was to examine the structural changes incurred in mitral/tufted, periglomerular and granule cells following focal denervation. We showed that removal of afferent contact caused structural changes in mitral/tufted cells that were reminiscent of less mature cells. In addition, denervation appears to have a less drastic effect on periglomerular and granule cell structure, perhaps due to increased plasticity or the survival of more robust subpopulations of these cells. Although the effects of denervation on mitral/tufted cells have been studied in developing animals [50], to our knowledge this is the first study to examine the effects of long-term, neonatal denervation on olfactory bulb cells.

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