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# The Morphological Change of Supporting Cells in the Olfactory Epithelium after Bulbectomy

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## Abstract

Transmission electron microscopy was used to study the responses of the supporting cells of the olfactory epithelium at 1–5 days after surgical ablation of the olfactory bulb (bulbectomy). In intact olfactory epithelium, lamellar smooth endoplasmic reticulum and rod-shaped mitochondria were distinctly observed in the supporting cells. On the first day after bulbectomy, bending of the microvilli and an increase in the smooth endoplasmic reticulum were observed. Cristae of the mitochondria became obscure, and the density of the mitochondrial matrix decreased. On the second day after bulbectomy, the number of microvilli decreased, broad cytoplasmic projections that contained cytoplasmic organelles protruded into the luminal side, and the mitochondria were swollen. On the fifth day after bulbectomy, microvilli seemed to be normal and some cells had large cytoplasmic projections that protruded toward the lumen of the nasal cavity. Within the cytoplasmic projections of the supporting cells, a large lamellar and reticular-shaped smooth endoplasmic reticulum was evident. Mitochondria exhibited almost normal morphology. The current findings demonstrate that morphological changes occur in the supporting cells after bulbectomy. This new evidence hypothesizes that these changes represent events that contribute to the regeneration of the olfactory epithelium after bulbectomy.

**Key words:** mice, microvilli of the supporting cells, mitochondria, regeneration, smooth endoplasmic reticulum, transmission electron microscope

## Introduction

The olfactory epithelium consists of olfactory receptor neurons, supporting cells, and basal cells (Farbman 1992). There is a region of continuous neurogenesis, cell proliferation, migration, and differentiation within the olfactory epithelium. Throughout the lifespan of vertebrates, there is a continuous replacement of the olfactory receptor neurons by basal cells (Graziadei and Graziadei 1979). In addition, the replacement of the olfactory receptor neurons also occurs in response to neuronal injury (Schwob 2002). Despite the relatively large number of studies that have examined the degeneration and regeneration of the olfactory epithelium following neuronal injuries (Monti Graziadei and Graziadei 1979; Costanzo 1991; Suzuki and Takeda 1993), many questions still remain unanswered. Additionally, there is very little known about the morphological changes that occur within the supporting cells.

Bulbectomy triggers a massive wave of degeneration of the olfactory receptor neurons (Costanzo and Graziadei 1983; Suzuki et al. 1996; Calof et al. 1998). Scanning microscopy has shown that there are no changes in the supporting cells after bulbectomy (Morrison and Costanzo 1989). However, with transmission electron microscopy (TEM), Suzuki et al. (1996) reported the presence of phagocytizing apoptotic bodies within the supporting cells. This study indicated that after bulbectomy in newborn mice, as many as 30% of the supporting cell profiles contained apoptotic bodies, cellar debris, and phagosomes in the cytoplasm. In addition, Monti Graziadei and Graziadei (1979) reported that there were membranous-bound bodies or crystalline materials within the supporting cells of the adult rat after axotomy. In contrast, Yaku and Saruta (1986) reported that there were no changes in the supporting cells of rat from 4 days to 1 year

after a bulbectomy. To date, there have been no TEM reports that have examined the early morphological changes in the supporting cells of adult mice after bulbectomy. Because these supporting cells have an important function in the regeneration of the olfactory epithelium, we focused our attention on the morphological changes that occur in these cells after bulbectomies.

## Materials and methods

### Animals and surgeries

This study used 12- to 16-week-old C57BL6 mice. All surgical procedures were carried out in accordance with guidelines approved by the Jichi Medical University Animal Care and Concern Committee. The mice were anesthetized with 2.4% isoflurane inhalation (180 ml/min). After cutting the right olfactory nerves under a stereomicroscope, vacuum aspiration with a fine stainless tube was used to remove the right olfactory bulb (bulbectomy).

The mice were underwent morphological examinations on days 1, 2, 3, and 5 after the unilateral bulbectomy. There were 4 mice in each group. A sham operation was performed in one group, which served as the control group.

### Tissue preparations for light and electron microscopy

After cardiac perfusion of cold physiological saline, the mice were perfused with a mixture containing 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 M cacodylate solution (pH 7.4) via the same route. Nasal septum epithelium and the neighboring epithelium covering the dome of the nasal cavity were carefully dissected out and immersed into the same fixative for 12 h. After washing with the cold cacodylate buffer, the specimens were postfixed with 1% osmium tetroxide solution buffered with cacodylate solution for 2 h on ice. Specimens were treated with 0.5% uranyl acetate for 2 h at room temperature, followed by dehydration with an ascending ethanol series. Specimens were then embedded in an Epon mixture.

For light microscopy, 0.5–0.7 μm thick Epon-embedded sections were stained with toluidine blue. For electron microscopy, ultrathin sections were mounted on a copper grid and then stained with uranyl acetate and lead citrate. Sections were observed using a JEM-2000EX electron microscope.

### Measurement of the thickness of the microvilli

To determine the effect of the olfactory bulbectomy, the thickness of the microvilli in the supporting cells was measured on the intact epithelium and the epithelium 1 day after bulbectomy using ×3000 magnification images. In 4 mice per day, distances were measured between the surface of the epithelium and the top of the microvilli layer.

## Results

### Light microscopy

Mouse olfactory epithelium was composed of tall, pseudostratified columnar epithelium (Figure 1A). The free surface of the epithelium was covered with a faintly stained brush border of supporting cells, olfactory vesicles, and mucus. Pale nuclei and unknown structures (which coincided with the lamellar smooth endoplasmic reticulum that was observed with electron microscopy) stained by toluidine blue were observed in the cytoplasm of the supporting cells. Heterochromatin was sometimes observed in the dark-stained nuclei of the olfactory receptor neurons.

On the first day after bulbectomy, the nuclei of the olfactory receptor neuron became homogeneous (Figure 1B). Although the number of unknown structures increased within the supporting cells, their nuclei presented almost identical profiles to that which was seen for the control epithelium. The stainability of the olfactory vesicles was somewhat reduced, and the thickness of the brush border of the supporting cells increased in the free surface of the epithelium.

On the second day after bulbectomy (Figure 1C), the number of olfactory vesicles reduced in the free surface of the epithelium. Numerous small empty vesicles also appeared in the apical area of the supporting cells. In the middle and lower parts of the epithelium, clearly stained apoptotic bodies induced by bulbectomy were observed among the olfactory receptor neurons. A few of them were observed in the supporting cells.

On the third day after bulbectomy (Figure 1D), olfactory vesicles were rarely recognized in the free surface of the epithelium. There was a widening of the intercellular space among the olfactory epithelial cells, especially in the lower part of the epithelium. The dark nuclei of the olfactory receptor neurons disappeared. The position of the supporting cells looked random, and the smooth endoplasmic reticulum in the cytoplasm became distinct.

On the fifth day after bulbectomy (Figure 1E), novel structures appeared in the free surface of the epithelium. Many cytoplasmic projections were frequently observed within the brush borders. In the epithelium, the shape of the epithelial cells became thinner, and the volume of intercellular space increased. There were no olfactory vesicles observed in the free surface of the epithelium.

### Electron microscopy

#### *Control (prebulbectomy) olfactory epithelium*

Figure 2A is an electron micrograph of the intact olfactory epithelium, which includes the free surface of the epithelium and the underlying olfactory receptor neurons and supporting cells. Ciliated olfactory vesicles extended above the free surface of the epithelium. Distinct tight junctions were

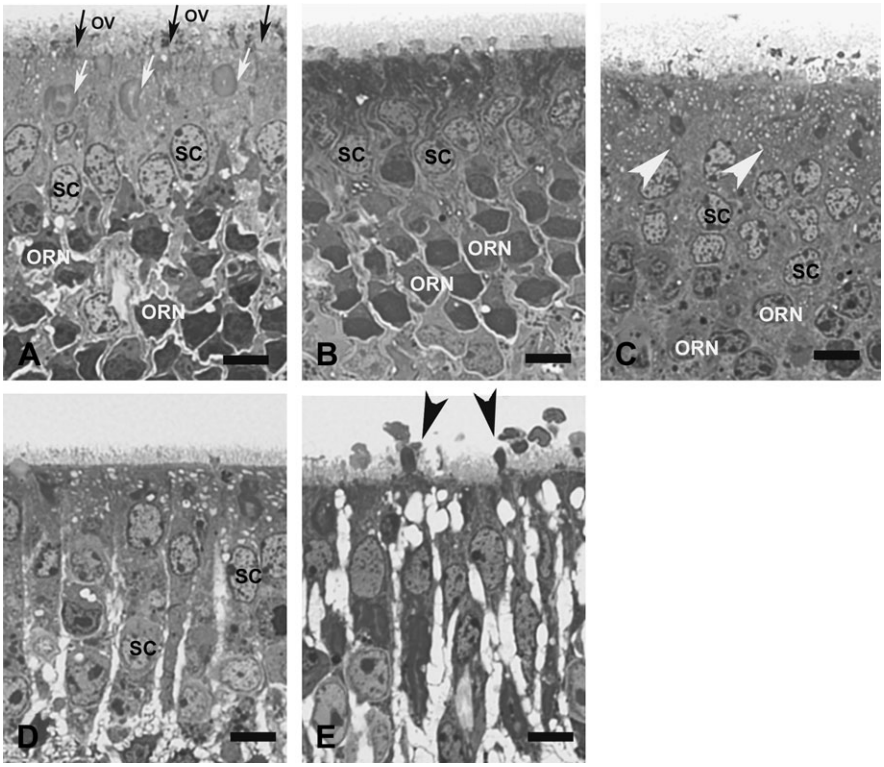


**Figure 1** Light micrograph of the olfactory epithelium. **(A)** Intact. At the free surface of the epithelium, the faintly stained brush border of the SC and the olfactory vesicle (OV, black arrow) were seen. In SC, unknown structures were observed (white arrow). **(B)** One day after bulbectomy. Unknown structures increased in number. The stainability of the olfactory vesicles remained unchanged. The thickness of the brush border of SC increased. **(C)** Two days after bulbectomy. Olfactory vesicles in the free surface of the epithelium were reduced in number. In the apical area, numerous small empty vesicles appeared (white arrowhead). **(D)** Three days after bulbectomy. Olfactory vesicles were barely recognized. The volume of the intercellular space increased, especially in the lower part of the epithelium. The dark nuclei of the ORNs disappeared. SC: supporting cell. **(E)** Five days after bulbectomy. Novel structures (black arrowhead) appeared in the free surface of the epithelium. Many cytoplasmic projections were frequently observed in the brush borders. The intercellular space was also very wide. No olfactory vesicles were seen in the free surface of the epithelium. SC, supporting cell; ORN: olfactory receptor neuron. Scale bars: 5 μm.

observed between the olfactory vesicles and the supporting cells. Microvilli were observed on the free surface of the supporting cells, while underlying this surface, an organelle-free terminal web area of cytoplasm was noted (Figure 2A, black arrow). At the apical portion of the cells, there were many rod-shaped mitochondria and membranous structures. The rod-shaped mitochondria were usually oriented along the long axis of the cell, and they contained an electron dense matrix (Figure 2B,C).

Close observations revealed that the membranous structures consisted of stacked lamellar smooth-surfaced endoplasmic reticulum. In the outer zone of these lamellae, 2 or 3 lamellae at the outer zone extended from this structure and encircled the rod-shaped mitochondria (Figure 2B). Careful observations revealed that at the edge of the stacked lamellar smooth endoplasmic reticulum, there was a continuance of the lamellar structures that extended to the dilated endoplasmic reticulum. These formed the reticular-shaped smooth endoplasmic reticulum (Figure 2C). In this study, we focused our attention on the relationship of the mitochondria and the smooth endoplasmic reticulum during the early stages of the degeneration. Lysosomes or secretory granules were rarely found during our observations.

### *Observations at 1 day after bulbectomy*

The free surface of the olfactory epithelium exhibited a complex structure. An increased thickness of the microvilli was noted in the supporting cells (Figure 3A). Total microvilli thickness (7.5 ± 0.75 μm) was increased as compared with that seen for the intact epithelium (4.8 ± 0.45 μm). The microvilli layer appeared to be 2 separate layers. One layer consisted of microvilli derived from the supporting cells that were arranged longitudinally (Figure 4B), whereas the other layer was located nearer to the mucus layer, with the microvilli appearing to be positioned perpendicular to the first layer. There were many vacuoles evident within the second layer (Figure 4A). These vacuoles were most likely derived from the degenerating cilia. Olfactory vesicles were overlaid with the degenerating microvilli and cilia.

Reticular-shaped smooth endoplasmic reticulum that was contiguous with the stacked lamellar smooth endoplasmic reticulum invaded into the organelle-free terminal web area

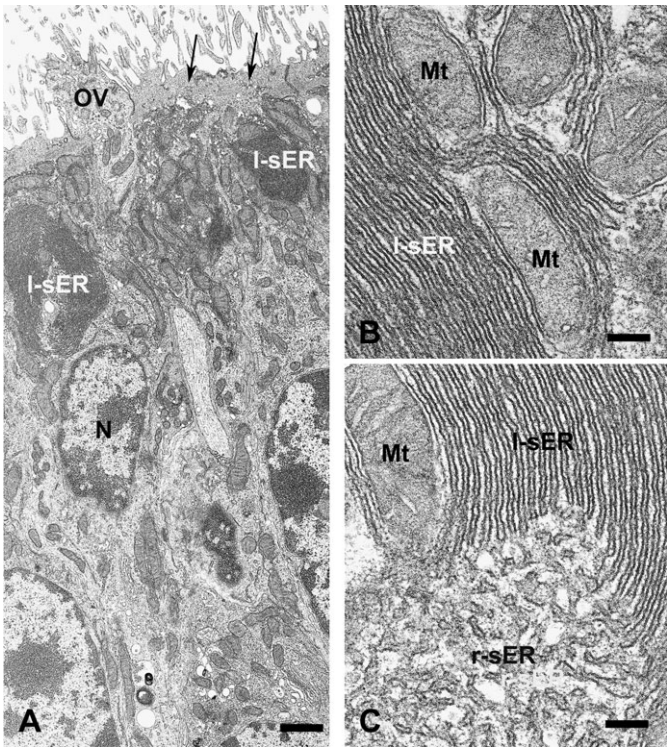


**Figure 2** **(A)** An electron micrograph of the intact olfactory epithelium. The ciliated olfactory vesicles (OVs) were recognized. There were many rod-shaped mitochondria, large l-sER, and nuclei (N) in the supporting cells. An organelle-free terminal web area was observed (black arrow). **(B)** Mitochondria (Mt) have an electron dense matrix. In the outer zone of the l-sER, 2 or 3 lamellae encircled the rod-shaped Mt. **(C)** At the edge of the l-sER, the lamellar structures were contiguous with the reticular-shaped smooth endoplasmic reticulum (r-sER). l-sER, lamellar smooth endoplasmic reticulum. Scale bars: **(A)** 1 μm; (**B** and **C**) 100 nm.

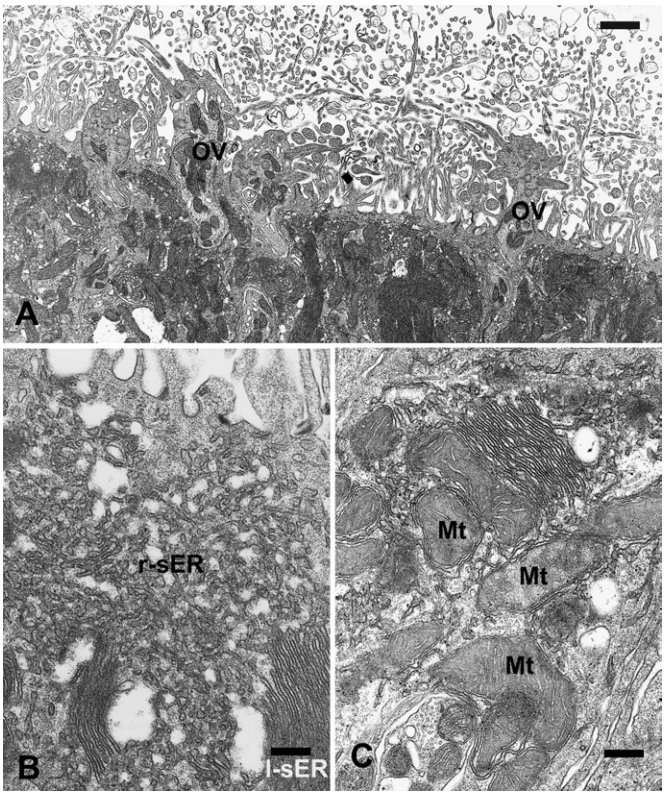


**Figure 3** An electron micrograph of the olfactory epithelium on the first day after bulbectomy. **(A)** The thickness of the microvilli from the supporting cells was increased. Apart from the free surface of the olfactory epithelium, the microvilli appeared to be located perpendicularly. **(B)** Reticular-shaped smooth endoplasmic reticulum (r-sER) invaded an organelle-free terminal web area of the cytoplasm. **(C)** Cristae of some of the mitochondria (Mt) became obscure and density of the matrix lowered. OV: olfactory vesicle. Scale bars: **(A)** 1 μm; (**B** and **C**) 200 nm.

of the cytoplasm that was underlying the free surface of the supporting cells (Figure 3B). The lamellar space of the smooth endoplasmic reticulum appeared to be more irregular than that seen in the intact areas. The cristae of the mitochondria become obscure, and there was a decrease in the density of the mitochondrial matrix (Figure 3C).

### *Observations at 2 days after bulbectomy*

At the free surface of the olfactory epithelium, the number of microvilli from the supporting cell surfaces decreased, and small dense material appeared within the transverse sections of the microvilli (Figure 5B). Instead of microvilli, we observed broad cytoplasmic projections with wide cytoplasmic bases protruding into the luminal side (Figure 5A). These projections contained undefined cytoplasmic organelles (Figure 5A, black arrow). In the organelle-free area under the apical cytoplasmic membrane of the supporting cells, a few unidentified cell organelles were also noted (Figure 5A, black arrow). Lamellar smooth endoplasmic reticulum was also observed (Figure 5A).

In the cytoplasm of the supporting cells, mitochondria were swollen, there was a decrease in the electron opacity, and the cristae became obscure. Closer observation revealed that the matrices of the swollen mitochondria contained amorphous materials and fragments of mitochondrial cristae. A close association existed between the lamellar smooth endoplasmic reticulum and the mitochondria. In some cases, multiple layers of stacked lamellar smooth endoplasmic reticulum encircled the degenerating mitochondria (Figure 5C).

In the lower part of the olfactory epithelium, many apoptotic bodies were observed. On occasion, these appeared to be involved with the supporting cells (Figure 6).

### *Observations at 3 days after bulbectomy*

The free surface of the olfactory epithelium was more prominent than that of the previous stage. The lamellar smooth endoplasmic reticulum became loose, and there was clear evidence of reticular-shaped smooth endoplasmic reticulum (Figure 7A). Underneath the cytoplasmic membrane of the free surface of the supporting cells, small

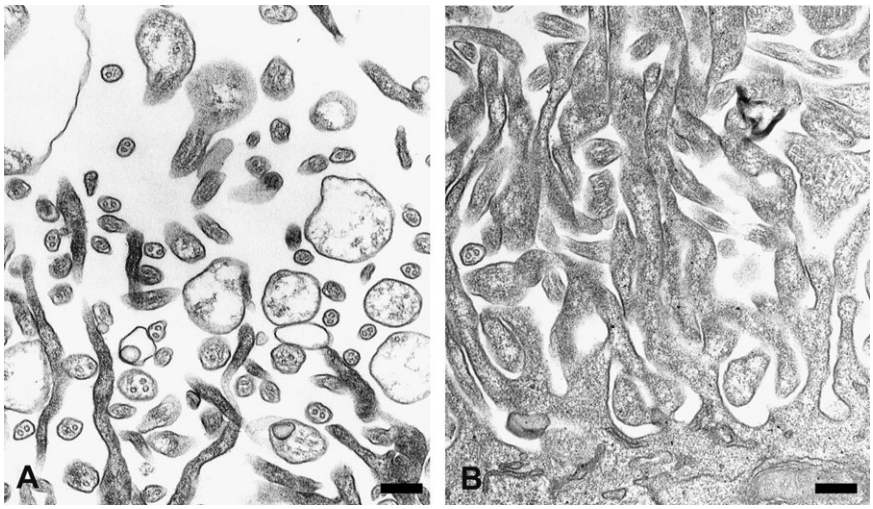


**Figure 4** An electron micrograph of the olfactory epithelium on the first day after bulbectomy. **(A)** Upper part of the microvilli from the supporting cells. The microvilli appeared to be located perpendicularly. There were many vacuoles derived from the degenerating cilia. **(B)** Lower part of the microvilli from the supporting cells. The microvilli appeared to be located longitudinally. Scale bars: 200 nm.

vesicular or reticular-shaped smooth endoplasmic reticulum was clearly observed (Figure 7B). With higher magnification, a close association between the smooth endoplasmic reticulum and the mitochondria was observed (Figure 7C). However, as compared with that seen in the previous stages, the interstices of each lamella of the lamellar smooth endoplasmic reticulum appeared to be somewhat wider. In addition to these findings, the reticular-shaped smooth endoplasmic reticulum was found to be contiguous with the lamellar structures (Figure 7D).

### *Observations at 5 days after bulbectomy*

As compared with the third day after bulbectomy, on the fifth day, there were drastic changes noted in the morphology of the olfactory epithelium. The overall size and shape of the supporting cells became smaller and thinner. Although there were extremely wide intercellular spaces between the adjacent cells, the junctional complex was maintained. At the free area of the cell lining, microvilli seemed to be normal and there were large cytoplasmic projections that protruded toward the lumen of the nasal cavity in some cells. At the mucus layer, a large lamellar smooth endoplasmic reticulum was evident within the cytoplasmic projections of the supporting cells. No olfactory vesicles or receptor neuron dendrites were visible (Figure 8A).

Examination of the supporting cell projections at a higher magnification indicated the presence of clear lamellar smooth endoplasmic reticulum and reticular-shaped endoplasmic reticulum, which were contiguous with each other. However, no secretory granules or lysosomes were observed (Figure 8B).

In the cytoplasm of the apical portion of the supporting cells, the large lamellar smooth endoplasmic reticulum and the small lamellar structure adjacent to the mitochondria appeared to have normal morphologies. In addition, a reticular-shaped smooth endoplasmic reticulum was also observed (Figure 8C).

## Discussion

Many researchers have reported morphological studies on the olfactory epithelium after bulbectomy (Costanzo and Graziadei 1983; Costanzo 1991; Suzuki and Takeda 1993; Calof et al. 1998). However, these studies focused on the degeneration and regeneration of the olfactory receptor neurons. Other than this information, little else is known about the morphological changes taking place during this time that involve the supporting cells in the olfactory epithelium. Therefore, the aim of this study was to examine the changes that occur in the supporting cells of the olfactory epithelium after bulbectomy.

Although little information has been reported with regard to what role the supporting cells play within the olfactory epithelium, it has been suggested that these cells are involved in mucus production or in regulation (Frisch 1967). Kern et al. (1991) and Fong et al. (1999) reported finding $Na^+/K^+$ATPase immunoreactivity in the supporting cells, and thus, they suggested that the supporting cells participate in the maintenance of $Na^+$ in the olfactory mucus. Robinson and Kern reported finding glucocorticoid receptors (1998) and mineralocorticoid receptors (1999) in the supporting cells. Based on a scanning electron microscopy study, Morrison and Costanzo (1989) suggested that the supporting cells filled the space for the newly developing neurons after bulbectomy. Based on a TEM study, Monti Graziadei and Graziadei (1979) reported the presence of membrane-bound bodies and crystalline materials

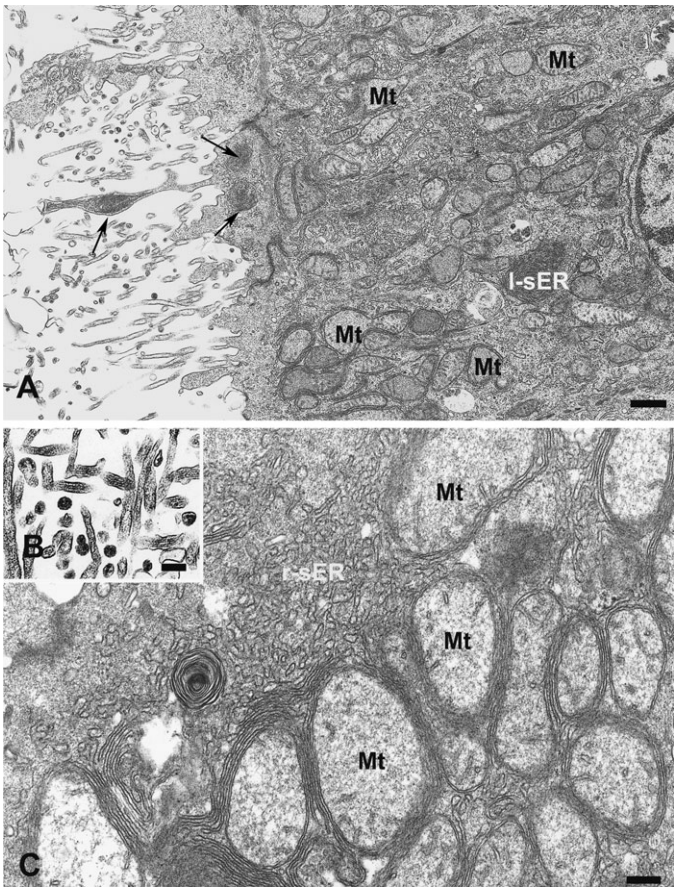


**Figure 5** An electron micrograph of the olfactory epithelium on the second day after bulbectomy. **(A)** Only a small number of microvilli and broad cytoplasmic projections were present. In the organelle-free area under the apical cytoplasmic membrane of the supporting cells, undefined cell organelles sometimes appeared (black arrow). **(B)** Microvilli with small dense materials began to appear. **(C)** Mitochondria (Mt) were swollen. The electron opacity decreased in addition to the cristae becoming obscure. Lamellar smooth endoplasmic reticulum (l-sER) encircled the degenerating Mt. Scale bars: **(A)** 1 μm; (**B** and **C**) 200 nm.

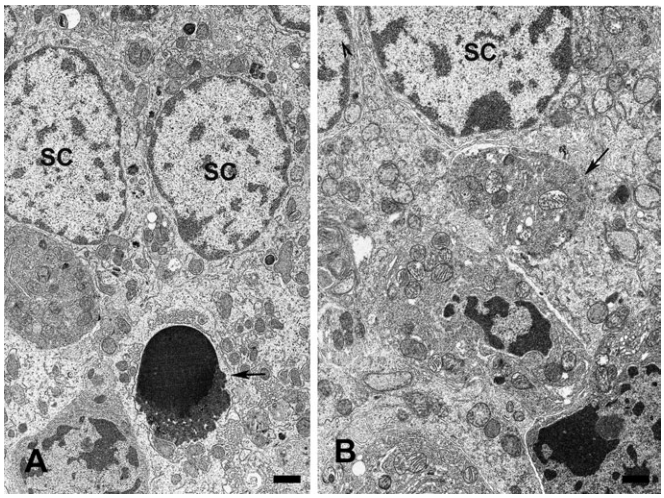


**Figure 6** An electron micrograph of the olfactory epithelium on the second day after bulbectomy. **(A)** An apoptotic body (black arrow) was seen in the supporting cell. SC: nuclei of the supporting cells. **(B)** A part of the degenerating olfactory receptor neuron cytoplasm (black arrow) was seen in the supporting cell. SC: nuclei of the supporting cell. Scale bars: 1 μm.

within the supporting cells at the third day after axotomy. Suzuki et al. (1996) also reported the morphological changes that occur in the olfactory epithelium after bulbectomy. This study demonstrated that after bulbectomy in mice at approximately 1 day after birth, the resultant degeneration of the olfactory receptor neuron leads to the supporting cells acting as phagocytes. In the current study, we also observed unusual phagocytic activity by the supporting cells and found new evidence for the morphological changes that are observed during the early degenerative changes that occur following bulbectomy in adult mice.

Mouse olfactory epithelium is classified as pseudostratified epithelium. In our light microscopic study, we used plastic embedded materials so that it was possible to prepare 0.5–0.7 μm thick sections that could be stained with toluidine blue. This technique provided us with clear morphological details of the olfactory epithelium. Our findings indicated that there were unknown structures within the apical portion of the supporting cells. This structure might possibly coincide with the lamellar smooth endoplasmic reticulum that was depicted in our electron micrographs. On the second day after bulbectomy, we observed small empty vesicles in the apical portion of the supporting cells. These vesicles might represent the ballooned degenerative mitochondria that were documented by electron microscopy on the second day after bulbectomy. Our findings also indicated that the free surface of the olfactory epithelium exhibited morphological changes. Olfactory vesicles that were clearly identified in intact olfactory epithelium were reduced and barely recognizable on the third day after bulbectomy. On the other hand, novel structures appeared in the free surface of the epithelium on the fifth day after the bulbectomy. These structures may represent cytoplasmic projections of the supporting cells that contain the clear lamellar smooth endoplasmic reticulum and the contiguous reticular-shaped smooth endoplasmic reticulum.

Furthermore, ultrathin sections that were stained with uranyl acetate also provided full details on the olfactory epithelium. In intact olfactory epithelium, we were able to recognize the large lamellar smooth endoplasmic reticulum of the supporting cells. Generally, smooth endoplasmic reticulum has a central role in lipid and protein biosynthesis. It is abundant in cells that synthesize steroid hormones from cholesterol.

Another cell known to have abundant smooth endoplasmic reticulum is the hepatocyte. In this cell, the smooth endoplasmic reticulum contains enzymes that are responsible for carrying out detoxification reactions,

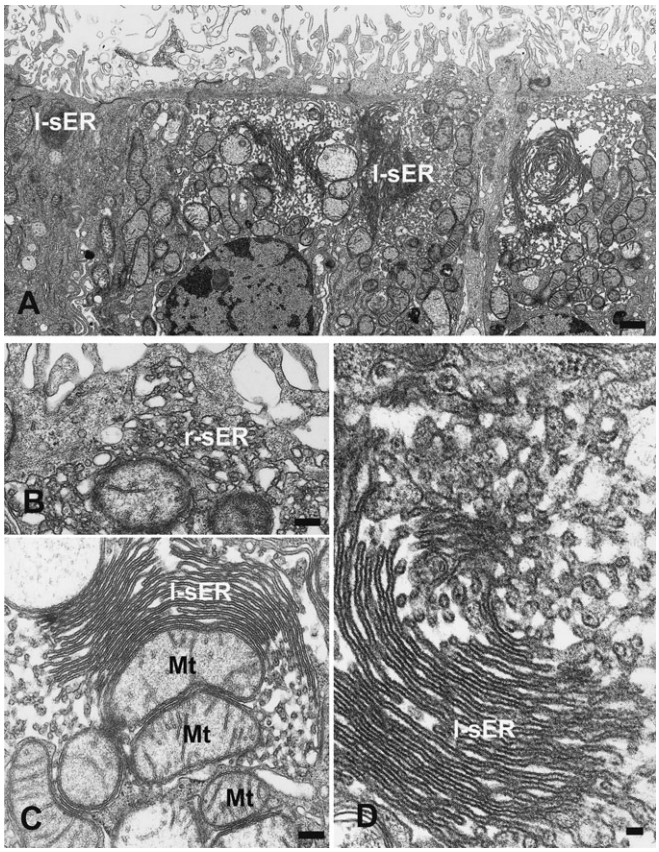


**Figure 7** An electron micrograph of the olfactory epithelium on the third day after bulbectomy. **(A)** The lamellar smooth endoplasmic reticulum (l-sER) became loose, and the reticular-shaped smooth endoplasmic reticulum (r-sER) was evident. **(B)** Under the cytoplasmic membrane of the free surface under the supporting cells, the r-sER was clearly observed. **(C)** The l-sER, the r-sER, and the mitochondria (Mt) were closely associated. **(D)** The r-sER was contiguous with the lamellar structures. Scale bars: **(A)** 1 μm; **(B–D)** 200 nm.

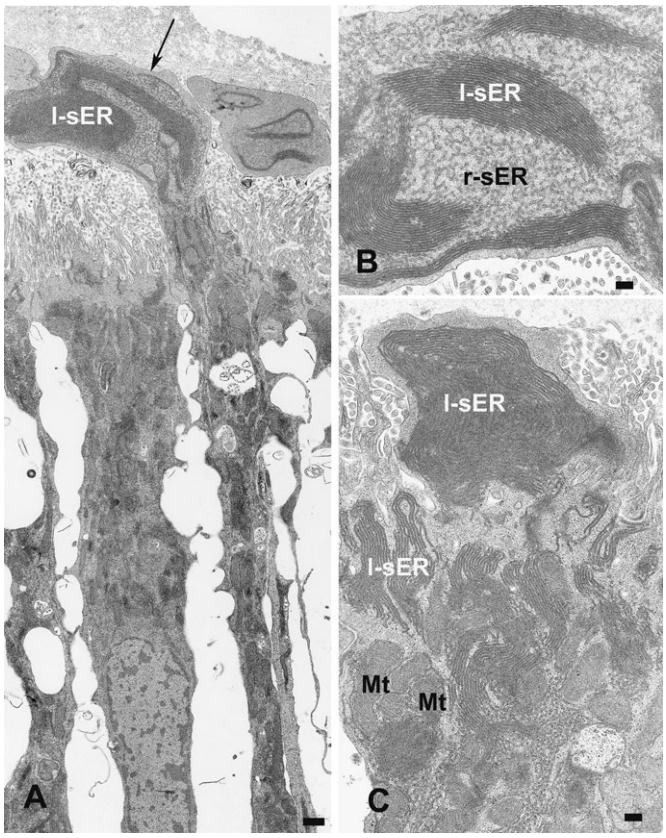


**Figure 8** An electron micrograph of the olfactory epithelium on the fifth day after bulbectomy. **(A)** The supporting cells became thinner, and the intercellular spaces widened. Microvilli seemed to be almost normal, and some cells had a large cytoplasmic projection that protruded toward the lumen of the nasal cavity (black arrow). **(B)** The projections of the supporting cells illustrated the clear lamellar smooth endoplasmic reticulum (l-sER) and the reticular-shaped smooth endoplasmic reticulum (r-sER), which was contiguous with the l-sER. **(C)** In the cytoplasm of the apical portion of the supporting cells, the l-sER was adjacent to almost normal mitochondria and the r-sER was observed. Scale bars: **(A)** 1 μm; (**B** and **C**) 200 nm.

such as the cytochrome P450 family of enzymes (Alberts et al. 2002). It has also been reported that the supporting cells express high levels of the cytochrome P450 isoform (Zupko et al. 1991; Rodriguez et al. 2008). Therefore, the primary role of the large smooth endoplasmic reticulum in the supporting cells might involve detoxification reactions.

On the first day after bulbectomy, the thickness of the degenerating microvilli of the supporting cells increased. In addition, the lamellar smooth endoplasmic reticulum also increased. These results suggest that after bulbectomy, changes to the smooth endoplasmic reticulum itself or changes in its surrounding environment might be the factors responsible for the subsequent reaction that takes place.

Olfactory receptor neurons are connected to the olfactory bulb. Even though the supporting cells do not have a direct connection with the olfactory bulb, there is a direct effect on these neurons after a bulbectomy. Thus, the question that needs to be answered is what kind of mechanism is responsible for the induction of the morphological changes that occur in these supporting cells. One obvious area that needs to be considered is the tight junction that is found between the supporting cells and the olfactory receptor neurons. It is well known that the surface of the epithelium is lined with olfactory receptor neurons and supporting cells. They are bound together and attached via the use of distinct attachment devices, such as tight junctions (Frisch 1967; Graziadei 1971; Wang and Halpern 1980; Morrison and Costanzo 1989). Many researchers have reported that bulbectomy induces olfactory receptor neuron degeneration. This olfactory receptor neuron degeneration involves both olfactory vesicles and dendrites and subsequently causes the destruction of the tight junctions between the supporting cell and the olfactory receptor neurons (Masukawa et al. 1985). This particular type of destruction may be one of the important factors that contribute to the

induction of the morphological changes that are seen in the supporting cells.

On the second day after bulbectomy, mitochondria of the supporting cells began to balloon and degenerate. We also noted a similar change of the mitochondria in the olfactory receptor neurons that began at relatively the same time (data not shown). However, the increase in the reticular-shaped and lamellar smooth endoplasmic reticulum around the ballooned degenerative mitochondria was only seen in the supporting cells, and by the fifth day after the bulbectomy, these changes were completely replaced by a normal morphology. On the other hand, death of olfactory receptor neurons occurs by apoptosis that associated with condensation of the cell nucleus and fragmentation of DNA (Kerr et al. 1972). In this study, we observed evidence of phagocytosis of olfactory receptor neuron debri following bulbectomy, but our impression is that these events were fewer in number than that reported by Suzuki. The difference in ages, neonates versus adults, might explain this difference. Nevertheless, our results suggest that the supporting cells in adult mice might also have the phagocytizing ability.

Therefore, our data might suggest that the increased smooth endoplasmic reticulum within the supporting cells can either lead to the rescue of degenerated mitochondria or provide protection from exogenous materials, such as apoptotic bodies.

Normally, when large quantities of certain compounds, such as phenobarbital, enter the circulation, within a few days the hepatocytes synthesize detoxification enzymes. During the time that this enzyme synthesis occurs, the smooth endoplasmic reticulum doubles in size. Once the drug disappears, a lysosome-dependent process called autophagocytosis rapidly removes the excess smooth endoplasmic reticulum (Venditti et al. 1998; Alberts et al. 2002). In the current study, protrusions were seen from some supporting cells on the fifth day after the bulbectomy. These large cytoplasmic projections protruded into the lumen of the nasal cavity and were found to contain large lamellar smooth endoplasmic reticulum. Therefore, it might very well be that it is the smooth endoplasmic reticulum within the supporting cells that is responsible for much of the P450 produced for these protection reactions. However, because we found very little if any lysosome within the supporting cells, it might be that in this case the excess smooth endoplasmic reticulum is removed via a mechanism that involves the cytoplasmic projections moving toward the lumen of the nasal cavity.

It is well known that olfactory receptor cells begin to proliferate and differentiate at approximately 5–7 days after bulbectomy (Morrison and Costanzo 1989). Because regeneration of the olfactory receptor neurons begins after the recovery of the supporting cells, our results suggest that the supporting cells contribute to the regeneration of the olfactory epithelium after bulbectomy.

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