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A New Surgical Approach to the Study of Vomeronasal System Regeneration

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The vomeronasal system, including the vomeronasal organ, the accessory olfactory bulb and higher centers, functions to detect substances associated with social and reproductive behavior (Wysocki, 1979; Halpern, 1987). The vomeronasal epithelium has a structure similar to that of the olfactory epithelium, consisting of receptor neurons, supporting cells and precursor cells (Matsuoka *et al.*, 2000). The axons of vomeronasal sensory cells terminate in the accessory olfactory bulb, while axons of olfactory sensory cells project directly to the main olfactory bulb (Matsuoka *et al.*, 1998; Rodriguez *et al.*, 1999). In rodents, the accessory olfactory bulb is located at the posterocaudal region of the main olfactory bulb.

The olfactory and vomeronasal systems provide ideal models for the study of neural degeneration and regeneration, because a continual neurogenesis of olfactory and vomeronasal neurons occurs during development and under normal physiological conditions in adult animals (Moulton, 1974; Graziadei and Monti-Graziadei, 1978). However, more dramatic increases in the number of the sensory cells have been observed after injury. Previous studies have examined regeneration and recovery of both systems following different methods of injury, including removal of the olfactory and accessory olfactory bulbs, chemical lesion of the sensory epithelium and transection of nerve sensory fibers (Costanzo and Graziadei, 1983; Samanen and Forbes, 1984; Costanzo, 2000; Matsuoka *et al.*, 2002). Different surgical methods have their advantages and disadvantages. Methods developed to cut the olfactory nerves at the cribriform plate also cut the vomeronasal nerves. Selective transection of the vomeronasal nerve at the level of the cribriform plate is not possible using current methods. Removal of the olfactory bulbs provides complete degeneration in the vomeronasal system. However, this method is not reversible, since there is no target tissue remaining for the regenerated nerves to reestablish functional connections and enable complete recovery. To study vomeronasal system function and recovery, a method is needed to selectively cut the vomeronasal nerves while leaving the olfactory system intact. In this study, we tried to establish a new method to selectively cut only the vomeronasal nerves.

Using adult male mice, we developed a new surgical approach that cuts the vomeronasal nerves at a point just anterior to the accessory olfactory bulb. After anesthesia, the frontal bone over the olfactory bulb is removed. This provides good access to the olfactory bulbs and frontal cortex. We then cut the vomeronasal nerve at the borderline between the olfactory bulb and the frontal cortex using vannis micro dissecting scissors. Next we examined the vomeronasal epithelium, the olfactory epithelium and their sensory nerve terminals within the main and accessory olfactory bulb at 6, 20, 60 and 120 days after surgery. In order to study the degeneration and regeneration of vomeronasal neurons, we used an olfactory cell marker called the olfactory marker protein (OMP). OMP is an acidic protein which is expressed in both olfactory and vomeronasal neurons including cell bodies, axons and nerve terminals. Although its function is unknown, OMP has been used extensively as a marker for mature chemosensory cells in the olfactory system (Margolis, 1980).

We observed OMP reactivity of vomeronasal nerve terminals in the accessory olfactory bulb at several time points after vomeronasal nerve transection. A rapid reduction in OMP positive nerve terminals within the accessory olfactory bulb was observed 6 days after surgery and no OMP positive terminals were present in any of the animals examined at 20 and 60 days. Interestingly, in some animals examined on day 120, OMP positive nerve terminals were observed in the accessory olfactory bulb. But, the size and distribution of these terminals was reduced compared with those in control animals. In addition, we observed the expression of OMP reactivity in the vomeronasal epithelium after surgery. In control animals, OMP positive neurons were present throughout the vomeronasal epithelium. A reduction in the number of OMP positive neurons was observed in the vomeronasal epithelium at 6 days after surgery. By 20 and 60 days, there were no OMP positive neurons present in the epithelium. At 120 days after surgery, OMP positive neurons were observed in some animals while others showed no signs of recovery. Interestingly, in those animals where recovery was observed, OMP positive nerve terminals were also observed in the accessory olfactory bulb.

We also examined the olfactory epithelium and the main olfactory bulb after surgery. OMP positive glomeruli in the main olfactory bulb were present at all recovery times (in controls and 6, 20, 60 and 120 days after surgery). There was little or no damage to olfactory bulb connections. OMP positive neurons in the olfactory epithelium at the same time points in recovery showed no changes following the vomeronasal nerve transection procedure. These results are consistent with the intact olfactory nerve terminals observed in the main olfactory bulb.

In the present study, we observed a complete reduction of OMP positive neurons in the vomeronasal epithelium and terminals within the accessory olfactory bulbs after surgery. This finding suggested that this new approach to cut the vomeronasal nerve was very effective. We confirmed the reduction of OMP reactivity in the accessory olfactory bulbs of all animals examined at 20 and 60 days after surgery. The number of mice in each group was >15, so we are confident that our surgery performed a complete transection of the vomeronasal nerves. An additional advantage of this method is that there was minimal or no damage to the olfactory system, including the olfactory epithelium and main olfactory bulb. Our findings suggest that this new surgical method is effective in making a complete and selective transection of the vomeronasal nerves.

Using this new surgical method, we can observe regeneration and recovery in the vomeronasal system. In some animals we observed recovery of OMP reactivity in the accessory olfactory bulb. Interestingly, where epithelial recovery was observed on one side but not the other, recovery of OMP positive nerve terminals in the accessory olfactory bulb was observed only on that same side. These findings suggest that recovery of the vomeronasal epithelium may require successful retargeting of nerve terminals in the accessory olfactory bulb. Retargeting of the vomeronasal nerves to the accessory olfactory bulb may depend on several factors, for example, tissue damage within the surgery area, the position of the cutting blades and postoperative bleeding. Tissue damage and gliosis within the surgery area

may be one of the most important factors. It is likely that if there is significant tissue damage and gliosis in the surgery area, regenerated fibers will not be able to proceed through the damaged area. Other factors may affect the regenerative capacity of the vomeronasal nerves, however, specific factors have not yet been identified.

The surgical approach reported in this study provides a new method for the complete and selective transaction of vomeronasal nerves. It should prove useful in future studies of vomeronasal system function, since it has the distinct advantage of leaving the olfactory system intact.

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