

Vomeronasal Systems in Aquatic Salamanders: Axolotls Smell Better Than Fish

**Heather Eisthen, Dale Sengelaub,
and Jeff Alberts**

**Department of Psychology and Program in
Neural Science, Indiana University
Bloomington, IN 47405**

Axolotls possess numerous sensory systems, including gustation, electro- and mechanoreception, vision, tactile senses, and olfaction. But axolotls also have another chemosensory system that current theories predict they shouldn't have: axolotls have a vomeronasal system.

Most mammals, reptiles, and amphibians possess both olfactory and vomeronasal systems. Although both detect chemical stimuli, these systems are morphologically and functionally different. Olfactory and vomeronasal systems can generally be distinguished by receptor type and projection site (reviewed in Wysocki and Meredith, 1987; Price, 1987). In vertebrates the olfactory receptors are ciliated and project to the main olfactory bulb at the rostral end of the telencephalon. In animals that possess both olfactory and vomeronasal systems, the receptors are located in physically separate regions of the nasal cavity. Vomeronasal receptors have microvillar processes and project to the accessory olfactory bulb, dorsal and caudal to the main olfactory bulb. Secondary projections of the vomeronasal system include the amygdala and hypothalamus; unlike the olfactory system, the vomeronasal system does not project to cortex.

Olfactory and vomeronasal systems can also be distinguished functionally, based on the type of stimuli to which they respond. In terrestrial animals, only volatile molecules have access to the olfactory epithelium, and so olfactory receptors respond to low-molecular-weight compounds. In contrast, the receptors of the vomeronasal system have been shown to respond to high-molecular-weight, or nonvolatile, molecules (Wysocki, Wellington, and Beauchamp, 1980; Wang, Chen, Jiang, and Halpern, 1988). In mammals the vomeronasal system is involved in reproductive and social

behaviors (Wysocki, 1979), whereas in snakes the system is also involved in prey detection (Halpern and Kubie, 1984).

Given the different functions of these systems, which came first? To answer this question, most researchers have looked to fish and amphibia to determine which systems are possessed by which animals. Attempts to trace the phylogenetic history of olfaction and vomeronasal chemoreception have rested largely on comparative morphology of nasal cavities and receptor types, bolstered by the prevailing view of the function of each of the systems. Thus Broman (1920) compared the nasal epithelium in fish to the vomeronasal and olfactory epithelia in lizards and mammals and concluded that the fish nasal epithelium was vomeronasal. Broman also mistakenly believed that the same nerve innervates the fish nasal epithelium and the tetrapod vomeronasal epithelium. After he observed that both that the fish nasal cavity and the mammalian vomeronasal organ are fluid filled, Broman proposed that the vomeronasal system is phylogenetically older than the olfactory system. As recently as 1970, Parsons supported Broman's hypothesis in his review of the morphology of the nasal cavity and chemosensory epithelia.

Bertmar (1981), however, has examined the morphology of the nasal cavity in a variety of tetrapods and has proposed that the evolution of the vomeronasal organ was closely associated with the evolution of terrestriality in vertebrates. Recent studies of the ultrastructure of nasal chemosensory epithelia (Derivot, 1984) have found that fish possess a ciliated epithelium, indicating that the fish possess an olfactory but not a vomeronasal system. Combined with information indicating that the vomeronasal system responds to nonvolatile molecules, Bertmar's hypothesis forms a plausible argument. Because many nonvolatile molecules can be dissolved or suspended in water, perhaps the olfactory epithelium detects high-molecular-weight compounds in aquatic animals such as fish; the vomeronasal system then arose to detect such compounds on land. Not surprisingly, then, the vomeronasal system has been found to be absent in secondarily-aquatic mammals and reptiles (Mackay-Sim, Duvall, and Graves, 1985; Parsons, 1967).

Given this view, we would expect that an aquatic amphibian like the axolotl would also lack a vomeronasal system. Much of our understanding of the peripheral vomeronasal system in salamanders comes from the work of

comparative anatomists around the turn of the century. Seydel (1895) and Anton (1908) described the anatomy of the nasal cavity in representatives of six families of salamanders. In terrestrial salamanders, the vomeronasal epithelium is located in a diverticulum, or pouch, in the lateral wall of the nasal cavity. The primary and secondary projection sites of the vomeronasal receptors in the tiger salamander, *Ambystoma tigrinum*, have been described in great detail by C.J. Herrick (1921, 1924, 1948). Herrick reported that the accessory olfactory bulb of salamanders, unlike that of other tetrapods, does not contain glomeruli, and that the accessory olfactory bulb projects largely to the amygdala. More recently, Dawley and Bass (1988) have described the vomeronasal epithelium and nasal cavity in the terrestrial Eastern red-backed salamander, *Plethodon cinereus*. When presented with stimuli from conspecifics, plethodontids display a stereotyped nose-tapping behavior. This behavior appears to facilitate the entry of heavy molecules into the nasolabial groove, which draws stimuli into the lateral pouch (Dawley & Bass, 1989). The mechanism of stimulus access to the lateral pouch in salamanders that lack a nasolabial groove remains unknown.

We have examined sections of the nasal cavity and forebrain in adult axolotls and have found both the peripheral components of a vomeronasal system and an accessory olfactory bulb. The nasal cavity appears similar to that of other salamanders: the cavity extends from the external nostril on the dorsal part of the snout to the internal nostril in the roof of the mouth and is essentially an elongated tube with a lateral pouch. In cross sections that have been stained with cresyl violet, we have observed physically separate receptor populations in the nasal cavity. The medial wall of the cavity appears to contain ciliated olfactory receptors, and the lateral pouch contains a non-ciliated sensory epithelium, corresponding to the locations of the olfactory and vomeronasal epithelia in other salamanders. We have also traced the forebrain projections of these receptor populations using horseradish peroxidase (HRP). In animals in which we placed HRP into the medial nasal cavity we found labeled fibers throughout the olfactory nerve, and these fibers terminated in discrete glomeruli in the main olfactory bulb. In contrast, after placement of HRP into the lateral pouch we found label restricted to fibers on the lateral edge of the olfactory nerve. These fibers terminated in a small, aglomerular region lateral and caudal

to the main olfactory bulb, corresponding to the accessory olfactory bulb described by Herrick (1921, 1948) for the tiger salamander. Although we have not confirmed the identity of the vomeronasal receptors by demonstrating that they are microvillar, our data indicate that axolotls possess a vomeronasal system.

Axolotls are probably descended from terrestrial tiger salamanders, which are known to possess a vomeronasal system (Herrick, 1921, 1948). The presence of the vomeronasal system in axolotls may be a larval characteristic, or may be a trait associated with sexual maturation. To distinguish between these possibilities, we have examined the nasal cavity and forebrain of larval tiger salamanders and of larval Jefferson's salamanders, *Ambystoma jeffersonianum*, a transforming ambystomid that is distantly related to axolotls and tiger salamanders (Shaffer, 1984). As in the axolotl, we have found a lateral pouch in the nasal cavity of the larvae of both species and have found that the wall of this pouch appears to be lined with sensory receptors. These receptors are physically separate from the receptors located in the medial portion of the nasal cavity. The forebrain of these larvae is like that of adult tiger salamanders and axolotls and appears to contain both main and accessory olfactory bulbs. From these data, we conclude that the presence of a vomeronasal system in axolotls is characteristic of ambystomid larvae in general.

If it is true that the vomeronasal system responds to nonvolatile molecules and is therefore well-suited for terrestrial life, as Bertmar (1981) has suggested, then its presence in aquatic larvae is a paradox. Perhaps the system is present in aquatic larvae simply because the system will be used by these animals in their terrestrial habitats after metamorphosis. Following this line of reasoning, we could argue that the vomeronasal system in nontransforming axolotls is unnecessary but has not yet been eliminated. Vomeronasal systems have been described in non-transforming adults of the families Sirenidae (Seydel, 1895), Cryptobranchidae, and Amphiumidae (Anton, 1908), and may exist in nontransforming Proteidae (Jurgens, 1971), yet the vomeronasal system may be present in each of these animals as a vestige from the larvae of their transforming ancestors.

In addition to nontransforming axolotls and ambystomid larvae, we have examined the adults of a different type of aquatic salamander. Larval red-spotted newts, *Notopthalmus*

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viridescens, are aquatic and metamorphose into terrestrial subadults, or efts. Efts undergo a second metamorphosis and return to an aquatic habitat as adult newts. We have found that the nasal cavities and forebrains of adult newts are similar to those of ambystomids. The nasal cavity of the red-spotted newt contains a receptor-lined lateral pouch, and sensory receptors can also be found in the medial portion of the cavity. The forebrain appears to contain both main and accessory olfactory bulbs.

We have found evidence for the existence of a vomeronasal system in several types of aquatic salamanders: nontransforming axolotls, larvae of two other ambystomid species, and secondarily aquatic newts. These data contradict Bertmar's (1981) proposal that the vomeronasal system evolved as an adaptation to terrestrial life.

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