SUGGESTED EVOLUTION OF TONOTOPIC ORGANIZATION IN THE FROG AMPHIBIAN PAPILLA

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As one progresses from the most primitive to the most derived frogs, one observes remarkable changes in that peculiarly amphibian auditory organ, the amphibian papilla. In all but the most primitive frog, the papilla comprises two patches with separate innervation and apparently corresponding to a spatial separation of frequency sensitivity (i.e. tonotopic organization). The caudal patch is quite variable and in the more derived frogs exhibits an elongation that apparently corresponds to extension of auditory sensitivity to higher frequencies.

The amphibian papilla, an inner-ear sensory surface peculiar to amphibians, was examined with the scanning electron microscope and the light microscope in 70 anuran species distributed over 11 families. The papilla resides on the ceiling of a separate chamber that is open laterally to the fluid (endolymph) space of the saccule, sealed medially or caudally by a thin membrane separating the fluid in the chamber from that in the amphibian periotic canal, and otherwise bounded by a thick wall, the postero-lateral part of which is formed by the papillar branch of the VIIIth nerve as it courses around the chamber to innervate the papilla [2, 8, 19]. In frogs with tympanums and middle ears, the amphibian periotic canal contacts directly the middle-ear apparatus [9, 16]. In *Ascaphus truei,* the most primitive frog in this study [20, 21], the papilla comprises one patch of sensory epithelium (Fig. 1, bottom), innervated by a single, undivided branch of the VIIIth nerve. Hanging from the patch is a bulky tectorium that fills much of the papillar chamber and exhibits a ridge running rostrolaterally along its distal surface. In all other species examined, the papilla is more complex and conveniently can be considered to comprise two contiguous patches (Fig. 1) [12]. The papillar branch of the VIIIth nerve is divided into two or three distinct branchlets, depending on the species, with one branchlet innervating the caudal-most patch and either one or two branchlets innervating the rostral-most patch. The boundary between the two patches is signalled not only by this abrupt change in innervation, but also by an abrupt change in the morphological polarization of the hair cells. Furthermore, in the two species (*Rana catesbeiana* and *Xenopus laevis*) in which we examined the papillae of
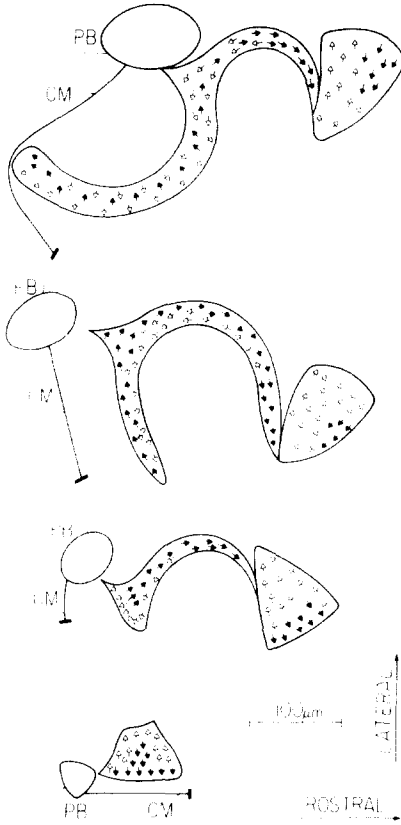
Fig. 1. Outlines of the amphibian papillae of 4 anurans, all drawn to the same scale. Each arrow depicts the polarization of many hair cells, pointing toward the side of the luminal surface at which the kinocilium resides. PB, transected papillar branch of the VIIIth nerve; CM, contact membrane between the papillar chamber and the amphibian periotic canal. On the basis of phylogenetic relationships deduced from traits independent of the amphibian papilla, the outlines are presented in phylogenetic order, with that of the most primitive frog (*Ascaphus truei*) at the bottom and that of the most derived frog (*Kassina senegalensis*) at the top [20]. Second from the bottom is the discoglossid, *Bombina orientalis*; second from the top is the pelobatid, *Scaphiopus couchi*. In *Ascaphus, Bombina* and *Scaphiopus*, the papilla is confined to the ceiling of the papillar chamber; in *Kassina* and other hyperoliids as well as in the ranids, rhacophorids and some microhylids, the long caudal extension projects beyond the limits of the chamber ceiling and well down the posterior wall of the chamber.

larvae, we found that the two patches are not contiguous in early larval stages (i.e. are separated by a field of standard endolymphatic epithelial cells) but gradually come together in later larval stages [15]. Hanging from the two-patch papilla is a single tectorium (Fig. 2) that is bulky under the rostral patch, filling completely the rostral end of the papillar chamber, and thin under the caudal patch, filling a small fraction of that end of the chamber. Extending from the caudal portion of the tectorium, and possibly derived from the ridge on the *Ascaphus* tectorium, is a thin diaphragm that spans the papillar chamber [11, 23].
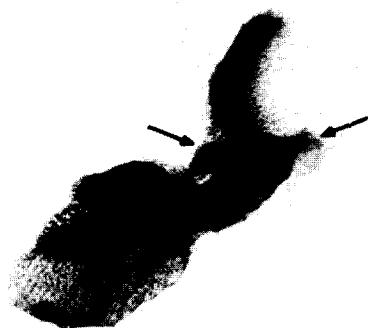
Fig. 2. Tectorium from the microhylid *Kaloula pulchra,* viewed with phase contrast while immersed in sodium cacodylate buffer solution. Rostral end is at lower left and caudal end at upper right. Entire inner ear was fixed with buffered glutaraldehyde and post-fixed with osmium prior to removal of tectorium. Residual outline of papillar sensory epithelium appears darkly stained, apparently reflecting osmophylic nature of the tectorium immediately adjacent to papillar surface. Arrow points to the diaphragm that extends from the part of the tectorium adjacent to the caudal patch and spans the papillar chamber. Width of micrograph (left to right) = 0.8 mm.

The rostral patch has a relatively constant topography among the species studied; the caudal patch is remarkably variable. In the two early larval forms examined (i.e. those of *Rana catesbeiana* [15] and *Xenopus laevis*) and in the adults of *Bombina bombina, Bombina orientalis* and *Pipa pipa,* the caudal patch exhibits the shape of a narrow, curved triangle with its base adjacent to the portion of the chamber wall formed by the papillar branch of the VIIIth nerve (see *Bombina* papilla in Fig. 1). In adults of the other species examined, the caudal patch extends beyond that part of the wall: either the curve of the triangle continues past the branch, leading to rostromedial extension (see *Scaphiopus* papilla in Fig. 1); or the curvature reverses, leading to caudal extension (see *Kassina* papilla in Fig. 1). Hair cells of rostromedial extensions are polarized along axes parallel to the papillar margins; those of caudal extensions are polarized predominantly along axes normal to the papillar margins. Among the species studied, caudal extensions occur in none of the ascaphids, discoglossids or pipids, in one out of six pelobatids, in 15 out of 16 bufonids, and in all hylids, dendrobatids, microhylids, ranids, rhacophorids and hyperoliids. In the pelobatid (*Palobates fuscus*), the caudal extension is very short, encompassing less than 5% of the papillar hair cells. Among the bufonids and microhylids its length is quite variable; the bufonid lacking the caudal extension is *Bufo boreas,* the only bufonid among the 16 examined that lacks a mating call [1]. Among the remaining families, the caudal extension is long, encompassing 40% or more of the papillar hair cells. Thus the caudal extension is either absent or short in families considered

primitive, is long in families considered most derived, and is variable in length in one intermediate family (Bufonidae) and one family (Microhylidae) whose phylogenetic position is debated [6, 21].

Physiological evidence from several species has shown that the amphibian papilla in anurans is an auditory sensor [3, 4, 7]. Additional physiological studies have suggested the presence of a place mechanism underlying frequency analysis in the papilla [5]. The results of the comparative physiological studies, taken together with morphological results for the same species (*Scaphiopus couchi, Bufo debilis, Bufo americanus* and *Rana catesbeiana*), provide strong circumstantial evidence in support of that suggestion, showing that the proportion of papillar afferent nerve fibers exhibiting high-frequency sensitivity increases almost directly with increasing length of the caudal extension [13]. The correspondence between the caudal extension and high-frequency sensitivity subsequently was confirmed in one of these species, *Rana catesbeiana,* by functional mapping with a fluorescent intracellular dye [14]. In this species, low-frequency sensitivity resides in the rostral patch, high-frequency sensitivity in the caudal extension, and sensitivity to intermediate frequencies in the intervening region. In birds and mammals, such tonotopic organization often has been attributed in part to structural gradations in the basilar membrane – a very thin specialization of the labyrinthine wall underlying the sensory epithelium. In reptiles, the structural gradations of the basilar membrane generally appear not to be sufficient to account for the observed ranges of auditory tuning [22]. In the alligator lizard, although the auditory afferent fibers exhibit sharp tuning and tonotopic organization, the basilar membrane itself has been found to be very broadly tuned and not to exhibit tonotopic organization [17]. Thus the basis of tonotopic organization, at least in the lizard ear, is not clear. The thick wall underlying the amphibian papilla in frogs is not analogous to the basilar membrane [8, 23]. On the other hand, the papillar tectorium does exhibit structural gradations appropriate for tonotopic organization. The most bulky (therefore presumably the most massive) part of the tectorium is associated with the low-frequency region of the papilla; the thin (presumably least massive) part is associated with the high-frequency region. These are precisely the associations expected if the tectorium were serving as part of a distributed acoustical filter, tuned to low frequencies at its rostral end and high frequencies at its caudal end. Thus it seems reasonable to conjecture that the observed tonotopic organization in *Rana catesbeiana* is attributable in part to structural gradation of the tectorium, and that all anurans exhibiting such structural gradation (i.e. all but *Ascaphus truei*) will exhibit tonotopic organization. It also is possible that tonotopic organization is related to the patterns of hair-cell polarization. Since hair cells are most sensitive to shearing motion along their polarization axes [10], those in the caudal extension will be sensitive to a mode of tectorial motion different from that which excites the more rostral hair cells. In the anurans exhibiting caudal extensions, the diaphragm of the tectorium lies directly over the transition from longitudinal to transverse

polarization, perhaps providing separation of two modes of tectorial motion.

Capranica and Moffat (unpublished) found the frequency-sensitivity range of the one-patch amphibian papilla of *Ascaphus truei* to be essentially the same as that of the two-patch papilla of *Scaphiopus couchi* [4], with both lacking the higher-frequency sensitivity of *Rana catesbeiana* and the other more derived species. Thus, without the caudal extension (i.e. even in the presence of a rostromedial extension), the two-patch papilla seems not to have the advantage of extended frequency range. If one accepts the proposition that the one-patch papilla of *Ascaphus* is primitive relative to the two-patch papilla of *Scaphiopus,* then one must ask what selective advantage led to derivation of the two-patch state. All two-patch papillae (with or without caudal extensions) exhibit structurally graded tectoria and presumably, therefore, some form of tonotopic organization. The sorting of frequency sensitivity among two or three separate branchlets of the VIIIth nerve presumably would facilitate tonotopic mapping of first-order afferents in the brain, which in turn might facilitate auditory signal processing. Thus the two-patch papilla may have arisen as a result of a selective advantage of tonotopic organization itself. The extraordinarily variable caudal patch may then have been shaped by the coevolution of diverse vocal repertoires and concomitantly diverse auditory sensitivity. In particular, extension of vocal range and auditory sensitivity to previously unused frequencies would be advantageous in the face of competition among anuran species for communication bands in the acoustical frequency spectrum. Thus the caudal extension may have arisen as a result of a selective advantage of extension of papillar sensitivity to higher frequencies.

The caudal extension in the more derived anurans shares at least three features with the sensory epithelium of the organ of Corti in mammals: (1) both are long and narrow, but their lengths are compressed by extreme curvature; (2) both exhibit high-frequency sensitivity at the end most directly in contact with the middle ear and lower-frequency sensitivity at the other end; (3) both exhibit transverse hair-cell polarization. Thus we have evidence of convergence. The analogy is spoiled, however, by the lack of basilar membrane in association with the amphibian papilla. The two-patch papilla also shares one feature with the sensory epithelium of the primary auditory organ (saccule) of non-ostariophysine teleosts: both have two pairs of oppositely polarized hair-cell populations [18]. The analogy here is spoiled by the lack of an otolith in the amphibian papilla.

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