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Research Note

The Saccule May Be the Transducer for Directional Hearing of Nonostariophysine Teleosts*

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Summary. The hearing of fishes is transduced by the otolithic end-organs of the eighth nerve. In several nonostariophysine fish, the nerve innervation and hair cell orientation in the saccule, one otolithic organ, suggest that directionality is encoded by a set of mutually perpendicular sensory epithelia. The anterior saccular branch innervates only the hair cell groups oriented along the rostrocaudal body axis which are located at the anterior of the saccule. The posterior saccular branches innervate the hair cell groups oriented along the dorsoventral body axis and are found at the posterior of the saccule.

Key words: Ear – Hearing – Localization – 8th nerve

Fishes and elasmobranches can behaviorally localize the position of a sound source (Nelson 1967; Schuijf 1975, 1981). The mechanism used by fishes for sound localization (Schuijf 1981) appears to differ substantially from that used by aquatic mammals and non-aquatic vertebrates (Erulkar 1972), perhaps because sound in water is associated with both displacement and pressure fields, whereas sound in air is primarily associated with a pressure field. A fish may determine the direction of an acoustic signal by comparing response levels of morphologically polarized receptors that analyze the directional component of the acoustic signal, particle displacement (Dikgraaf 1960; Schuijf 1981). This transducer for directional analysis is likely to be the sensory epithelium of the saccule, and perhaps the lagena, of the fish ear (Platt and Popper 1981; Popper et al. 1982).

A detailed examination of the saccules of a number of nonostariophysine fishes (fishes without Weberian ossicles) has revealed that the saccule is constructed as a potential directional transducer with four sensors maximally responsive to mutually perpendicular directions (Platt and Popper 1981; Popper et al. 1982). We compared the innervation patterns of the saccules of several fishes (*Helostoma temincki, Hemichromis bimaculatus, Pantadon buchholzi, Trichogaster leeri,* and *T. trichopterus*) with the orientation pattern of their hair cells as seen in scanning electron microscopy. In all cases, saccular components of the eighth nerve terminated as branches that separately innervate paired regions of orthogonally oriented hair cells. The more anterior division innervates hair cells morphologically polarized along the rostrocaudal body axis of the fish; the posterior division innervates hair cells polarized along the dorsoventral body axis.

Hair cell orientation patterns were mapped by SEM using standard procedures (Popper and Hoxter 1981). Fish were heavily anesthetized with MS-222, the crania opened, the saccules rinsed with saline, and fixed by immersion in 4% glutaraldehyde in 0.2 M s-Collidine buffer, pH 7.4. The saccules were then removed, trimmed, and further fixed overnight. The tissue was rinsed in buffer, post-fixed with 1% OsO_4, dehydrated with graded ethanols, critical point dryed, mounted on a stub, coated with gold-palladium, and examined in an Etec Autoscan SEM operated at 20 kv. The number of hair cells was estimated using a planimetric graphics program on an Apple II Plus microcomputer.

The nerve innervation patterns were observed following two procedures, osmium staining to distinguish the numbers of saccular branches in the eighth nerve and the reduced silver protocol of Winkelmann and Schmitt to distinguish the termination patterns of individual fibers within each branch (Winkelmann

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and Schmitt 1957; Bone 1972). This technique stains the entire axon to its synaptic contacts with the sensory hair cells. The saccules, the attendant eighth nerve branches, and part of the medulla fixed in either 10% formalin or 4% glutaraldehyde were used for both procedures. The medulla was subsequently excised, the remaining tissue dehydrated, cleared in xylene and mounted in Permount.

Because our observations are similar across the species examined, we illustrate them with details from only one species, *Trichogaster leeri,* the pearl gourami (Family: Anabantidae). A 1.8 mm saccule of *T. leeri* contains an estimated 15,900 sensory hair cells unevenly distributed in an area of about 0.49 mm^2. Each hair cell is morphologically polarized with the direction defined by the position of a single, eccentrically-placed kinocilium within the ciliary bundle. The hair cells are divided into four orientation groups (Fig. 1A) as in the saccules of other nonostariophysines (Popper and Coombs 1982). The rostral region of the macula has two horizontally oriented groups, with one group oriented anteriorly and the other posteriorly (Fig. 1A) in the opposed pattern (Popper and Coombs 1982). Sensory cells in the caudal region of the saccular sensory epithelium are oriented vertically, relative to the animal's body axis, with the ciliary bundles on the dorsal side of the macula oriented dorsally, and those on the ventral side, oriented ventrally (Fig. 1A). The pattern is nearly identical to the congeneric species, *T. trichopterus* (Popper and Hoxter 1981).

Figure 1B illustrates the innervation of the saccular otolith organ by the saccular branches of the eighth nerve. The region of innervation of each saccular branch was determined by camera lucida drawing (at ×100) of a number of fibers from each branch (Fig. 1C). Each branch divides and subdivides several times before penetrating the basement membrane of the saccular epithelial region. Most axons terminate in a number of small fibers, each fiber synapsing on a different hair cell (Fig. 2A). The terminal fibers of axons near each other typically overlap (Fig. 2B). Axons which terminated on only one hair cell were not seen.

The terminations of fibers from the different branches divide the saccular sensory epithelium into two regions. The posterior saccular branch (PSB) innervates the thin mid-region and caudal end of the saccular epithelium. Only vertically oriented sensory cells are found in these regions of the sensory epithelium. The anterior saccular branch (ASB) innervates only the rostral region of the epithelium where the horizontally oriented hair cells are located. A few fibers from the PSB terminated more rostral than the caudalmost fibers of the ASB. Within the

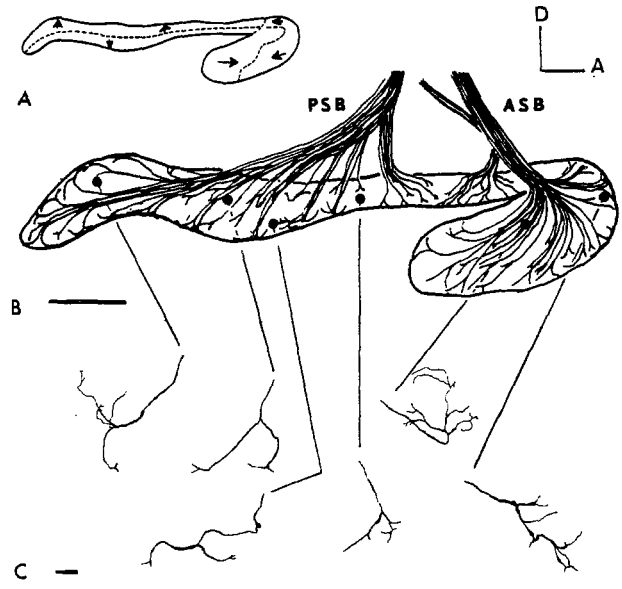

Fig. 1. A Diagram of the saccular hair cell orientation patterns in *Trichogaster leeri.* The sensory epithelium is divided into four regions. The ciliary bundles of all hair cells in each region are oriented in the same general direction. The *dashed lines* indicate the approximate boundaries between each region, and the *arrows* show the direction toward the kinocilium of the ciliary bundles in each orientation group (see Fig. 2C). A – anterior; D – dorsal. **B** Schematic illustration of the innervation pattern of the saccular sensory epithelium determined by staining with the Winkelmann-Schmitt technique. The saccular component of the eighth nerve divides into two branches. The anterior saccular branch (*ASB*) innervates the rostral region of the sensory epithelium containing cells oriented horizontally. The posterior saccular branch (*PSB*) innervates epithelial regions containing only vertically oriented sensory cells. (Note that a small branch of the *PSB* runs with the *ASB* for a short distance.) For clarity, nerve fibers that travel along the border between the two vertically oriented cell groups have been omitted. Bar = 225 μm. **C** Camera lucida drawings of selected nerve terminations. The *solid lines* connect the terminations to their sites on the saccular epithelium. Bar = 10 μm

limits of resolution of the Winkelmann-Schmitt stained preparations, the boundary between the ASB and the PSB regions of innervation corresponds to the border between orthogonal hair cell groups. Because hair cells of orthogonal polarity overlap at this border (Fig. 2C), the interdigitation of axon terminals from the ASB and the PSB does not necessarily indicate single fiber innervation of hair cells in orthogonally polarized orientation groups.

The cues involved in a binaural comparison of signals by terrestrial vertebrates are not available to fishes (van Bergeijk 1964) because the wavelength of sound in water, and the proximity of the two ears, preclude binaural temporal differences for sound localization. To determine the position of a sound source, the mechanism for detection of directional information must, therefore, necessarily differ from the mechanism found in terrestrial vertebrates.

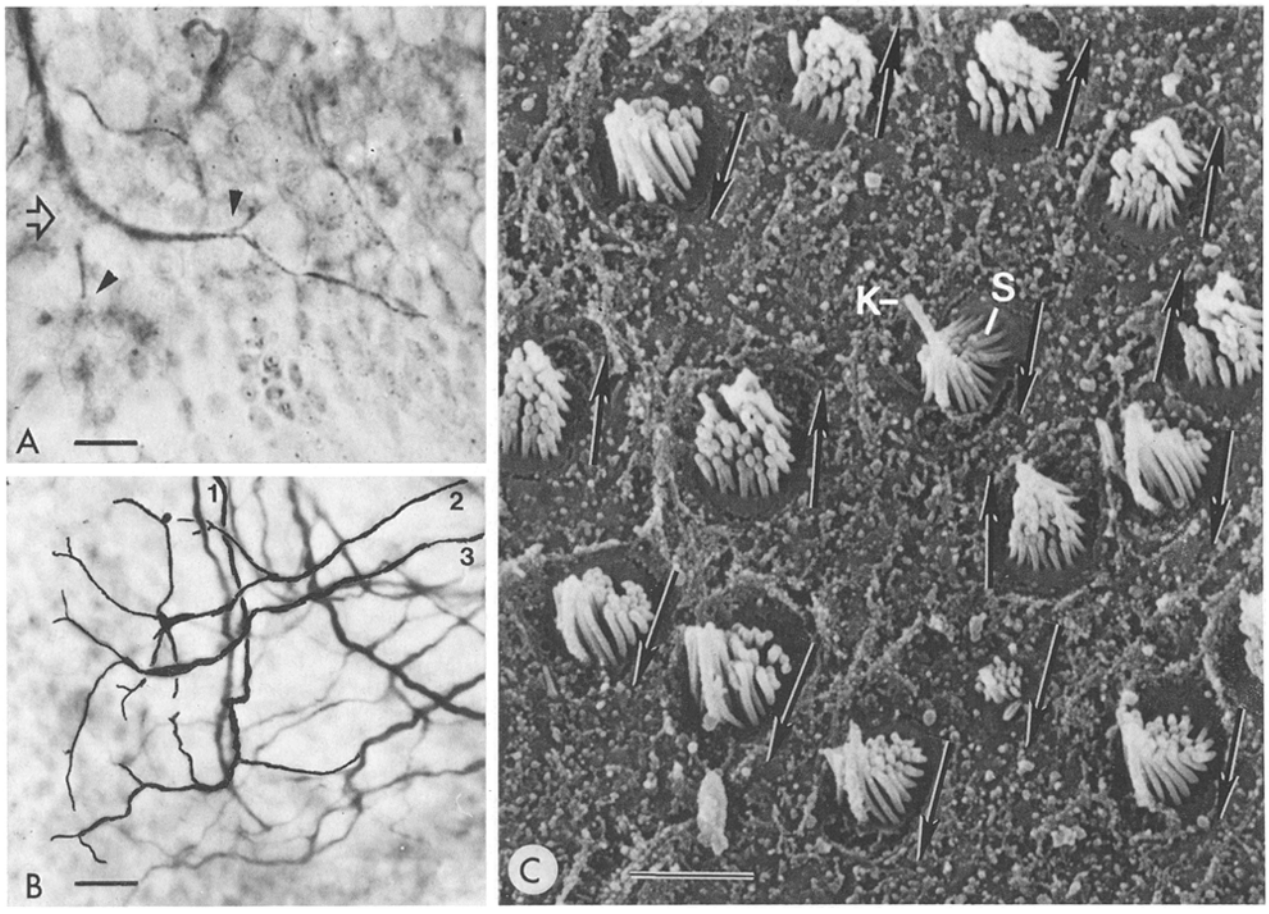

Fig. 2. A Photomicrograph of the terminal arborizations of a saccular fiber from a wholemount of the saccular epithelium showing the multiple terminations in the epithelium. The *open arrow* indicates where the fiber is outside the plane of focus; the closed arrows point to fiber bifurcations. Bar = 10 μm. **B** Photomicrograph of several nerve terminals illustrating the overlap among the terminal arborizations of individual fibers. Three (1, 2, 3) fibers have been retouched for clarity. Bar = 25 μm. **C** Scanning electron micrograph illustrating the interdigitation of ciliary bundles at the border between two orientation groups. The *arrows* indicate the orientation of each of the ciliary bundles. K – kinocilium; S – stereocilia. Bar = 2.0 μm

Theoretically, were a fish able to determine the direction of the vectorial component of a sound (particle displacement), it should be able to determine the direction of the signal (Dikgraaf 1960). Recently, the fish ear has been shown capable of detecting both particle displacement and pressure (Buwalda and Van der Steen 1979; Buwalda 1981; Fay et al. 1982), both of which interact in the otolith organs to produce characteristic otolith motions (or orbitals, Popper et al. 1982) for signals from different directions (Schuijf 1981). Each characteristic orbital will produce a stimulus pattern over the four hair-cell groups so the direction of a sound source could be extracted by centrally integrating the inputs.

In each region on the saccule, the polarized ciliary bundles on the hair cells bend as a consequence of the otolith orbital and generate physiological responses in the hair cells characteristic of the particular orbital (Sand 1974; Schuijf 1981; Platt and Popper 1981; Popper 1983). The response level from each group of oriented receptor cells depends upon the vector of the orbital of the otolith due to the stimulus taken in relationship to the axis of ciliary bundle symmetry. This axis is defined for cells in each orientation group by the line from the kinocilium that symmetrically bisects the stereocilia (Hudspeth and Corey 1977; Hudspeth 1982). Thus, horizontally oriented cells would respond best to the horizontal component of the otolith orbital, and vertically oriented cells would best respond to the vertical component of the orbital. Stimulation at an angle to the preferred axis would generate a response that is graded as a cosine of stimulus direction (Flock 1971; Shotwell et al. 1981).

The results of our studies lend morphological

support to a model of vectorial weighing (Schuijf and Buwalda 1975, 1980). In this model, signals from every direction (with the exception of those 180° apart) will generate a unique ratio of response levels from the orthogonally organized cell groups (Sand 1974). Since the peripheral nerves innervating the saccular sensory epithelium are organized to independently relay information from orthogonally oriented cell groups of the saccular epithelium, the simplest interpretation of our data indicates that directional information is the derived result of central interactions of orthogonally derived inputs from different ears or ear regions. Thus, the nerve fibers that innervate each orientation group must convey directional as well as frequency (or temporal) information (Enger 1981). However, nothing is known about the physiologic mechanism by which inputs from the various receptor cell groups are compared to one another or where in the central nervous system (CNS) this interaction occurs. One locus may be the torus semicircularis where some single cells respond best to signals originating from both ears (Horner et al. 1980). Localization by fishes using a vectorial weighing scheme requires signals from both ears, as well as from different regions of the same ear. We suspect that, with appropriate directional stimulus conditions, individual neurons will be found in the teleost CNS that respond to orthogonal inputs from a single ear.

The peripheral mechanisms and strategy for determining sound localization apparently differs between fishes and tetrapods, but some aspects of the CNS pathways of aquatic and terrestrial groups may well be homologous (Northcutt 1981). Thus, localization of sound sources in nonostariophysine fishes as compared to terrestrial vertebrates represents the remarkable situation of different peripheral input parameters to potentially homologous nervous pathways.

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References

Bergeijk WA van (1964) Directional and non-directional hearing in fish. In: Tavolga WN (ed) Marine bio-acoustics. Pergamon Press, Oxford, pp 281–299

Bone Q (1972) Some notes on histological methods for peripheral nerves. Med Lab Tech 29: 319–324

Buwalda RJA (1981) Segregation of directional and non-directional acoustic information in the cod. In: Tavolga WN, Popper AN, Fay RR (eds) Hearing and sound communication in fishes. Springer, Berlin Heidelberg New Yor, pp 139–171

Buwalda RJA, Steen J van der (1979) Sensitivity of the cod sacculus to directional and non-directional stimuli. J Comp Biochem Physiol 64A: 467–471

Dikgraaf S (1960) Hearing in bony fishes. Proc R Soc Lond [Biol] 152: 51–54

Enger PS (1981) Frequency discrimination in teleosts-central or peripheral? In: Tavolga WN, Popper AN, Fay RR (eds) Hearing and sound communication in fishes. Springer, Berlin Heidelberg New York, pp 243–253

Erulkar SD (1972) Comparative aspects of spatial localization of sound. Physiol Rev 52: 237–260

Fay RR, Hillery CM, Bolan K (1982) Representation of sound pressure and particle motion information in the midbrain of the goldfish. Comp Biochem Physiol 71A: 181–191

Flock Å (1971) Sensory transduction in hair cells. In: Lowenstein WR (ed) Principles of receptor physiology. Springer, Berlin Heidelberg New York (Handbook of sensory physiology, vol I, pp 396–411)

Horner K, Sand O, Enger P (1980) Binaural interaction in the cod. J Exp Biol 85: 323–331

Hudspeth AJ (1982) Extracellular current flow and the site of transduction by vertebrate hair cells. J Neurosci 2: 1–10

Hudspeth AJ, Corey DP (1977) Sensitivity, polarity, and conductance change in the response of vertebrate hair cells to controlled mechanical stimuli. Proc Natl Acad Sci USA 74: 2407–2411

Nelson DR (1967) Hearing thresholds, frequency discrimination, and acoustic orientation in the lemon shark, *Negaprion brevirostris* (Poey). Bull Mar Sci 17: 741–768

Northcutt RG (1981) Audition and the central nervous system of fishes. In: Tavolga WN, Popper AN, Fay RR (eds) Hearing and sound communication in fishes. Springer, Berlin Heidelberg New York, pp 331–355

Platt C, Popper AN (1981) Fine structure and function of the ear. In: Tavolga WN, Popper AN, Fay RR (eds) Hearing and sound communicaion in fishes. Springer, Berlin Heidelberg New York, pp 3–38

Popper AN (1983) Organization of the inner ear and processing of acoustic information. In: Northcutt RG, Davis RE (eds) Neurobiology of fish. Michigan Press, Ann Arbor (in press)

Popper AN, Coombs S (1982) The morphology and evolution of the ear in actinopterygian fishes. Am Zool 22: 311–328

Popper AN, Hoxter B (1981) The fine structure of the sacculus and lagena of a teleost fish. Hear Res 5: 245–263

Popper AN, Platt C, Saidel WM (1982) Acoustic function in the fish ear. Trends Neurosci 5: 276–280

Sand O (1974) Directional sensitivity of microphonic potentials from the perch ear. J Exp Biol 60: 881–899

Schuijf A (1975) Directional hearing of cod (*Gadus morhua*) under approximate free field conditions. J Comp Physiol 98: 307–322

Schuijf A (1981) Models of acoustic localization. In: Tavolga WN, Popper AN, Fay RR (eds) Hearing and sound communication in fishes. Springer, Berlin Heidelberg New York, pp 267–310

Schuijf A, Buwalda RJA (1975) On the mechanism of directional hearing in cod (*Gadus morhua* L.). J Comp Physiol 98: 333–343

Schuijf A, Buwalda RJA (1980) Underwater localization – a major problem in fish acoustics. In: Popper AN, Fay RR (eds) Comparative studies of hearing in vertebrates. Springer, Berlin Heidelberg New York, pp 43–77

Shotwell SL, Jacobs R, Hudspeth AJ (1981) Directional sensitivity of individual vertebrate hair cells to controlled deflection of their hair bundles. Ann NY Acad Sci 374: 1–10

Winkelmann RK, Schmitt RW (1957) A simple silver method for nerve axoplasm. Proc Mayo Clinic 32: 217–222

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