

REFERENCES

- Barry, M.A. (1987) Afferent and efferent connections of the primary octaval nuclei in the clearnose skate, *Raja eglanteria*. *J. Comp. Neurol.* **266**, 457-477.
- Bass, A.H., Bodnar, D.A. & Marchaterre, M.A. (2000) Midbrain acoustic circuitry in a vocalizing fish. *J. Comp. Neurol.* **419**, 505-531.
- Corwin, J.T. (1981) Audition in elasmobranchs. In *Hearing and Sound Communication in Fishes* (W.N. Tavolga, A.N. Popper and R.R. Fay, eds). Springer-Verlag; New York, pp. 81-105.
- Kozloski, J. & Crawford, J.D. (1998) Functional neuroanatomy of auditory pathways in the sound-producing fish *Pollimyrus*. *J. Comp. Neurol.* **401**, 227-252.
- McCormick, C.A. (1999) Anatomy of the central auditory pathways of fish and amphibians. In *Comparative Hearing: Fish and Amphibians* (R.R. Fay and A.N. Popper, eds). Springer-Verlag; New York, pp. 155-217.
- Montgomery, J.C., Coombs, S., Conley, R.A. & Bodznick, D. (1995) Hindbrain sensory processing in lateral line, electrosensory, and auditory systems: a comparative overview of anatomical and functional similarities. *Aud. Neurosci.* **1**, 207-231.
- Popper, A.N. & Fay, R.R. (1999) The auditory periphery in fishes. In *Comparative Hearing: Fish and Amphibians* (R.R. Fay and A.N. Popper, eds). Springer-Verlag; New York, pp. 43-100.
- Striedter, G.F. (1991) Auditory, electrosensory, and mechanosensory lateral line pathways through the forebrain in channel catfishes. *J. Comp. Neurol.* **312**, 311-331

OTOLITHIC ENDORGAN PROJECTIONS OF THE INNER EAR IN A VOCAL FISH

JOSEPH A. SISNEROS¹, MARGARET A. MARCHATERRE² AND
ANDREW H. BASS³

Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA. ¹jas226@cornell.edu ²mm43@cornell.edu ³ahb3@cornell.edu

INTRODUCTION

The plainfin midshipman *Porichthys notatus* is a vocalising species of teleost fish that generates acoustic signals for intraspecific social communication. The inner ear of the midshipman and other teleost fishes includes three otolithic endorgans (sacculle, lagena, and utricle) that are proposed to be either vestibular or auditory in function (Popper and Fay 1993). Although the sacculle is well developed in the midshipman, and is considered to provide the major auditory input from the inner ear to the medulla, the position and extent of auditory input from the lagena and utricle remain undetermined. Previous studies in the goldfish and trout suggest that central integration of auditory input from all three otolithic endorgans may be involved in sound localisation (Fay 1984, Schellart and Popper 1992). Thus, the purpose of this study was to examine the otolithic endorgan projec-

tions of the midshipman inner ear to delineate possible auditory pathways for each endorgan.

METHODS

Midshipman were first deeply anaesthetised by immersion in a 0.2% ethyl *p*-aminobenzoate saltwater bath followed by an intramuscular injection of pancuronium bromide (approximately 0.5 mg/kg) and fentanyl (approximately 1 mg/kg) for immobilisation and analgesia, respectively. Primary afferents from the inner ear endorgans were exposed by a dorsal craniotomy. Neurobiotin or biocytin was applied to each endorgan's (sacculae, lagena, and utricle) afferents near the sensory macula in both juvenile (3.5-5.5 cm SL) and adult (>12 cm) fish. Survival times after biotin labelling were 12-24 hrs. Following survival, animals were transcardially perfused with cold teleost Ringer solution followed by cold 4% paraformaldehyde and 1% glutaraldehyde dissolved in 0.1 M phosphate buffer, pH 7.2. The brain and the labelled endorgans were removed, postfixed for 1 hr in fixative, and stored in phosphate buffer. Prior to sectioning, brains were transferred into 30% sucrose-phosphate buffer for 12-24 hrs and then frozen sections were cut at 50 μ m. The biotin product was visualised using an Elite Kit (Vector Laboratories). After visualisation, sections were mounted on chrom-alum coated slides and counterstained with cresyl violet prior to being coverslipped.

RESULTS AND DISCUSSION

All three otolithic endorgans have projections to the descending, magnocellular, and anterior octaval nuclei in the medulla. Endorgan input to the five recognised zones of the descending octaval nucleus (Bass et al. 2000) were generally segregated: saccular projections to intermediate, rostral intermediate, dorsolateral and dorsomedial zones; lagenar projections to intermediate, rostral intermediate, dorsolateral and ventral zones; utricular projections to intermediate, rostral intermediate and ventral zones. Neurobiotin-labelled terminals and fibres from each endorgan also extended to the eminentia granularis and to the torus semicircularis. Transneuronal labelling of terminals in the auditory region of the torus semicircularis suggests that each endorgan may provide input to the auditory midbrain and thus all have an auditory function.

In contrast to the overlapping endorgan projections to octaval nuclei, neurobiotin-labelled cells and terminals in the posterior and tangential octaval nuclei were found to have exclusive projections from the sacculae and utricle, respectively. Exclusive input of the utricle to

the tangential octaval nucleus and from the saccule to the posterior octaval nucleus suggests that the tangential octaval nucleus may have a vestibular function while the posterior octaval nucleus has an auditory function.

Endorgan projections of the saccule and lagena extended caudally to the medulla at the level of the sonic motor nucleus where neurobiotin-labelled cells and terminals were found in the inferior olive following labelling of the saccule and in the lateral reticular nucleus following labelling of either the saccule or lagena. The octavolateralis efferent nucleus in the medulla was also labelled by projections from each endorgan while the efferent nucleus in the diencephalon was only labelled by projections from the saccule.

These results contribute to a more complete overview of the organisation of inner ear endorgan projections in the midshipman and will allow comparisons with that of other teleosts.

ACKNOWLEDGEMENTS

This research was supported by a NIH postdoctoral fellowship (1F32DC00445) to JAS and a NIH grant (DC00092) to AHB.

REFERENCES

- Bass, A.H., Bodnar, D.A. & Marchaterre, M.A. (2000) Midbrain acoustic circuitry in a vocalising fish. *J. Comp. Neurol.* **419**, 505-531.
- Fay, R.R. (1984) The goldfish codes the axis of acoustic particle motion in three dimensions. *Science* **225**, 951-954.
- Popper, A.N. & Fay, R.R. (1993) Sound detection and processing by fish: critical review and major research questions. *Brain Behav. Evol.* **41**, 14-38.
- Schellart, N.A.M. & Popper, A.N. (1992) Functional aspects of the evolution of the auditory system of actinopterygian fish. In *The Evolutionary Biology of Hearing* (D.B. Webster, R.R. Fay, and A.N. Popper, eds). Springer-Verlag; New York, pp. 295-322.
-