5. M. Tatibana and K. Shigesada, *ibid.* **10**, 249 (1972).
6. R. C. Jackson, M. S. Lui, T. J. Boritzki, H. P. Morris, G. Weber, *Cancer Res.* **40**, 1286 (1980).
7. M. Mori, H. Ishida, M. Tatibana, *Biochemistry* **14**, 2622 (1975).
8. W. T. Shoaf and M. E. Jones, *Biochem. Biophys. Res. Commun.* **45**, 796 (1971).
9. M. Mori and M. Tatibana, personal communication (28 November 1979).
10. M. J. Sweeney, J. W. Parton, D. H. Hoffman, *Adv. Enzyme Regul.* **12**, 385 (1974).
11. M. C. M. Yip and W. E. Knox, *J. Biol. Chem.* **245**, 2199 (1970).
12. K. Ito and M. Tatibana, *Biochem. Biophys. Res. Commun.* **23**, 672 (1966).
13. M. S. Lui, R. C. Jackson, G. Weber, *Biochem. Pharmacol.* **28**, 1189 (1979).
14. S. F. Queener, H. P. Morris, G. Weber, *Cancer Res.* **31**, 1004 (1971).
15. R. C. Jackson, H. P. Morris, G. Weber, *Nature (London)* **256**, 331 (1975).
16. H. L. Elford, M. Freese, E. Passamani, H. P. Morris, *J. Biol. Chem.* **245**, 5228 (1970).
17. G. Weber, E. Olah, M. S. Lui, H. Kizaki, D. Y. Tzeng, E. Takeda, *Adv. Enzyme Regul.* **18**, 3 (1980).
18. P. Ove, J. Laszlo, M. D. Jenkins, H. P. Morris, *Cancer Res.* **29**, 1557 (1969).
19. M. Tatibana and K. Shigesada, *J. Biochem. (Tokyo)* **72**, 537 (1972).
20. M. Mori and M. Tatibana, *Biochem. Biophys. Res. Commun.* **54**, 1525 (1973).
21. M. Tatibana and K. Ito, *J. Biol. Chem.* **244**, 5403 (1969).
22. Supported by PHS grants CA-05034 and CA-13526 to G.W.
* Correspondence should be sent to G.W.

1 October 1980; revised 3 December 1980

Electroreception in Lampreys: Evidence That the Earliest Vertebrates Were Electroreceptive

Abstract. *Evoked potential and unit responses from the lamprey brain to weak electric fields demonstrate that lampreys have an electrosensory system as sensitive as those of other electroreceptive fishes. Electrosensory responses were recorded in the dorsal medulla, the midbrain torus semicircularis, and the optic tectum. Similarities in the structure of the anterior lateral line nerves and medullary organization between lampreys and several primitive jawed fishes indicate that the electroreceptive systems are homologous in these taxa. Thus electroreception was probably present in the earliest vertebrates ancestral to both agnathans and gnathostomes.*

Marked similarities between lampreys and cartilaginous fishes in the morphology of the lateral line nerves and the specific projections of these nerves in the brain suggest that lampreys, like sharks, skates, and rays, may be electroreceptive (*1*). The octavolateralis area of the lamprey medulla, like that in cartilaginous fishes, is divided into dorsal, medial, and ventral nuclei (*2, 3*) (Fig. 1). The anterior lateral line nerve in both lampreys and cartilaginous fishes is divided into dorsal and ventral roots, which enter the dorsal and medial octavolateralis nuclei, respectively. However, the posterior lateral line nerve has a single root and enters only the medial nucleus. The ventral octavolateralis nucleus does not receive lateral line input, but is the main target of the entering eighth nerve fibers (*4–6*).

Boord and his colleagues (*4, 7*) have suggested that the dorsal root of the anterior lateral line nerve in sharks is composed solely of electroreceptive fibers innervating head ampullary organs, whereas the ventral root is composed of mechanoreceptive fibers innervating head neuromast organs. This anatomical hypothesis was recently confirmed by electrophysiological recordings from single units in the dorsal and ventral roots of the anterior lateral line nerve of the thornback ray (*8*). Additional physiological evidence revealed that the dorsal octavolateralis nucleus in these rays is the primary medullary target of the entering electroreceptive fibers. Thus, the presence of a dorsal octavolateralis nucleus in the medulla of fishes should indicate the presence of electroreception, and the existence of this nucleus in lampreys suggests that these fishes are electroreceptive.

To test this hypothesis, we have studied an anadromous lamprey (*Lampetra tridentata* Richardson) that spawns in rivers from Alaska to southern California, but spends most of its adult life in the Pacific Ocean. Adult animals (60 to 70 cm in total length, 0.5 to 0.7 kg) were captured in the course of their upstream spawning migration in the Columbia River and transported to the laboratory, where they were held and tested in spring water (resistivity, 9.0×10^3 ohm-cm).

Electrophysiological responses to sensory stimuli were recorded after briefly anesthetizing animals by immersing them in tricaine methanesulfonate (0.01 percent) and surgically exposing the dorsal surface of the brain. Animals were then paralyzed by an intramuscular injection of tubocurarine chloride (4 mg per kilogram of body weight) and posi-

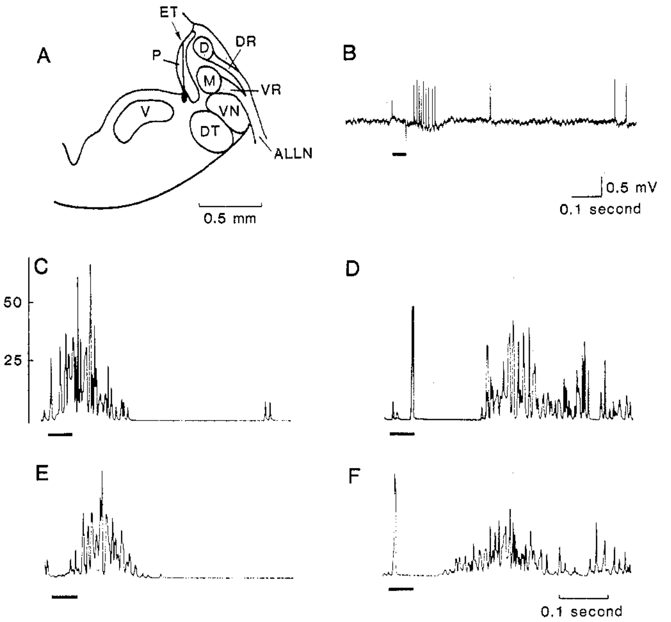

Fig. 1. Single unit responses to weak electric field stimuli. (A) Transverse section through the medulla oblongata at the level of entry of the anterior lateral line nerve. The cell in (B to F) was recorded at the surface of the indicated electrode track in the periventricular layer of cells, the processes of which form the neuropil of the dorsal octavolateralis nucleus. (B) Evoked potential and superimposed single unit response to an electric field stimulus presented as a 50 msec d-c pulse indicated by the line beneath the record. The 50 μV/cm field was oriented parallel to the longitudinal axis of the fish, with the caudal end positive relative to rostral. (C to F) Poststimulus-time histograms of responses (20 in each case) of the unit in (B) to four different orientations and polarities of the stimulus field. (C) Transverse to the longitudinal body axis, left side positive. (D) Transverse, right positive. (E) Parallel to the longitudinal axis, caudal end positive. (F) Parallel, rostral positive. Abbreviations: *ALLN*, anterior lateral line nerve; *D*, neuropil of dorsal octavolateralis nucleus; *DR*, dorsal root of anterior lateral line nerve; *DT*, descending trigeminal tract and nucleus; *ET*, electrode track; *M*, neuropil of medial octavolateralis nucleus; *P*, plate of cells whose processes form the neuropils of the octavolateralis nuclei; *VN*, ventral octavolateralis nucleus; *VR*, ventral root of anterior lateral line nerve; *V*, trigeminal motor nucleus.

0036-8075/81/0424-0465$00.50/0

tioned in the center of a large plexiglass aquarium filled with spring water (16° to 20°C), with only the dorsal surface of the head and exposed brain above water level. Animals were ventilated with oxygenated spring water through a mouth tube.

Electric field stimuli were presented as d-c pulses 5 to 150 msec long between pairs of carbon rod electrodes on the ends or sides of the aquarium so that electric fields were parallel or transverse to the longitudinal axis of the fish; the intensities of the fields varied from 0.01 to 1000 µV/cm, depending on the experiment (*9*). Electrophysiological responses to visual stimuli presented as 10-µsec light flashes were also recorded. The large and easily recorded visual evoked responses from the midbrain served as a measure of the condition and responsiveness of the experimental animal.

Electrophysiological activity in the form of evoked potentials, multiple unit activity, and (rarely) isolated single-unit impulses was recorded from the brain through the use of glass micropipettes (5 to 10 µm tip diameter) or stainless steel electrodes (1 to 5 megohms). When steel electrodes were used, specific recording sites were marked by the Prussian blue technique (*10*) and identified in subsequent histology. With glass microelectrodes, recording sites were determined on the basis of electrode depth and information provided by adjacent tracks with steel electrodes. Signal averaging was used to improve the signal-to-noise ratio of evoked potentials.

Positive evidence of electroreception was found in five of the six lampreys studied. Evoked potential and multiple-unit responses to electric fields as weak as or weaker than 20 µV/cm (2.2×10^{-3} µA/cm^2) were recorded in each of these fish; in the best case, reliable responses to field intensities as low as 0.1 µV/cm (1.1×10^{-5} µA/cm^2) were measured. This establishes a sensitivity threshold for lampreys as low as thresholds reported in freshwater fish with electrosensory systems (*11, 12*). Only marine elasmobranchs have been shown to be more sensitive, responding to uniform fields as weak as 0.01 µV/cm (*13, 14*).

We recorded electrophysiological responses to electric field stimuli from three different areas of the lamprey brain. The responses of shortest latency were from the dorsal medulla, where the onset of the evoked potential response to a moderately intense field (100 µV/cm), in the best orientation and polarity, had a latency of about 40 msec, and the major peak in the evoked wave occurred 65 msec after stimulus onset. Single unit responses from the dorsal octavolateralis nucleus had latencies as short as 25 msec (Fig. 1C).

Electrosensory responses were readily recorded from the midbrain, and electrode marks indicated two distinct sources of electroreceptive activity—(i) an area deep to the optic tectum that represents the lamprey torus semicircularis and (ii) the optic tectum itself (Fig. 2). Responses from the torus semicircularis had shorter latencies than tectal responses, with the earliest peak of the evoked responses at about 75 to 85 msec, compared with 140 msec for the earliest tectal responses. In all cases, the latencies of electric evoked responses of lampreys were longer than those of elasmobranchs (for example, 65 msec versus 20 to 50 msec for medullary evoked potentials) (*15*). This may, in part, be a result of the absence of myelin from the agnathan nervous system (*16*).

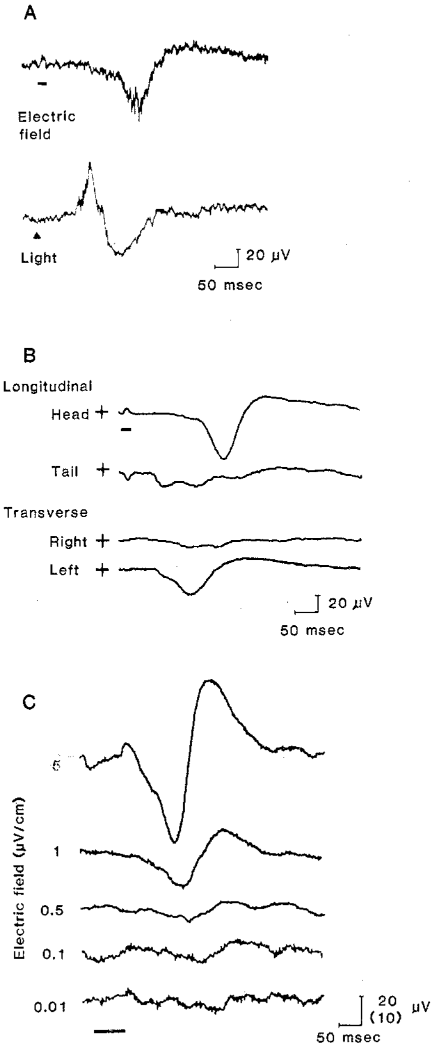

Fig. 2. Evoked potential responses from a single recording site in the optic tectum to electric and photic stimuli. (A) Single unaveraged evoked potential responses to an electric field of 50 µV/cm and 20-msec duration (indicated by the line below the record) and a 10-µsec light flash. In each case the evoked potential is accompanied by a multiple unit response near the peak of the evoked wave. The results indicate convergence of visual and electrosensory modalities in the tectum. (B) Average evoked potential responses to an electric field of 100 µV/cm illustrate the selectivity of the response for field orientation and polarity. The field was aligned with or transverse to the longitudinal axis of the lamprey. (C) Evoked potential response versus the electric field intensity. Repeatable responses were elicited by fields as weak as 0.1 µV/cm. Each record is the average of 64 responses. The vertical calibration bar is 20 µV for the top three records and 10 µV for the remaining two records. Positive potentials are upward in all records.

Evoked potential responses to visual stimuli were found throughout the midbrain, with latencies to the peak of the response varying from 95 to 140 msec, depending on the recording site. At several locations multiunit responses were elicited by both light and electric field stimuli (Fig. 2A). Although it is not certain that the same cells were affected by both stimuli, the results indicate convergence of visual and electrosensory information in both the torus semicircularis and the optic tectum.

In all areas studied, the profile and latency of the electrosensory evoked response varied greatly depending on the orientation and polarity of the electric field (Fig. 2B). The best orientation and polarity were different at different recording locations, suggesting that sensory maps of field orientation and polarity may exist in the brain.

Our results indicate that lampreys are electroreceptive and that their sensitivity is comparable to that of cartilaginous and electroreceptive teleost fishes (*13*). This is, to our knowledge, the first demonstration of electroreception in the vertebrate class Agnatha, although it has been suspected (*1, 17*).

The adaptive significance of electroreception in lampreys remains to be determined, but behavioral studies of similarly sensitive cartilaginous fishes demonstrate that low-frequency electric fields can guide close-range prey localization and suggest that electric field information may also be used in orientation (*18*).

Lampreys and several groups of primitive jawed fishes (Chondrichthyes, Chondrostei, Dipnoi, and Crossopterygii) have anterior lateral line nerves divided into dorsal and ventral roots; in each case the dorsal root terminates in the dorsal octavolateralis nucleus (*6*). The high degree of similarity between the lateral line system of lampreys and that of jawed fishes suggests that these anatomical features are homologous (*1*). Electroreception has probably evolved independently in the teleosts; living representatives of the holostean fishes, the group from which teleosts evolved, lack a dorsal nucleus (*1*) and are not electroreceptive (*19*), and only 3 of 35 teleost orders are electroreceptive (*20*).

Although only a single type of lateral line receptor has been reported for lampreys (*21*), we have histologically identified at least two populations that differ with respect to their depth from the epidermal surface and the diameter of their canals. We have not yet positively identified the electroreceptors. The homologies suggest, however, that the receptors, like those of chondrichthian and chondrostean fishes (*22*), will be ciliated and excited by cathodal electric stimuli—that is, negative electrical potential at the outer face of the receptor relative to the rest of the fish. In contrast, the electroreceptors of teleosts bear microvilli on their apical surfaces and are excited by anodal stimuli (*23*).

If electrosensory systems in lampreys and primitive jawed fishes are indeed homologous, by definition they have been inherited from the common ancestor of agnathans and gnathostomes. Thus, the earliest vertebrates were probably electroreceptive, and vertebrate electroreceptors may be as ancient as lateral line mechanoreceptors.

DAVID BODZNICK*
Department of Neurosciences,
University of California at San Diego,
La Jolla 92095
R. GLENN NORTHCUTT
Division of Biological Sciences,
University of Michigan,
Ann Arbor 48109

References and Notes

1. C. A. McCormick, thesis, University of Michigan (1978).
2. A. A. Pearson, *J. Comp. Neurol.* **64**, 235 (1936); W. J. A. J. Smeets and R. Nieuwenhuys, *ibid.* **165**, 333 (1976); R. G. Northcutt, in (*3*), p. 117.
3. E. S. Hodgson and R. F. Mathewson, Eds., *Sensory Biology of Sharks, Skates, and Rays* (Office of Naval Research, Department of the Navy, Arlington, Va., 1978).
4. R. L. Boord and C. B. G. Campbell, *Am. Zool.* **17**, 431 (1977).
5. D. M. Koester and R. L. Boord, *ibid.* **18**, 587 (1978).
6. R. G. Northcutt, *Brain Res.* **167**, 163 (1979); in *Comparative Studies of Hearing in Vertebrates*, A. N. Popper and R. R. Fay, Eds. (Springer-Verlag, New York, 1980).
7. P. J. McCready and R. L. Boord, *J. Morphol.* **150**, 527 (1976).
8. D. Bodznick and R. G. Northcutt, *Brain Res.* **195**, 313 (1980).
9. Voltage pulses were supplied by a square-pulse stimulator and isolation unit, and a 10^5 to 10^6 ohm series resistance was used to minimize electrode polarization effects. Electric field intensities were monitored with a pair of electrodes in the water. The fields were only approximately homogeneous in the central area of the tank where the fish were held.
10. J. D. Green, *Nature (London)* **182**, 962 (1958).
11. A. Fessard, Ed., *Handbook of Sensory Physiology* (Springer, New York, 1974), vol. 3, part 3.
12. A. J. Kalmijn, in (*11*), p. 147.
13. ———, *Nature (London)* **212**, 1232 (1966).
14. T. H. Bullock and J. T. Corwin, *J. Comp. Physiol.* **129A**, 223 (1979).
15. C. J. Platt, T. H. Bullock, G. Czéh, N. Kovaĉević, D. Konjević, M. Gojković, *ibid.* **95**, 323 (1974); T. H. Bullock, *J. Physiol. (Paris)* **75**, 397 (1970).
16. R. Schultz, E. C. Berkowitz, D. C. Pease, *J. Morphol.* **98**, 251 (1956); J. K. Moore and T. H. Bullock, personal communication.
17. H. Kleerekoper and K. Sibakin, *J. Fish, Res. Board Can.* **14**, 145 (1957); E. A. Stensiö, in *Traite de Zoologie*, P. Grassé, Ed. (Masson, Paris, 1958), vol. 13, part 1, p. 173; K. S. Thomson, in *Problems in Vertebrate Evolution*, S. M. Andrews, R. S. Miles, A. D. Walker, Eds. (Academic Press, New York, 1977), p. 247.
18. A. J. Kalmijn, in (*3*), p. 507.
19. R. G. Northcutt, D. Bodznick, T. H. Bullock, paper presented at the 28th International Congress of Physiological Sciences, Budapest, 13 to 19 July 1980.
20. T. H. Bullock, in (*11*), p. 1.
21. J. B. Johnston, *J. Comp. Neurol.* **12**, 1 (1902).
22. R. W. Murray, in (*11*), p. 125; J. H. Teeter, R. B. Szamier, M. V. L. Bennett, *J. Comp. Physiol.* **138**, 213 (1980).
23. T. Szabo and A. Fessard, in (*11*), p. 60.
24. We thank T. H. Bullock for support throughout the project and C. C. Bell for valuable comments on the manuscript. We also thank B. Monk and the National Marine Fisheries Service for animal collection. This study was supported by an NIH fellowship to D.B., and NIH and NSF research grants to R.G.N. and T. H. Bullock. R.G.N.'s participation was made possible by a Guggenheim Foundation fellowship.

* Present address: Department of Biology, Wesleyan University, Middletown, Conn. 06457.

16 July 1980; revised 25 November 1980

Insects as Selective Agents on Plant Vegetative Morphology: Egg Mimicry Reduces Egg Laying by Butterflies

Abstract. *Experiments show that* Heliconius *butterflies are less likely to oviposit on host plants that possess eggs or egglike plant structures. These egg mimics are an unambiguous example of a plant trait evolved in response to a host-restricted group of insect herbivores.*

The idea of coevolution between insects and plants is attractive to biologists attempting to account for patterns of plant chemistry and the use of plants by insects (*1*). However, it is difficult to demonstrate a causal connection between a plant characteristic and a particular selective agent (*2*) because most plants have been exposed through time to a multitude of pathogens and herbivores, any complement of which may have helped to drive the evolution of the presumed defensive chemistry. Thus, instead of a gene-for-gene coevolution, there may be a more diffuse process (*3*) that is intractable to experimental analysis.

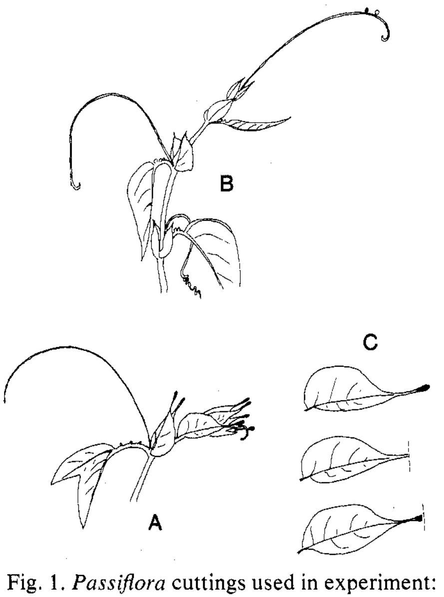

Fig. 1. *Passiflora* cuttings used in experiment: (A) *Passiflora cyanea*, showing display of egg mimics on stipule tips. (B) *Passiflora oerstedii*, showing yellow egg (open circle) placed near green egg (closed circle) on tendril. (C) Enlarged view of *P. cyanea* stipules showing (top) unaltered stipule, (middle) stipule with egg mimic removed, and (bottom) stipule cut but retaining egg mimic for control. *Passiflora cyanea* stipules are 3 to 4 cm in length.

One approach is to study plant groups that support only one or a few important herbivore taxa and that may therefore have traits attributable to coevolution with such taxa. For neotropical vines of the genus *Passiflora*, heliconiine butterflies are likely selective agents on several features of morphology (*4*). Plants such as *Passiflora* may have effectively "filtered out" most potential herbivores at an early stage of the evolution of their defensive chemistry, so that the insects remaining as significant herbivores of these plants are those that circumvent the chemical defenses of these plants. Morphological rather than chemical innovation is the effective evolutionary response to such herbivores (*5*), and herbivore behavior rather than counterdefensive chemistry shapes the course of evolution (*4*).

Structures resembling the yellow eggs of *Heliconius* butterflies have arisen independently on a number of *Passiflora* species in several subgenera and are derived from several distinct structures (*6*). That these structures have evolved specifically to mimic *Heliconius* eggs is indicated by the facts that (i) heliconiines are important defoliating agents of *Passiflora* (*7*); (ii) larvae of many *Heliconius* feed on congeneric eggs and larvae (*6*); and (iii) females exhibit great care in inspecting oviposition sites (*6, 8*). In this report we provide experimental evidence

0036-8075/81/0424-0467$00.50/0