that electrotonically couple cells and underlie the phenomenon of dye coupling (*10*). If our hypothesis of coupling between MNC's is correct, we predict that gap junctions occur on the cell membrane of MNC's (*19*). In freeze-fracture replicas of the PVN and SON, MNC's were identified by the criteria of large cell body diameter (> 15 μm) and cytoplasmic content of neurosecretory vesicles (160 to 240 nm). Morphology typical of gap junctions in other mammalian nerve tissue (*11, 20*) was observed (Fig. 3). Aggregates of 15 to several hundred particles were occasionally (*21*) found on the protoplasmic face of membrane surrounding neurosecretory cytoplasm. Particle aggregates were surrounded by a partial halo of nonparticulate membrane. We conclude that gap junctions provide the channels by which MNC's are dye-coupled.

Neuronal populations that fire action potentials synchronously (*8, 9*) and endocrine populations stimulated to secrete hormone in unison (*22*) are usually composed of electrotonically coupled cells (*23*). Our results show that some neuroendocrine cells in the rat hypothalamo-neurohypophysial system are dye-coupled and possess gap junctions. Since ionic current can flow through gap junctions that mediate dye coupling (*10*), some mammalian neuroendocrine cells should also be electrotonically coupled. Two hypotheses are suggested. First, the recruitment of vasopressinergic MNC's into a phasic firing pattern in the rat could be enhanced by electrotonic coupling. Second, the spike burst of oxytocinergic MNC's before the milk ejection reflex could be synchronized by electrotonic coupling. Studies with immunohistochemical techniques (*24*) should reveal the identity of coupled neuropeptidergic cells.

R. DAVID ANDREW*
Department of Zoology and Erindale College, University of Toronto, Mississauga, Ontario, Canada,
and *Department of Physiology, Tulane University School of Medicine, New Orleans, Louisiana 70112*
BRIAN A. MACVICAR
Department of Zoology and Erindale College, University of Toronto,
and *Neuroscience Program, Michigan State University, East Lansing 48824*
F. EDWARD DUDEK
Department of Zoology and Erindale College, University of Toronto, and
Department of Physiology, Tulane University School of Medicine
GLENN I. HATTON
Neuroscience Program, Michigan State University

References and Notes

1. W. Bargmann and E. Scharrer, *Am. Sci.* **39**, 255 (1951); J. Rossier, E. Battenberg, Q. Pittman, A. Bayon, L. Koda, R. Miller, R. Guillemin, F. Bloom, *Nature (London)* **277**, 653 (1979); M. Sar *et al.*, *J. Comp. Neurol.* **182**, 17 (1978).
2. M. J. Brownstein, J. T. Russell, H. Gainer, *Science* **207**, 373 (1980).
3. F. Vandesande and K. Dierickx, *Cell Tissue Res.* **164**, 153 (1975); P. Micevych and R. Elde, *J. Comp. Neurol.* **190**, 135 (1980); J. Rossier, Q. Pittman, F. Bloom, R. Guillemin, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 2555 (1980).
4. J. B. Wakerley and D. W. Lincoln, *Endocrinology* **57**, 477 (1973); D. W. Lincoln and J. B. Wakerley, *J. Physiol. (London)* **242**, 533 (1974).
5. E. Arnauld, B. Dufy, J. D. Vincent, *Brain Res.* **100**, 315 (1975); D. A. Poulain, J. B. Wakerley, R. E. J. Dyball, *Proc. R. Soc. London Ser. B* **196**, 367 (1977).
6. B. H. Gähwiler and J. J. Dreifuss, *Brain Res.* **177**, 95 (1979); R. M. Gulrajani and F. A. Roberge, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 2146 (1978).
7. C. D. Tweedle and G. I. Hatton, *Cell Tissue Res.* **181**, 59 (1977); W. A. Gregory, C. D. Tweedle, G. I. Hatton, *Brain Res. Bull.* **5**, 301 (1980).
8. P. A. Getting, *J. Neurophysiol.* **37**, 846 (1974); _____ and A. O. D. Willows, *ibid.*, p. 858; M. Merickel and R. Gray, *J. Neurobiol.* **11**, 73 (1980).
9. M. V. L. Bennett, G. D. Pappas, M. Giménez, Y. Nakajima, *J. Neurophysiol.* **30**, 236 (1967); M. V. L. Bennett, in *Structure and Function of Synapses*, G. D. Pappas and D. P. Purpura, Eds. (Raven, New York, 1972), p. 357.
10. M. V. L. Bennett and D. A. Goodenough, *Neurosci. Res. Program Bull.* **16**, 373 (1979); N. B. Gilula, in *The Neurosciences: Fourth Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1979), p. 359; S. B. Kater and N. J. Galvin, *J. Cell Biol.* **79**, 20 (1978); A. N. Spencer and R. A. Satterlie, *J. Neurobiol.* **11**, 13 (1980).
11. These areas include mesencephalic trigeminal nucleus of rat [R. Baker and R. Llinás, *J. Physiol. (London)* **212**, 45 (1971)], lateral vestibular nucleus of rat [H. Korn, C. Sotelo, F. Crepel, *Exp. Brain Res.* **16**, 225 (1973)], and inferior olive of cat [C. Sotelo, R. Llinás, R. Baker, *J. Neurophysiol.* **37**, 541 (1974); R. Llinás, R. Baker, C. Sotelo, *ibid.*, p. 560]. Gap junctions have been observed at dendrodendritic contacts in sensory cortex [J. J. Sloper, *Brain Res.* **44**, 641 (1972)], olfactory bulb [A. J. Pinching and T. P. S. Powell, *J. Cell Sci.* **9**, 347 (1971)], and cerebellar cortex [C. Sotelo and R. Llinás, *J. Cell Biol.* **53**, 271 (1972)].
12. In the invertebrates, neuroendocrine bag cells of *Aplysia* are weakly electrotonically coupled [J. E. Blankenship and J. T. Haskins, *J. Neurophysiol.* **42**, 347 (1979)]. These cells exhibit dye coupling, presumably by gap junctions found on neurosecretory neurites [L. K. Kaczmarek, M. Finbow, J. P. Revel, F. Strumwasser, *J. Neurobiol.* **10**, 535 (1979)]. Neuroendocrine cells in the snail, *Lymnaea* [T. A. De Vlieger, K. S. Kits, A. Ter Maat, J. C. Lodder, *J. Exp. Biol.* **84**, 259 (1980)] and in the lobster, *Homarus* [S. Konishi and E. A. Kravitz, *J. Physiol. (London)* **279**, 215 (1978)] are electrotonically coupled.
13. W. W. Stewart, *Cell* **14**, 741 (1978).
14. Dye coupling has been demonstrated between rat hippocampal neurons [B. A. MacVicar and F. E. Dudek, *Brain Res.* **196**, 494 (1980)] subsequently shown to be electrotonically coupled [B. A. MacVicar and F. E. Dudek, *Soc. Neurosci. Abstr.* **6**, 407 (1980)].
15. E. Scharrer, *Z. Vgl. Physiol.* **7**, 1 (1928).
16. F. E. Dudek, G. I. Hatton, B. A. MacVicar, *J. Physiol. (London)* **301**, 101 (1980).
17. A percentage of somata in dorsomedial PVN innervates extrahypothalamic centers [T. Ono *et al.*, *Neurosci. Lett.* **10**, 141 (1978)]. We did not impale this area.
18. Our findings with Lucifer Yellow do not support the suggestion [U. Kuhnt, M. J. Kelly, R. Schaumberg, *Exp. Brain Res.* **35**, 371 (1979)] that intracellular Procion Yellow crosses chemical synapses, since we found secondary staining only in MNC's and not processes with abundant synapses on MNC somata.
19. Thin sections show close membrane apposition between some MNC's (*8*) without glial intervention.
20. We observed many gap junctions with similar morphology between ependymal cells of the third ventricle, medial to the PVN. We found them less commonly between glial cells within the PVN and SON.
21. Gap junctions are difficult to find on MNC's, possibly because only 1 to 2 percent of the soma membrane directly contacts other MNC's (*7*). However there may simply be a low incidence of identifiable channels. For example, regenerating hepatocytes still display dramatic electrotonic coupling and faint dye coupling even after gap junction numbers are greatly reduced, suggesting that electrotonic coupling can be mediated by very few channels [D. J. Meyer, S. B. Yancey, J. P. Revel, *J. Cell Biol.* **83**, 84a (1979); for further discussion of this point, see C. Peracchia, *Int. Rev. Cytol.* **66**, 81 (1980), p. 98].
22. P. Meda, A. Perrelet, L. Orci, *J. Cell Biol.* **82**, 441 (1979).
23. Spiking populations are not restricted to nerve cells. Some exocrine cells such as those in salivary gland of *Helisoma* [S. B. Kater, J. R. Rued, A. D. Murphy, *J. Exp. Biol.* **72**, 77 (1978)] generate action potentials that propagate through the gland by electrotonic synapses.
24. Difficulties with impalement and dye injection of single hypothalamic neurons of mammals in vivo have prevented correlation of single-cell physiology with neuropeptide content. In the preoptic nucleus of the goldfish, Lucifer Yellow has been injected into antidromically identified MNC's that were subsequently shown by immunohistochemistry to contain isotocin [T. A. Reaves and J. N. Hayward, *Cell Tissue Res.* **202**, 17 (1979)] and enkephalin [T. A. Reaves and J. N. Hayward *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6099 (1979)]. Dye coupling was not apparent. A similar approach in the rat should indicate the content of dye-coupled MNC's.
25. We are grateful to R. R. Shivers of the University of Western Ontario for assistance with the freeze-fracture technique, W. W. Stewart for numerous gifts of Lucifer Yellow, W. G. Filion for providing fluoresence microscopy facilities, and G. Weir for technical assistance. This research was supported by grants from the Connaught Foundation, National Science and Engineering Research Council of Canada (NSERC) (A0395), and NIH (NS 16683) to F.E.D.; by NIH grant NS 01940 to G.I.H.; and by Canadian fellowships from MRC and NSERC to R.D.A. and B.A.M., respectively.

* Correspondence should be sent to R.D.A. at Tulane University School of Medicine.

17 September 1980; revised 4 November 1980

Unique Eye of Probable Evolutionary Significance

Abstract. *The eyes in the brain of a larval flatworm studied by electron microscopy are dissimilar. Light-sensitive structures in the right eye are microvilli; those in the left eye, the unique one, are both microvillar and ciliary. Perhaps this is evidence for the origin of a microvillar line of photoreceptoral evolution from a more primitive ciliary line.*

Larvae of certain marine flatworms have two symmetrically situated cerebral (that is, brain associated) eyes, called ocelli (*1*). In the larva of *Pseudoceros canadensis* studied by us (*2*) the two eyes are unlike each other. The right eye (Fig. 1) is composed of one cup-shaped pigmented cell (eyecup), whose cavity is directed laterally, and three sensory cells. Each sensory cell extends an array (rhabdomere) of straight, cylindrical, tightly packed microvilli into the

0036-8075/81/0313-1189$00.50/0

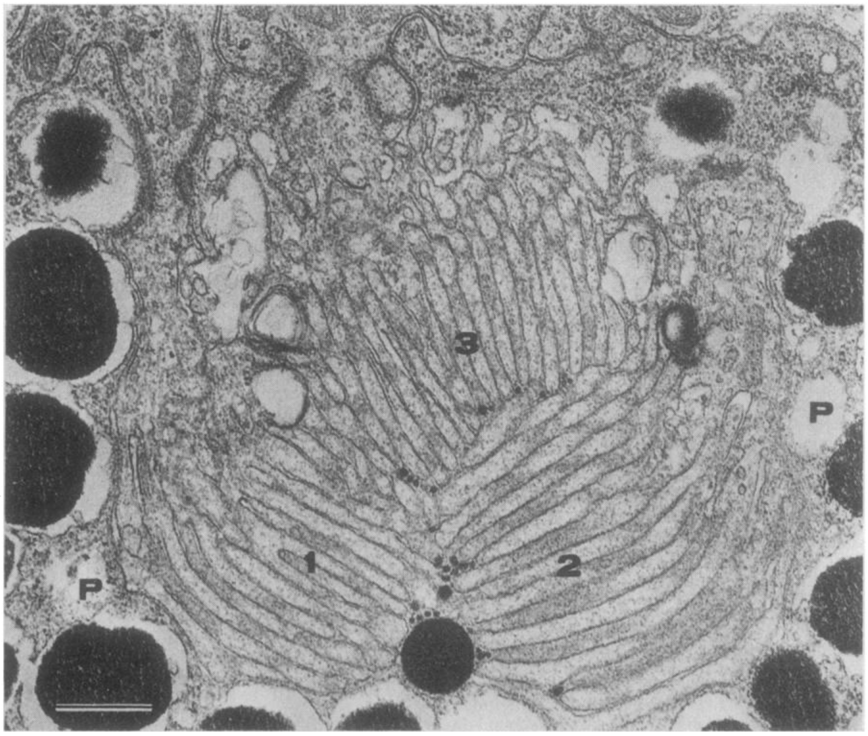
eyecup. One rhabdomere projects dorsally, another ventrally, and the central one toward the base of the pigmented cup. The bodies of the sensory cells, containing the nuclei, lie outside the opening of the eyecup.

The left eye (Fig. 2) is similarly oriented and constructed of one pigmented and four sensory cells. Three of the sensory cells bear cylindrical microvilli (right inset) like those in the right eye. The central sensory cell, however, sends unusually large arching cilia into the eyecup among the three rhabdomeres. Because of the curvature of the cilia a given section will show them cut longitudinally, obliquely, or transversely (left inset). In one ocellus we estimated about 50 cilia and 480 microvilli. Incidental cilia occur in many rhabdomeric eyes (*3*), but we know of no other cerebral eye with prominent arrays of both microvilli and cilia. Moreover, we know of no animal exhibiting bilateral asymmetry in its photoreceptors. Because of the positions of the microvilli and cilia within the eyecup we infer that both are photosensitive.

What is the possible evolutionary significance of the eyes, especially the left one, in this marine flatworm? The senior author theorized that there are two lines of photoreceptoral evolution: ciliary and rhabdomeric (*4*). The former is found in protists, coelenterates, bryozoans, arrowworms (chaetognaths), lower chordates, and vertebrates. The photoreceptor in these groups is usually a modified cilium. In arthropods, annelids, mollusks, and flatworms, the predominant photoreceptoral type belongs to a rhabdomeric lineage in which the photoreceptor is formed of microvilli or membranous lamellae from the cell surface, not from cilia. In both of these phyletic assemblages, however, photoreceptors of the opposite type are occasionally found (*3*).

There are other theories that attempt to describe the evolution of photoreceptors (*5*), but according to our theory the flatworms (Platyhelminthes) are near the divergence between those invertebrate phyla in the rhabdomeric line and those in which ciliary photoreceptors predominate. We suggest that the larva of *Pseudoceros canadensis* retains a ciliary photoreceptor, inherited from more primitive ancestors, plus an innovation in light sensors inherited from ancestral flatworms, namely, an array of microvilli. The rhabdomere then became the dominant photoreceptor in the evolutionary lineage that culminated in the eyes of cephalopods and arthropods.

RICHARD M. EAKIN
JEAN L. BRANDENBERGER
Department of Zoology,
University of California, Berkeley 94720



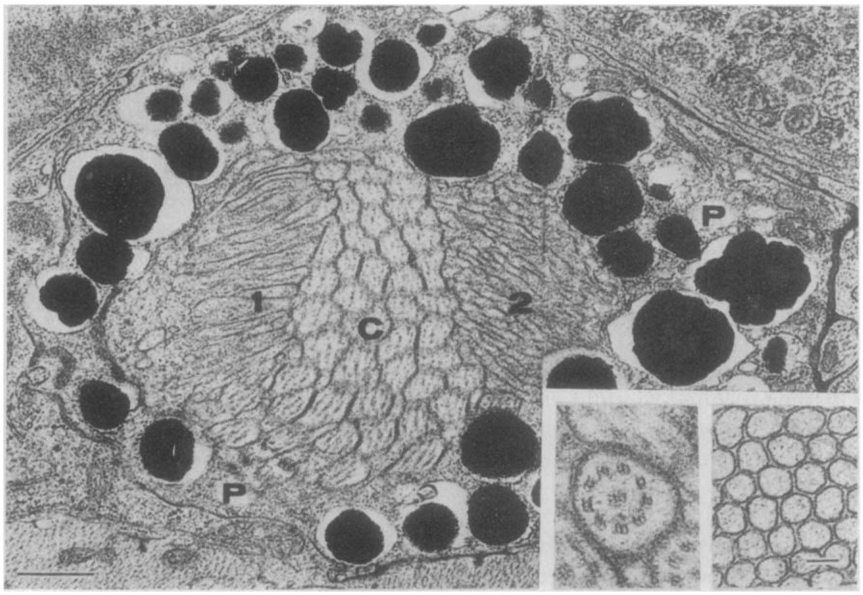


Fig. 1 (top). Electron micrograph of right eye composed of a pigmented eyecup (*P*) containing three rhabdomeres of microvilli (1, 2, and 3). The scale bar is 0.5 μm. Fig. 2 (bottom). Electron micrograph (montage) of left eye composed of three rhabdomeres of microvilli (1 and 2) and one central array of cilia (*C*) in a pigmented eyecup (*P*). The scale bar is 1.0 μm. (Right inset) Rhabdomeric microvilli in cross section; (left inset) a cilium in cross section. The scale bar is 0.1 μm.

References and Notes

1. E. E. Ruppert, in *Settlement and Metamorphosis of Marine Invertebrate Larvae*, F. Chia and M. E. Rice, Eds. (Elsevier, New York, 1978), pp. 65–81.
2. The Müller's larvae of the polyclad turbellarian *Pseudoceros canadensis* were obtained from cultures of adult worms at the Friday Harbor Laboratories of the University of Washington. The larvae were fixed in 1.5 percent glutaraldehyde in 0.2*M* sodium cacodylate, 0.1*M* NaCl, and 0.35*M* sucrose, *p*H 7.2. After being transported to Berkeley, they were postfixed in chilled 1 percent OsO_4, dehydrated, embedded in Spurr's resin, sectioned, and examined with RCA and Siemens electron microscopes.
3. R. M. Eakin, *Am. Zool.* **19**, 647 (1979).
4. ——, in *General Physiology of Cell Specialization*, D. Mazia and A. Tyler, Eds. (McGraw-Hill, New York, 1963), pp. 393–425; in *Evolutionary Biology*, Th. Dobzhansky, M. K. Hecht, W. C. Steere, Eds. (Appleton-Century-Crofts, New York, 1968), vol. 2, pp. 194–242.
5. L. v. Salvini-Plawen and E. Mayr, in *Evolutionary Biology*, M. K. Hecht, W. C. Steere, B. Wallace, Eds. (Plenum, New York, 1977), vol. 10, pp. 207–263; J. R. Vanfleteren and A. Coomans, *Z. Zool. Syst. Evolutionsforsch.* **14**, 157 (1976).
6. We thank Dr. T. C. Lacalli of the University of Saskatchewan whose personal communication about the eyes of the Müller's larva of *Pseudoceros canadensis* led us to make this study, and to Dr. R. L. Fernald and C. Young of the Friday Harbor Laboratories for culturing and fixing the larvae used by us. We thank D. R. Bentley, C. O. Hermans, and D. R. Pitelka, for criticisms of the work. Supported by USPHS grant EY02229.

28 July 1980