which incoming odors can be matched, allowing a special responsiveness to meaningful odors. The special neural responsiveness to a previously experienced odor may represent the functioning of an olfactory template.

We have shown that early olfactory experiences, of the type that reflect normal differences in sensory experience for the developing rats, can alter subsequent responses of olfactory bulbs. To date, our understanding of how mammalian sensory systems develop has been based largely on sensory deprivation or deafferentation (*26*). We hope that analyses like this one will enable us to learn more about how development is affected by normal variation in sensory experience.

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References and Notes

1. M. Leon and H. Moltz, *Physiol. Behav.* **7**, 265 (1971); *ibid.* **8**, 683 (1972).
2. J. S. Rosenblatt and D. S. Lehrman, in *Maternal Behavior in Mammals*, H. L. Rheingold, Ed. (Wiley, New York, 1963), p. 5.
3. J. Altman *et al.*, *Dev. Psychobiol.* **4**, 97 (1971).
4. M. Leon, *Physiol. Behav.* **13**, 441, (1974).
5. W. Harder, *Verh. Dtsch. Zool. Ges.* **2**, 95 (1949).
6. M. Leon, *Physiol. Behav.* **14**, 311 (1975).
7. B. S. Draser *et al.*, *Proc. Nutr. Soc.* **32**, 49 (1973); J. A. Lampen and H. R. Peterjohn, *J. Bacteriol.* **62**, 281 (1951); J. R. Porter and L. F. Rettger, *J. Infect. Dis.* **66**, 104 (1940).
8. J. R. Alberts, *J. Comp. Physiol. Psychol.* **92**, 220 (1978); P. C. Brunjes and J. R. Alberts, *ibid.* **93**, 548 (1979).
9. M. Leon, B. G. Galef, J. Behse, *Physiol. Behav.* **18**, 387 (1977).
10. C. R. Gallistel, C. T. Piner, T. O. Allen, N. T. Adler, E. Yadin, M. Negin, *Neurosci. Biobehav. Rev.* **6**, 409 (1982); L. Sokoloff, *J. Cereb. Blood Flow Metab.* **1**, 7 (1981); P. J. Yarowski and D. H. Ingvar, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 2353 (1981).
11. We used three procedures to minimize extraneous odor experience by the pups. (i) Their mothers were maintained on a sucrose-based diet sufficient in fat (Teklad Diet TD69446, Madison, Wis.) after parturition to suppress the dominant maternal odor (*5*, *6*). (ii) Litters were kept in two vivarium rooms separate from the rest of the colony. (iii) All of the pups in a litter received the same olfactory experience but only one pup was tested from each litter to avoid odor contamination and the statistical problems associated with using littermates in the same group (H. Abbey and E. Howard, *Dev. Psychobiol.* **6**, 329 (1973)]. Pregnant (16- to 18-day) Wistar rats were obtained from Simonsen Laboratories and allowed to give birth in polypropylene cages (34 by 29 by 17 cm) lined with wood chips. Lights remained on from 0800 to 2000 hours and all animal rooms were maintained at 20° ± 1°C. Wayne Lab Blox and water were freely available until parturition, at which time dams were given unlimited test diet. Litters were culled to eight pups on day of birth. During daily exposure sessions, a flow-dilution olfactometer was used to obtain either a 1:10 dilution of saturated peppermint vapor (Schilling, Baltimore) at a flow rate of 8 liter/min or a clean airstream at the same flow rate. The airstreams were routed through separate sets of tubing and exposure containers to avoid contamination. The outlet from each stream opened into a 30-cm Tupperware container with an outlet port in its side opposite the airstream inlet. Clean wood chips lined the floor of the container and the temperature in the container was 22° ± 1°C. Each day, the litters were removed from the home cage and placed in the exposure chamber, about 10 cm from the airstream. Each pup was then individually cupped in a gloved hand so that heat loss was minimized and was held with its nose in the airstream. At the same time, the pup's perineal region was stimulated with a small sable hair brush (*12*).
12. P. E. Pedersen and E. M. Blass, *Dev. Psychobiol.* **15**, 349 (1982).
13. P. T. Kelly and T. McCulloch, *Brain Res.* **260**, 172 (1983).
14. After being removed from the skull, the brain was mounted on a cryostat pedestal with embedding matrix (Tissue-Tek, Miles) and immersed in Freon-12 at −40°C until frozen, usually 1 to 2 minutes. The frozen brain was equilibrated to the cryostat temperature of −17°C for 45 minutes. We then cut coronal 20-μm sections through the entire olfactory bulb. Each section was immediately picked up on a cover slip and placed on a slide warmer at 60°C for 5 to 10 minutes. We glued the cover slips to a sheet of cardboard and exposed them to Kodak SB-5 x-ray film for 10 days at 22°C in an exposure cassette. A set of ^{14}C-labeled methylmethacrylate standards (Amersham), previously calibrated to ^{14}C uptake in 20-μm brain sections, was exposed with each sheet of film.
15. The image processing system consisted of a PDP 11/23 computer in conjunction with a De Anza video image processor and a high-resolution video camera. The video image of the autoradiograph was digitized by assigning an eight-bit gray level value to each element of a 512 by 512 pixel array. The digitized image was then stored for further analysis. Next, a calibration function was constructed by plotting the gray levels of the exposure standards against their previously determined ^{14}C-labeled tissue equivalents. This curve was then fitted to a linear function. A new image of the brain section was created from this calibration function. Concentrations of ^{14}C could then be determined for any specified area of the section.
16. F. Jourdan, A. Duveau, L. Astic, A. Holley, *Brain. Res.* **188**, 139 (1980).
17. F. R. Sharp, J. S. Kauer, G. M. Shepherd, *ibid.* **98**, 596 (1975).
18. T. O. Allen, N. T. Adler, J. H. Greenberg, M. Reivich, *Science* **211**, 1070 (1981).
19. This method allowed us to quantify our results on a ratio scale, rather than on a rank-order index. While the latter method is appropriate for many brain studies, several factors precluded our use of it. Since the areas of interest are the darkest areas of the autoradiographs even in the control animals, a ceiling effect would prevent any differences from being observed in a rank-order index. Second, olfactory bulb 2DG autoradiographs have very few discrete structures to be ranked, unlike sections taken from other parts of the brain. Rather, one observes a relatively homogeneous band, covering the glomerular layer and extending to the granule cell lamina. Occasionally, this band is punctuated by small loci of increased uptake that corresponds to individual groups of glomeruli.
20. F. R. Sharp *et al.*, *Brain Res.* **263**, 97 (1983).
21. The apparatus used to measure respiration rate was a glass canister (29 by 11 cm) with an airtight plastic lid that was fitted with a pressure-sensitive transducer, the output of which was fed to a respiration monitor (Columbus Instruments). Odor entered the chamber as a 2 liter/min stream of a 1:10 dilution of peppermint or a clean airstream at the same flow rate, through the flow-dilution olfactometer. The airstream was admitted for 1 minute, followed by 4.75 minutes in which no air was admitted and the respiration rate was measured. This cycle was repeated for 45 minutes. Respiration rate was integrated over successive 15-second periods, and these values were averaged over each of the eight measurement intervals.
22. W. B. Stewart, J. S. Kauer, G. M. Shepherd, *J. Comp. Neurol.* **185**, 715 (1979); M. H. Teicher, W. B. Stewart, J. S. Kauer, G. M. Shepherd, *Brain Res.* **194**, 530 (1980).
23. C. A. Greer, W. B. Stewart, M. H. Teicher, G. M. Shepherd, *J. Neurosci.* **2**, 1744 (1982).
24. B. Friedman and J. L. Price, *J. Comp. Neurol.* **223**, 88 (1984).
25. W. J. Freeman, *Perspect. Biol. Med.* **24**, 561 (1981); *Biol. Psychiatry* **18**, 1107 (1983).
26. R. W. Guillery, *J. Comp. Neurol.* **144**, 117 (1972).
27. We thank T. O. Allen, N. T. Adler, M. Berns, and R. Walter for their help in initiating and completing this experiment. We thank P. Yahr for her comments on the manuscript. Supported in part by NSF grant BNS 8123107 and Research Scientist Development Award MH 0037 from NIMH to M.L. The Laser Microbeam Project facility at the University of California, Irvine, is a National Institutes of Health Biotechnology Resource Program and is supported by NIH grant RRO 1192 to M. Berns.

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Illumination Induces Dye Incorporation in Photoreceptor Cells

Abstract. *Illumination of fly photoreceptors in the presence of the fluorescent dye Lucifer yellow initiates incorporation of the dye, which stains each cell down to its synaptic terminal. Unilluminated cells do not become stained. Experiments on animals in vivo show that selected cells can be stained without loss of viability. "Induced endocytosis" provides a plausible mechanism underlying this phenomenon.*

Several investigators have recently reported that certain classes of neurons autonomously take up various fluorescent dyes while other cells in the same matrix do not (*1*, *2*). Although this circumstance allows tagging of specific cells, the reason for its specificity remains obscure. We now report that illumination can induce photoreceptor cells to take up Lucifer yellow from the extracellular space. From a combination of optical experiments in living, unanesthetized animals and histological examinations, we infer that dye is internalized through "induced endocytosis." Beyond its use as a novel approach to the study of axonal wiring and visuotopic projection, this phenomenon may be used to enhance pharmacological treatment.

The retina of the fly's eye provides a decisive advantage in the study of a neural matrix because the array of photoreceptor inputs can be easily visualized in vivo. One method uses each corneal lenslet as a magnifying glass to observe the superimposed virtual images of the photoreceptors, an optical effect called the deep pseudopupil (DPP) (*3*). Alternatively, the corneal lenslets can be optically neutralized by a drop of water placed between the microscope objective and the cornea, which permits each individual photoreceptor cell to be seen (*4*). Using epi-illumination microscopy, one can simultaneously observe and selectively stimulate the photoreceptors.

Beneath each facet lens of the fly's eye, the unit structure called an ommatidium contains eight photoreceptor cells. Each cell has an isolated, special-

ized organelle called a rhabdomere, which houses the visual pigment and acts as an optical waveguide. Rhabdomeres of the six peripheral receptors (R1 to R6) form an asymmetric, trapezoidal pattern about a central pair of photoreceptors that lie in tandem (*5*) (inset in Fig. 1D).

Application of 0.5 percent Lucifer yellow CH (*6*) to the extracellular space of the retina, followed by 30 minutes of illumination produced a dramatic change in yellow emission from the DPP in the living animal (*7, 8*). Histological examination (*9*) readily confirmed the fate of the dye. Figure 1A shows a cross section of this retina cut perpendicular to the ommatidial axes in the region of the R1 to R6 nuclei (level a in Fig. 1D). In contrast to the unstained cells, which appear green due to their autofluorescence, some cells are intensely yellow, producing a mottled pattern of cell staining. This pattern is orderly and consistent with the known optics of the fly's eye, if one considers the inverted image produced by each facet lens onto its own set of photoreceptors (*10*). Staining was not produced by the mere presence of the dye, since application of an even higher concentration (suffusing the cut eye in vivo with 5 percent Lucifer yellow) followed by 4 hours of dark adaptation did not produce stained photoreceptors. Hence, illumination is necessary for dye uptake to occur.

Photoreceptor axons leave the retina

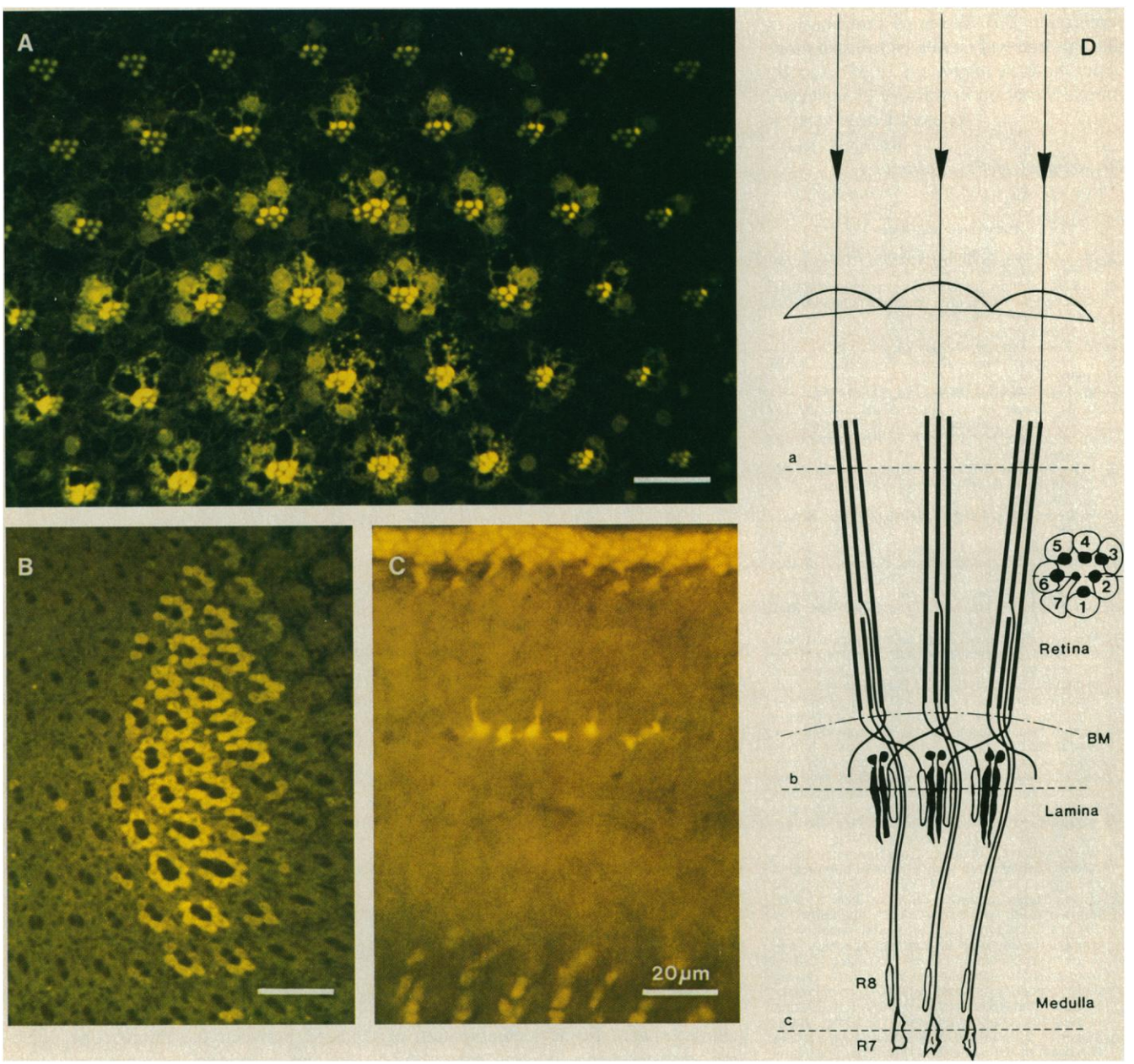

Fig. 1. Induced staining of photoreceptor cells in the compound eye of a female housefly *Musca domestica* (wild type), as revealed by epifluorescence microscopy (*7, 9*). (A) Cross section (1 μm thick) of the retina, corresponding to level a in (D). The bright yellow spots are stained rhabdomeres. The cells to which they belong are also stained, particularly the nuclei. (B) Cross section (1 μm thick) of the lamina of the same fly as in (A), corresponding to level b in (D). Each stained cartridge consists of two dark central elements (monopolar neurons L1 and L2) surrounded by a crown of six stained axon terminals whose receptors share the same direction of gaze. (C) Cross section (5 μm thick) of the medulla, corresponding to level c in (D). The stained profiles have the characteristic form of R7 photoreceptor axon terminals. (D) Schema of cellular arrangement, numbering system of photoreceptors in the retina, and projection of the various receptor axons to the lamina and medulla [adapted from (*13*)]. In the three adjacent lenses depicted, parallel light rays (arrowed lines), fall onto different photoreceptors whose axons project to a common lamina cartridge (*10, 12*). The two central cells R7 and R8 project directly to the corresponding medullary column and their axons terminate each within a specific sublamina. Abbreviation: BM, basement membrane.

through the basement membrane (Fig. 1D) and enter the first optic ganglion, the lamina. The short visual fibers of R1 to R6 decussate and project onto different unit structures called cartridges (*5, 11, 12*). The microdecussations are such that the axons of photoreceptors looking at a common point in space feed a common cartridge, where they make synaptic contact with its two large monopolar neurons L1 and L2 [principle of neural superposition (*10, 12*)]. A cross section through the lamina (level b in Fig. 1D) of the same fly shows an array of cartridges, each displaying a pair of dark central elements (L1 and L2) surrounded by a crown of six photoreceptor axon terminals (Fig. 1B). In contrast to the patchwork staining seen in the retina, virtually every stained cartridge has a complete crown of six yellow photoreceptor axon terminals, in agreement with the principle of neural superposition. Other lamina cartridges appear homogeneously green because of the weak autofluorescence of the unstained photoreceptor axons.

Axons from the two central cells R7 and R8 are long visual fibers (*5, 11*). They project pairwise to their respective columns in the second optic ganglion, the medulla, where they terminate, each in a specific sublamina, each with a characteristic synaptic ending (*11, 13*). Figure 1C shows stained profiles in the medulla of a fly that received the same treatment as the previous fly. The profiles terminate in the R7 sublamina and have the characteristic shape of R7 axon terminals (Fig. 1D). In this fly we could also follow the stained companion R8 throughout the chiasm to the outer layer of the medulla. In a cross section through the chiasm, these twin axons show up as a distinct double dot array (data not shown). Clearly, the described procedure stains the entire cell down to its synaptic terminal. Nineteen fully sectioned animals displayed distinctly stained photoreceptors and axon terminals; six other animals showed less clear results, which could be attributed later to some failure during the histological processing.

By generating a positive print of the outside world onto various neuropils, this staining method reveals not only retinotopic but also visuotopic projection. For example, the vertically oriented, elliptical patch of stained cartridges (Fig. 1B) resulting from exposure of the eye to a circular spot of light is direct evidence for the kind of anamorphism that characterizes the projection of the outside world onto the fly lamina. Moreover, the discrete staining of receptors in the retinal mosaic (Fig. 1A) and the full staining of the related cartridges (Fig. 1B) attests to the point-focusing property of each corneal lenslet.

To further explore the variables of the dye uptake mechanism, we performed experiments that did not require histological processing. Application of only 0.05 percent Lucifer yellow to the extracellular space followed by 15 minutes of illumination (*7*) led to brightly stained photoreceptors as assessed by in vivo observation of either the DPP or the individual cells. After 24 hours, even 60 minutes of illumination did not produce any other stained photoreceptors in the same eye, suggesting that the animal can clear the dye from the extracellular space within 24 hours. In contrast, within 3 hours of Lucifer yellow application, illumination of different regions of the same eye produced staining in each region.

Since dark-adapted photoreceptors are impermeable to the dye, How does it enter the illuminated ones? One possibility is that light-induced membrane damage could lead to leakage of Lucifer yellow into the cytoplasm. Such a mechanism has been proposed for merocyanine 540 (*2*). Photoexcitation of bound dye results in membrane damage leading to permeability and cell death (*2, 14*). Two arguments suggest that this is not the case in our experiments. (i) Immediately after staining the photoreceptors, application of trypan blue, a dye excluded by viable cells (*2*), completely quenched Lucifer yellow fluorescence in the extracellular space but not in the stained cells, suggesting that the cell membrane remained intact. (ii) During our experiments and even 7 days later (*15*), intracellular migration of pigment granules (*8*) was still functional in the stained cells, indicating that they were viable. This should not occur if the cell membrane had been ruptured, because membrane disruption at normal extracellular ion concentrations blocks organelle translocation (*16*).

We propose that the mechanism underlying dye uptake is a stimulated endocytosis for two reasons. (i) Some of the dye conspicuously appears to be contained in vesicles near the plasma membrane. In sections cut parallel to the ommatidial axis and observed at higher resolution, we could see these vesicles (diameter, approximately 0.4 μm) along the entire length of the receptor cell body. (ii) The normal recycling of shed rhabdomeric membrane in dipteran photoreceptors apparently proceeds by pinocytosis and phagocytosis at the plasma membrane (*17*), an effect that can be induced by local illumination, as demonstrated in the locust (*18*).

Perhaps a parallel exists between fly photoreceptors, which depolarize under photic stimulation and internalize stain, and vertebrate photoreceptors, which depolarize in darkness (*19*) and capture extracellular markers by endocytosis at the synaptic terminal (*20*). A similar parallel may exist with the neuromuscular junction, where electrical stimulation of the motoneuron induces endocytosis in the presynaptic terminal (*21*). Even chemically stimulated lymphocytes exhibit increased mitochondrial entrapment of the extracellularly applied dye rhodamine 123, which may reflect the increased energy requirements of these cells (*22*). Taken together these results suggest that an increase in either ionic conductance or metabolic activity may provoke dye integration in various kinds of cells.

This induced cellular ingestion can possibly be extended to other agents. In this context we have shown that illumination promotes colchicine incorporation into photoreceptors (*23*). Whatever its cellular mechanism, the uptake process is notable because it apparently does not affect cell viability.

Further studies will show how far the phenomenon can be extended to other neural and nonneural cells. If appropriate stimulation were to cause target cells to embody extracellularly applied agents, this would open new horizons not only in cell science but also in therapeutics.

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References and Notes

1. A. Holl, *Z. Naturforsch. Teil C* **35**, 526 (1980); F. M. de Monasterio, S. J. Schein, E. P. McCrane, *Science* **213**, 1278 (1981); P. Detwiler and P. Sarthy, *Neurosci. Lett.* **22**, 227 (1981); P. Layer and G. Vollmer, *ibid.* **31**, 99 (1982); ———, S. Kotz, *ibid.* **35**, 239 (1983). P. Sarthy *et al.*, *J. Comp. Neurol.* **206**, 371 (1982).
2. T. G. Easton *et al.*, *Cell* **13**, 475 (1978).
3. N. Franceschini and K. Kirschfeld, *Kybernetik* **9**, 159 (1971).
4. ———, *ibid.* **8**, 1 (1971).
5. O. Trujillo-Cenoz and J. Melamed, in *The Functional Organization of the Compound Eye*, C. G. Bernhard, Ed. (Pergamon, Oxford, 1966), p. 339.
6. Nonautoreactive dye [W. W. Stewart, *Cell* **14**, 741 (1978)].
7. One hundred nanoliters of solution with the given dye concentration (osmotically balanced with sucrose to 450 mosM) was ejected from a volumetric micropipette onto the surface of retinal tissue under a hole four facets in diameter, cut in the dorsal-parietal apex of the eye. The piece of cornea was replaced, and coagulated hemolymph sealed the wound, preventing dehydration. The fly was mounted on a goniometer and oriented under an epifluorescence microscope. Blue light (435-nm line) from a high-

pressure mercury bulb (HBO 100 W/2, Osram) was focused onto an aperture diaphragm imaged at the back focal plane of the objective (Zeiss Luminar 40 mm; numerical aperture, 0.13). This produced a luminous disk pattern imaged at infinity, subtending 7° of arc and having an intensity about three decades above the threshold for pigment granule migration (*8*). The fly observed this pattern for 30 minutes, with the dorsal-frontal part of its retina. Fluorescence increase in the photoreceptors was monitored by continuously observing the deep pseudopupil (*3*).

8. K. Kirschfeld and N. Franceschini, *Kybernetik* **6**, 13 (1969); N. Franceschini and K. Kirschfeld, *Biol. Cybern.* **21**, 184 (1976).
9. The animal was immediately dissected in 4 percent formaldehyde, fixed overnight at 4°C, dehydrated in an alcohol series, infiltrated with epoxy resin [J. R. Spurr, *J. Ultrastruct. Res.* **26**, 31 (1969)], embedded, and sectioned 1 or 5 μm thick. Sections were observed with an epifluorescence microscope (435-nm excitation) and photographed through a Zeiss Plan Neofluar ×25 objective with Kodak Ektachrome 400 ASA film.
10. K. Kirschfeld, *Exp. Brain Res.* **3**, 248 (1967).
11. S. R. Cajal and D. Sanchez, *Trab. Lab. Invest. Biol. Univ. Madrid* **13**, 1 (1915); J. A. Campos-Ortega and N. J. Strausfeld, *Z. Zellforsch. Mikrosk. Anat.* **124**, 561 (1972).
12. O. Trujillo-Cenoz and J. Melamed, *J. Ultrastruct. Res.* **16**, 395 (1966); V. Braitenberg, *Exp. Brain Res.* **3**, 271 (1967).
13. N. J. Strausfeld, *Atlas of an Insect Brain* (Springer-Verlag, Heidelberg, 1976).
14. Cell death following photoexcitation of merocyanine 540 incorporated into the cell membrane is also consistent with the rapid killing of photoexcited neurons injected with procion dyes or Lucifer yellow [J. P. Miller and A. I. Selverston, *Science* **206**, 702 (1979)].
15. After induced staining of photoreceptors, the flies were kept in darkness except during feeding and observation periods.
16. W. Z. Cande, K. McDonald, R. L. Meeusen, *J. Cell Biol.* **88**, 618 (1981); M. E. Stearns and R. L. Ochs, *ibid.* **94**, 727 (1982); D. S. Forman, *Exp. Cell Res.* **141**, 139 (1982); T. G. Clark and J. L. Rosenbaum, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4655 (1982).
17. D. S. Williams and A. D. Blest, *Cell Tissue Res.* **205**, 423 (1980).
18. D. S. Williams, *Science* **218**, 898 (1982).
19. H. B. Barlow and P. Fatt, Eds., *Vertebrate Photoreception* (Academic Press, London, 1977).
20. S. Schacher, E. Holtzman, D. C. Hood, *J. Cell Biol.* **70**, 178 (1976); S. Schaeffer and E. Raviola, *ibid.* **79**, 802 (1978).
21. J. E. Heuser and T. S. Reese, *ibid.* **57**, 315 (1973).
22. Z. Darzynkiewicz, L. Staiano-Coico, M. R. Melamed, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2383 (1981).
23. M. Wilcox and N. Franceschini, *Invest. Ophthalmol. Visual Sci.* **24**, 177 (1983).

24. We are grateful to A. Totin-Yvard for technical assistance and to J. P. Changeux, J. A. Coles, S. S. Easter, Jr., H. M. Gerschenfeld, T. E. Ogden, and W. L. Pak for critically reading the manuscript. We thank W. W. Stewart for his gift of Lucifer yellow CH. This work was supported by grants from Centre National de la Recherche Scientifique (CNRS), Délégation Générale à la Recherche Scientifique et Technique, Délégation à l'Aménagement du Territoire et à l'Action Régionale, and Fondation pour la Recherche Médicale to N.F. M.W. received a postdoctoral fellowship from the Fogarty International Center (NIH-CNRS), CNRS, and Fondation pour la Recherche Médicale.

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Identified Interneurons Produce Both Primary Afferent Depolarization and Presynaptic Inhibition

Abstract. *Crayfish interneurons were identified that appear to be directly responsible for presynaptic inhibition of primary afferent synapses during crayfish escape behavior. The interneurons are fired by a polysynaptic pathway triggered by the giant escape command axons. When directly stimulated, these interneurons produce short-latency, chloride-dependent primary afferent depolarizations and presynaptically inhibit primary afferent input to mechanosensory interneurons.*

Presynaptic inhibition decreases synaptic efficacy by reducing the release of transmitter substances from presynaptic terminals (*1, 2*). In the central nervous system of invertebrates and vertebrates, presynaptic inhibition of the release of transmitters in primary afferent fibers is correlated with primary afferent depolarization (PAD) (*3, 4*). The mechanisms for PAD and presynaptic inhibition in the central nervous system have not been determined, in part because the interneuronal pathways effecting PAD are unknown (*5*). We have studied the polysynaptic pathway by which the giant, escape command axons of the crayfish inhibit transmitter release in primary mechanosensory afferents. The synapses of these afferents are depression-prone and are responsible for behavioral habituation of the escape tail-flip (*6*). The pathway producing presynaptic inhibition is of special interest because it protects the afferent synapses from the depression that would result as a consequence of reafference during the tail-flip (*7*), and thus prevents habituation of the escape response caused by the animal's own movement. We have identified what appear to be the final inhibitory interneurons for command-derived presynaptic inhibition. These newly identified cells are fired by the giant escape command neurons and produce both PAD in mechanosensory afferents and presynaptic inhibition of primary afferent input to first-order mechanosensory interneurons.

Our experimental preparation was the isolated abdominal nerve cord of the crayfish *Procambarus clarkii* (*8*). Simultaneous intracellular recordings were obtained from interneurons and primary afferent axons in the neuropil of the sixth abdominal ganglion. Many of the primary afferent axons are of large diameter (6 to 30 μm) and can be routinely impaled with intracellular electrodes (*9*). With intracellular dye injections we determined that the afferents terminate within 300 μm of the margin of the neuropil after entering the sixth ganglion. Our electrodes were positioned in the afferent axons near the point where they entered the neuropil and thus were close to the sites of PAD production. This is supported by the fact that the amplitude of PAD could be altered by imposed polarization of the afferent membrane as well as intracellular chloride injection. In several experiments a sucrose gap recording of PAD from the fifth sensory root was combined with intracellular impalements of a PAD-producing inhibitory interneuron (PADI) in the sixth ganglion and an identified primary sensory interneuron. A simplified diagram of the pathways studied and the sites of recording and stimulation is presented in Fig. 1A.

To be classified as a PADI, a cell had to produce short-latency PAD and be fired by a single giant axon impulse. We studied a total of 34 PADI's in 31 animals. A single action potential in a giant escape command axon results in presynaptic inhibition of primary afferent synapses (*7*). Cells identified as PADI's always fired, and sometimes gave multiple spikes, after a single giant axon impulse (Fig. 1B). The time from the giant axon impulse to the first spike in a PADI was 11.4 ± 4.6 msec (mean ± standard deviation). Several lines of evidence (*10*) indicate that the pathway from the giant axons to the PADI's is polysynaptic and involves interganglionic interneurons. PADI's were never fired by stimulation of any of the sensory roots at stimulus intensities below the threshold of the lateral giant escape command axons (*11*).

When PADI's were fired directly by the injection of depolarizing current, their spikes produced constant PAD's with a mean latency of 0.97 ± 0.12 msec (n = 15) and a mean duration of 36.2 ± 9.2 msec (n = 15). These unitary PAD's were capable of following PADI impulses one-for-one at frequencies greater than 100 Hz, and their amplitude was increased by intracellular injection of chloride. Another notable aspect of the unitary PAD's was that their amplitude fluctuated in discrete steps (Fig. 1C). We have not analyzed these fluctuations, but the short, constant latency makes it highly unlikely that they are caused by the intermittent firing of an interposed interneuron.

In the seven cases tested, directly elicited PADI impulses produced both PAD and presynaptic inhibition of primary afferent input to identified sensory interneurons. An example from one experiment is presented in Fig. 1, D to G. Primary afferents were stimulated by