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## Light-Induced Changes in the Structure of Pigmented Granules in Aplysia Neurons


Abstract. *Pigmented granules in* Aplysia *neurons prepared in the dark contain material that appears to be composed of 50-angstrom globules and a precipitate, probably a calcium salt. On illumination the globules rearrange into paracrystalline arrays and membrane-like lamellae. The morphologic transformation may be related to calcium release from the granules, and the released calcium may mediate the light-evoked hyperpolarization described by others.*


The giant neurons of *Aplysia* hyperpolarize in response to light and have been studied as model photoreceptors (*1, 2*). Brown and Brown (*2*) have shown that the hyperpolarizing light response is due to an increase in the K conductance ($G_K$) of the membrane; they suggested that the action of light on $G_K$ may be mediated by an increase in the cytoplasmic Ca concentration for two reasons: (i) the effect of light on $G_K$ was mimicked by $CaCl_2$ injection (*3*), and (ii) pressure injection of the Ca chelator ethylene glycol-bis(aminoethylether)-*N,N'*-tetraacetic acid(EGTA) abolished the photoresponse. Since orange-yellow granules in the cytoplasm contain a pigment with an absorption maximum (*4*) at the same wavelength as the peak of the action spectrum of the light response, 490 nm (*1, 2*), it seems likely that the pigment mediating the light response is in the granules. I report here that the pigmented granules (*5–7*) are capable of accumulating or binding divalent cations, and that their contents undergo a light-induced change in substructure from globular particles about 50 Å in diameter to membrane-like lamellae. These morphological observations in combination with the physiological evidence suggest that release of Ca from the pigmented granules may mediate the hyperpolarizing light response and underlie the transformation from globule to lamella.

Dark-adapted neurons were prepared by dissecting the visceral or pleural ganglia from *Aplysia californica* under illumination by a photographic safe light with a dark red (Kodak 1A) filter; the light faced the ceiling about 2 m from the preparation. Whole ganglia were incubated for various lengths of time in artificial seawater (ASW) (*8*) in total darkness; they were then transferred under red light to fixative (*9*) for 1 hour before individual neurons were dissected out with usual illumination for the dissecting microscope. The isolated cells were fixed for an additional hour. Illuminated neurons were prepared by dissecting the ganglia from animals in room light. Ganglia were incubated in ASW in open beakers under a fluorescent desk lamp containing two Sylvania F15 T8-CW tubes positioned about 0.5 m from the ganglia. This supplied about 800 erg $cm^{-2}$ $sec^{-1}$ over the range of wavelengths from 435 to 570 nm. The temperatures of the solutions bathing ganglia in the light and in the dark were the same (about 22°C), and the illuminated solution did not become warmer with time. After incubation the illuminated whole ganglion was fixed for 1 hour, and the cell was then isolated and fixed for 1 hour longer.

Figure 1a shows a pigmented granule in a neuron prepared in the dark. It is bounded by a membrane, and its contents appear uniform in texture and in some areas seem to be made up of globules approximately 50 Å in diameter. A portion of the granule is more electron-opaque, and in many granules the darker area contains variable amounts of a scattered fine precipitate (*10*). The areas of different apparent densities and the precipitate are seen in unstained sections and are therefore not dependent on lead or uranyl stains. When unstained thin sections were floated on drops of 0.1*M* EGTA in tris(hydroxymethyl)aminomethane (tris) buffer, *p*H 8, the precipitate in the granules was removed, although floating sections on water or tris buffer alone did not remove the precipitate. This suggests that the precipitate contains Ca. In cells fixed in Ca-saturated phosphate buffer, a precipitate also was found just inside the limiting membrane of some granules (Fig. 1b). In cells that had been immersed for 1 hour in the dark in saline solution in which $SrCl_2$ had been substituted for $CaCl_2$, some of the pigmented granules contained a coarser precipitate often located just inside the limiting membrane. This apparent deposition of Sr in the granules further suggests that the granules are capable of binding or accumulating divalent ions (*11*). A high proportion of the pigmented granules of cells incubated in the light are converted to forms containing arrays of membrane-like lamellae (Fig. 1c). Images that can be interpreted as intermediate forms also occur (Fig. 1d), especially in cells illuminated for short times. These include paracrystalline arrays of globules (single arrow in Fig. 1d), small vesicular profiles embedded in areas of globules (double arrow in Fig. 1d), and small vesicles embedded in areas of globular units adjacent to membrane-like arrays in the same granule. In such composite forms there is often precipitate in the portion of the granule that retains the globular from, although precipitate is not visible among the membranous lamellae. The lamellae formed in pigment granules as a result of exposure to light have a typical double leaflet appearance, and pentalaminar regions of apparent membrane fusion also occur (inset, Fig. 1). The proportion of granules converted to membrane-like forms increases with the duration of illumination and decreases on return of cells from light to dark during incubation in ASW. It is not clear, however, whether the configuration of a single granule recovers from the effect of light or whether light-altered granules are replaced by newly formed granules in the dark.

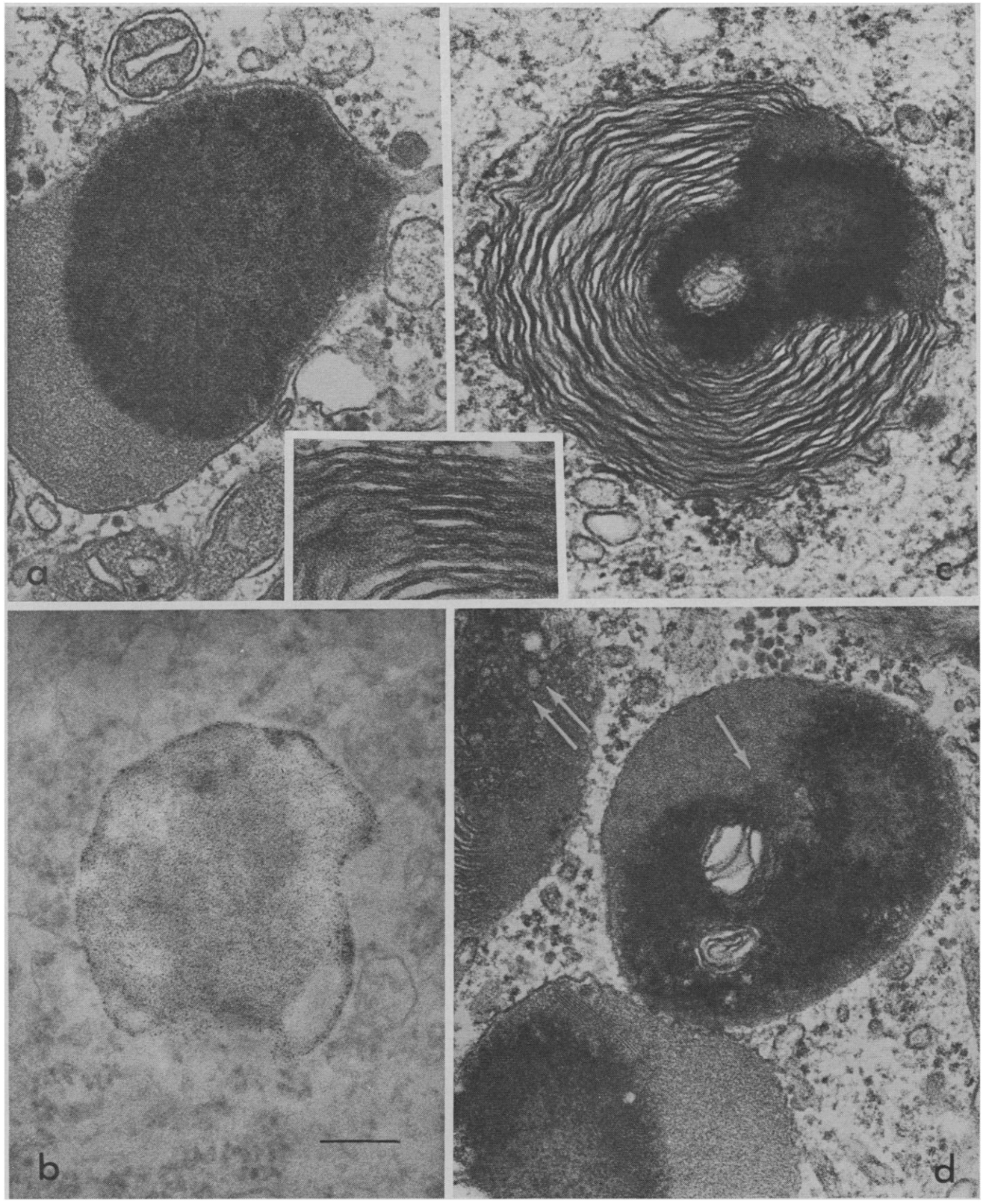


Fig. 1. Electron micrographs of pigmented granules in stages of the light-induced morphologic transformation. (a) Dark form. (b) Unstained section of a granule fixed in the dark in Ca-saturated phosphate buffer showing a precipitate scattered through the granule contents and just inside the limiting membrane. (c) Granule from a light-exposed neuron. The contents have been converted to lamellar arrays. (d) Intermediate forms in granules from a neuron exposed to light for 5 minutes. The single arrow indicates an example of 50-Å globules in a paracrystalline array. The double arrow indicates vesicular profiles in areas of globular units. (Inset) Bimolecular leaflet appearance of the membrane-like lamellae in light-exposed granules; (a), (c), (d), and the inset are from sections stained with uranyl acetate and lead citrate. Scale bar, 0.2 $\mu$m in (a) to (d) and 0.1 $\mu$m in the inset.

These observations combined with the physiological results of Brown and Brown (*2*) suggest that light absorption by the pigment contained in the granules causes release of Ca from the granules themselves. The 50-Å globular particles in the illuminated pigmented granules may be rearranged into membrane-like lamellae as a result of alteration of their ionic environment—in particular, a reduction in the intragranular Ca concentration. However, there is a possibility that some conformational change responsible for the transformation from globule to lamella precedes the release of Ca. These observations bear on several other aspects of cell biology, for example, mechanisms of membrane assembly (*12*), similar morphologic changes seen in lysosomes under certain conditions (*13*), and the possibility that a similar mechanism may exist in the vertebrate pigment epithelium (*14, 15*).

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4. N. Chalazonitis and A. Arvanitaki, *Arch. Sci. Physiol.* **10**, 291 (1956).
5. In previous morphologic studies of *Aplysia* neurons these granules have been called grains, lipid bodies, lipochondria, pigment-containing bodies, and pigmented granules [Arvanitaki and Chalazonitis (*1*); Rosenbluth (*6*); Coggeshall (*7*)]. I will call the whole structure a granule. Granules are of the order of 1 μm in diameter. The contents of granules can also be described as granular, but to avoid confusion I will call the substructure of the granule contents globular. The globular particles which make up the contents of dark-adapted granules are approximately 50 Å in diameter.
➡ J. Rosenbluth, *Z. Zellforsch. Mikrosk. Anat.* **60**, 213 (1963).
7. R. E. Coggeshall, *J. Neurophysiol.* **30**, 1263 (1967).
8. The composition of the ASW was (in millimoles per liter) 500 NaCl, 10 KCl, 10 $CaCl_2$, 50 $MgCl_2$, and 10 tris-Cl, *p*H 7.8.
9. The composition of the fixative was 3 percent glutaraldehyde, 0.1*M* cacodylate buffer, *p*H 7.6, and 0.8*M* sucrose. Cells were rinsed in buffer plus sucrose, postfixed in 1 percent $OsO_4$ in 0.1*M* cacodylate buffer, *p*H 7.6, dehydrated in ethanol, and embedded in Epon. Some cells were fixed in 3 percent glutaraldehyde, 0.1*M* phosphate buffer, *p*H 7.6, and 0.8*M* sucrose saturated with $CaCl_2$.
10. Rosenbluth (*6*), describing the pigment granules of *Aplysia* neurons after fixation in $OsO_4$ in veronal acetate, ASW, or saturated $CaCl_2$, noted that they sometimes contained crystalline material or precipitates.
11. When Ca is replaced by Sr in the ASW, these neurons generate bursting pacemaker potentia➡ [J. Barker and H. Gainer, *Brain Res.* **65**, 516 (1974)]. Since Ca acts as a carrier of current and measurable Ca influx occurs during the action potential in these cells [D. Geduldig and D. Junge, *J. Physiol. (Lond.)* **199**, 347 (19€➡ J. Stinnakre and L. Tauc, *Nat. New Biol.* **242**, 113 (1973)], and Sr can carry current in the Ca spike mechanism, at least in the barnacle mus➡ [S. Hagiwara and K. Naka, *J. Gen. Physiol.* **48**, 141 (1964)], cells that burst in Sr-ASW should become loaded with Sr.
12. Images that suggest the association of 50-Å globular units into various arrays including membrane-like lamellae are reminiscent of images seen in electron microscope studies of lipid-water systems and soaps [for example, see W. Stoeckenius, *J. Cell Biol.* **12**, 221 (1962); J. A. Lucy and A. M. Glauert, *J. Mol. Biol.* **8**, 727 (1964)]. Although it is not possible at this time to interpret the structural changes in *Aplysia* granules in molecular terms, they are interesting as an example of structural changes in a cell similar to phase changes described in model lipid systems.
13. As Rosenbluth (*6*) and Coggeshall (*7*) have pointed out, the pigmented granules resemble lysosomal dense bodies. Treatments that cause lysosomal dense bodies to be transformed into lamellar forms have been reviewed by H. Koenig [in *Lysosomes in Biology and Pathology*, J. T. Dingle and H. B. Fell, Eds. (American Elsevier, New York, 1969), vol. 2, pp. 150–154]. The mechanism of these transformations should be considered in terms of possible photosensitization or possible correlation of Ca release with the structural transformation.
14. The bases for this suggestion are (i) published electron micrographs of phagosome-derived granules in globular and lamellar forms strikingly similar to those I have described [M. Spitznas and M. J. Hogan, *Arch. Ophthalmol.* **84**, 810 (1970); H. Kolb and P. Gouras, *Invest. Ophthalmol.* **13**, 487 (1974)] and (ii) physiological studies showing that cells of the pigment epithelium hyperpolarize in response to light (*15*). Images of phagosomes in these configurations have been interpreted as stages in digestion of rod disk membranes. Part of the mechanism of membrane breakdown might include packaging of membrane subunits with Ca, the reverse of the process of membrane formation that occurs in *Aplysia* granules. The images of phagosome-related bodies in pigment epithelium could also be interpreted, however, as stages in light-induced conversion of uniformly globular forms to membrane-like lamellae. Thus, the structure of the phagosome contents after packing into globular form could undergo a light-induced change back to lamellar form, as in the *Aplysia* granules.
➡ R. H. Steinberg, R. Schmidt, K. T. Brown, *Nature (Lond.)* **227**, 728 (1970); R. Schmidt and R. H. Steinberg, *J. Physiol. (Lond.)* **217**, 71 (1971). The hyperpolarization of the pigment epithelium in response to light is thought to be rod-dependent principally because the action spectrum of the light response corresponds to the absorption spectrum of rhodopsin, but the available evidence is not unequivocal. The hyperpolarizing light response in the vertebrate pigment epithelium results in current flow sufficient to be recorded in the electroretinogram as the c-wave and may be an important component of retinal physiology.
16. I thank G. Neises for technical assistance and H. M. Brown and A. M. Brown for discussing their unpublished work with me.

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## Phototransduction in Aplysia Neurons: Calcium Release from Pigmented Granules Is Essential

Abstract. *Increased free intracellular calcium mediates increased membrane potassium conductance in illuminated* Aplysia *giant neurons. The calcium source was examined by microprobe analysis of cytoplasmic pigmented granules. Illumination markedly depleted granules of calcium and altered them structurally. Release of calcium from granules is essential for phototransduction.*

Henkart (*1*) has presented structural evidence suggesting that when an *Aplysia* giant neuron is illuminated Ca may be released from lipochondria or pigmented granules present in the cytoplasm. To investigate Ca release more directly, the Ca content of pigmented granules from nonilluminated neurons and from neurons illuminated with white light has been compared by using electron microprobe analysis. In addition, the concentration of free intracellular Ca required to mediate the light-evoked surface membrane hyperpolarization (*2*) known to occur in this neuron has been determined by pressure injection of Ca ethylene glycol-bis-(aminoethylether)-*N,N'*-tetraacetic acid (EGTA) buffers into the neuron before illumination. We found that the Ca content of illuminated ("light") granules is considerably less than that of nonilluminated ("dark") granules; this result is taken as direct evidence that illumination releases Ca from the granules. The light-evoked rise in free intracellular Ca then increased plasma membrane potassium conductance. Since the potassium equilibrium potential is more negative than the transmembrane potential, the neuron is hyperpolarized (*2*).

Table 1. Elemental peak integrals (mean ± standard deviation) from 36 nonilluminated granules (three neurons) and 12 illuminated granules (two neurons). Data are normalized to the chlorine peak integral (*5*).

| Granules | Counts per elemental peak integral | | | |
|---|---|---|---|---|
| | Ca | P | Na | S |
| Nonilluminated | 251 ± 100 | 1413 ± 606 | 377 ± 110 | 190 ± 89 |
| Illuminated | 63 ± 20 | 340 ± 231 | 186 ± 66 | 89 ± 18 |