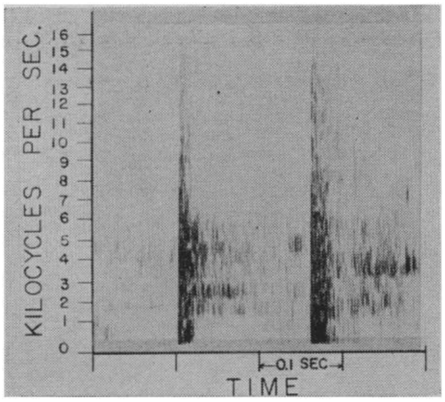

Fig. 1. Sound spectogram (sonagram) of a pair of high-energy "cracks" produced by a captive *T. truncatus* shortly after a model dolphin was introduced into the water with it. The "crack" is a rapid-onset, broad-band pulse with the bulk of the energy contained below 8000 cy/sec.

time was typified by flight, tight schooling, and hyperexcitability. Sometimes two or more of the "cracks" followed closely upon the first. We do not know whether one or both of the dolphins produced the sound.

The same sound was heard and recorded on another occasion in a tank containing three large *T. truncatus* juveniles: two females and one male, all recently captured. At night we abruptly shined a light into the eyes of a swimming dolphin just as it raised its head to breathe. It started, swam violently away, and the "crack" was heard concurrently through the underwater listening equipment. We repeated this test and the same physical reaction and sound were elicited.

In personal conversation, W. E. Evans, J. H. Prescott, and W. W. Sutherland told us that they once noted and recorded a similar sound. During the daytime, these workers projected sounds from a tank of five *T. truncatus* into a tank containing a single adult female of the same species. Picking up her sound emissions, they obtained whistles, echolocation clicks, barks, and, infrequently, "a single blast much louder than any of the other sounds." Tape recordings made during this experiment were made available to us. The infrequent blasts appear to be the same as the sound we obtained in our studies. Since the animal was alone in a small tank, observation opportunities were good and no jaw-clapping (*2*, *3*) was observed. A tail-slap against another dolphin obviously was not possible. The animal showed avoidance behavior toward the underwater speaker throughout the rest of the experiment.

The mechanism by which the sound is produced is not known, but apparently it is internal. Sonagrams of this sound and of a tail-slap, which conceivably could have produced the abrupt sound we heard, were compared and found to be different. We could not detect any evidence of jaw-clapping in our observations. However, as the animals were swimming violently at the time of the sound production and sonagrams of a jaw-clap (also an abrupt sound) were not available for comparison, we cannot completely rule out the possibility that this method may have been involved, although the evidence given us by Evans and his associates makes this seem unlikely.

Since the sound monitoring and recording equipment was set up primarily to study whistles, and to some extent, barks and echolocation clicks, the single extraordinarily loud and high-energy "cracks" easily overloaded the recording equipment. Consequently, it is impossible at present to make an accurate acoustical analysis of the sound. Plans are being made to investigate this sound with equipment better suited to the analysis of pulse-type sounds. However, preliminary examination of the sound revealed it to carry considerable energy in a lower frequency band (0.1 to 8.0 kcy/sec) than the echolocation click which, according to Evans and Prescott (*3*), has its bulk intensity at 20 to 35 kcy/sec.

Absorption of sound in sea water increases rapidly with increasing frequency (*4*). Conversely, sound of low frequency would be absorbed less and could be expected to carry for a greater distance. It has been known to both whalemen and biologists for a century or more that the sperm whale (*Physeter catodon* L.), and possibly other cetaceans, has a means of communicating alarm. The great distance of the communication is often noted (*5*). The sound we describe above thus would be very well suited to such communication of alarm, both because of its great energy (volume) at a low frequency and because of the startling impact of such a high-energy sound with its abrupt onset (*6*).

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6. This work was jointly supported by the Los Angeles County Museum and contract No. Nonr. 3680 (NR 301-604) between the Office of Naval Research and the Lockheed California Co. Facilities and animals for study were provided by Marineland of the Pacific Oceanarium, Los Angeles, through the courtesy of the curator of mammals, David H. Brown. William W. Sutherland of the Lockheed Aircraft Corporation kindly assisted with the technical problems of the monitoring and recording systems. For their critical examination of the manuscript, we thank Dr. Kenneth S. Norris of the University of California, Los Angeles, Dr. Winthrop N. Kellogg of Florida State University, and William E. Evans of the Lockheed Aircraft Corporation.

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Neurosecretion in the Ophiuroid Ophiopholis aculeata

Abstract. Neurocrine activity has been demonstrated for the first time in the Ophiuroidea and for only the second time in the phylum Echinodermata. Three types of neurosecretory cells have been shown, by means of histological staining techniques, to occur in the principal motor ganglia. The secretory products are carried away from the cell bodies by axon transport but cannot be traced to special terminal reservoirs. It is possible that the neurosecretory products diffuse into adjacent coelomic spaces for further transport. It is suggested that in the echinoderms neurocrine secretions may be associated with control functions which in other phyla are regulated by endocrine products.

Although neurosecretory cells have been demonstrated to occur in a wide range of invertebrate phyla, the echinoderms have been investigated scarcely at all in this respect. This report presents evidence for the occurrence of such cells in the Ophiuroidea, a class in which their presence had not previously been established. Neurosecretory cells have been shown to occur in the so-called motor ganglia (Fig. 1, bottom) of *Ophiopholis aculeata* (Retzius) var. kennerlyi, by means of the chrome hematoxylin-phloxin and paraldehyde fuchsin techniques (*1*). All of the motor ganglia of the disk and arm regions contain large numbers of neurosecretory cells, with the exception of the podial or ring ganglia surrounding the proximal ends of the buccal and locomotory tube feet. In these, neurosecretory cells appear to be completely lacking and the ganglia are composed exclusively of "unspecialized" neurons.

Within the neurosecretory ganglia there are three morphologically distinct cell types, designated A, B, and C cells. A cells (Fig. 1, top) are unipolar and pyramidal in shape, with cell bodies approximately 8 μ wide and 13 μ long. They are characterized by a cytoplasm filled with fine acidophile granules which are seen to be markedly phloxinophile when stained with chrome hematoxylin-phloxin. Small acidophile vacuoles are frequently found in the peripheral areas. Both the granules and the vacuoles run into and along the axons. B cells (Fig. 1, top) are characterized by a basophile and extensively vacuolated cytoplasm. These cells are also unipolar and pyramidal in shape and are about 8 μ wide and 17 μ long. The intensely basophile perinuclear zone is interpreted as probably representing a Nissl zone, since the basophilia in this region is removed by pretreatment with ribonuclease (*2*). The more peripheral cytoplasm is finely granulated and less intensely basophile. These granules stain heavily with paraldehyde fuchsin and with the hematoxylin of chrome hematoxylin-phloxin and are stable when treated with ribonuclease. Large vacuoles, filled with a faintly basophile or sometimes faintly acidophile colloid, are also found in the peripheral cytoplasm, commonly at the extreme basal end of the cell. The basophile granules and occasional small vacuoles can be seen passing along the axons. The C cells (Fig. 1, top) are multipolar, smaller cells, about 4 μ wide and 14 μ long, containing numerous large basophile granules which partially obscure a faintly acidophile cytoplasm. These granules stain heavily with paraldehyde fuchsin and with the hematoxylin of chrome hematoxylin-phloxin. They can be traced for considerable distances in the processes of the cells. The A and B cells occur in approximately equal numbers and show no special arrangement. The C cells are relatively infrequent and are usually

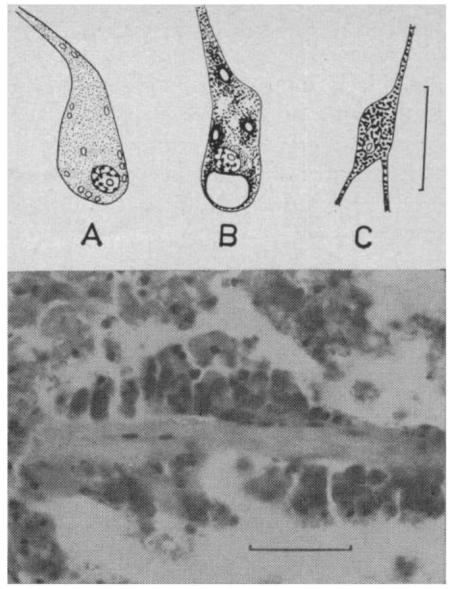

Fig. 1. (Top) Neurosecretory cells (A, B, and C) of *Ophiopholis aculeata,* drawn by projection from preparations stained with paraldehyde fuchsin. The scale represents 15 μ. (Bottom) Neurosecretory ganglion at the base of the arm spine, with nerve tract in center (stained with buffered azure II-eosin). The scale represents 35 μ.

located in the inner portion of the ganglion next to the central fiber tract.

There is no evidence that the release of any of the neurosecretory products occurs through the cell membranes. Axon transport is apparently the only method by which the products are carried away from the cell bodies. Although secretions can be followed along the axons within the nerve tracts for some distance, they have never been traced to a special terminal reservoir. The secretory products appear to diminish in volume, become faintly staining, and eventually disappear along the course of the nerve fiber. This suggests solution and diffusion of the products en route, along the axon, by rupture of the stainable granules, or, alternatively, the loss of a chromophilic carrier substance. The problem of transport of the secretions beyond the nervous system (if it occurs at all) is vexatious in view of the poorly developed hemal system characteristic of the Ophiuroidea. None of the secretory ganglia lie close to any part of the hemal system. Many ganglia are adjacent to coelomic spaces, however, and it is conceivable that diffusion into and transport within these heavily ciliated spaces is possible.

Preliminary investigations indicate that similar neurosecretory ganglia and cells occur in the ophiuroid species *Ophiocomina nigra* and *Ophiothrix fragilis,* suggesting that neurosecretion is widespread and probably ubiquitous in the class.

The target sites and functions of the neurosecretory products in these ophiuroids are not known at present. Neurohumoral and neurosecretory cells and products have been demonstrated recently in the Asteroidea (*3*), where they are associated with motor activity and probably with osmoregulatory and pigment physiology. With the limited techniques which have been applied to the ophiuroid material so far, it is impossible to distinguish between neurohumoral and true neurosecretory units. The occurrence of A, B, and C cells in ganglia which clearly innervate only the vertebral and oral musculature suggests that one or some of these cell types may be neurohumoral in function. On the other hand, the same cells occur in the ganglia of the arm spines and in other regions where the secretory products can be seen carried into regions totally devoid of musculature. It should be noted that in both cases the axons travel for at least part of their distance close to adjacent coelomic spaces. The demonstration of neurosecretion in the Ophiuroidea, as well as in the Asteroidea, suggests that it is common to the phylum Echinodermata. Since no specific endocrine organs have been detected previously, it is probable that all endocrine activity in the phylum is vested in complex neurosecretory systems. This concept provides a new angle of approach to some physiological problems which currently remain obscure (*4*).

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