The "Juxtaganglionic" Tissue and the Brain of the Abalone *Haliotis rufescens* Swainson

BY

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(4 Plates)

INTRODUCTION

THE MOLLUSCAN JUXTAGANGLIONIC organ was first described by MARTOJA (1965a) in the opisthobranch gastropod *Hydromyles globulosa* (Rang, 1852), a planktonic gymnosome. This organ consists of tissue of glandular appearance in direct contact with each cerebral ganglion; it is well developed at the time of gonocyte maturation but begins to atrophy at fertilization. MARTOJA (1965b) reported on a similar structure in the opisthobranch *Aplysia punctata* Cuvier, 1804 and suggested on the basis of histochemical tests that it elaborated peptides. Subsequently she (1965c) described similar tissue in several diotocardian (archeogastropod) prosobranchs, notably *Diodora mamillata* (Risso, 1826), *Patella lusitanica* Gmelin, 1791, and *Trochocochlea turbinata* (Born, 1780), and called attention to the eosinophilic, granular character of the cytoplasm and the large nucleolus. She reaffirmed cyclic changes in appearance, namely, advanced development at the time of gonocyte maturation and atrophy at the time of gamete release.

We wish to provide the evidence for a similar tissue in the eastern North Pacific abalone, *Haliotis rufescens* Swainson, 1822, and to call attention to its proximity to possible neurosecretory regions in the cerebral ganglia. Inasmuch as this tissue has not been subjected hitherto to examination with the electron microscope, we also include some ultrastructural observations. Preliminary, successful attempts at organ culture of the cerebral ganglion-juxtaganglionic tissue complex are also described.

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MATERIALS AND METHODS

Red abalones, ranging from 16 to 26mm in shell major diameter, were obtained by SCUBA diving at depths of 3 to 9m, at Ocean Cove, Horseshoe Cove, and Bodega Rock in the vicinity of the University of California Bodega Marine Laboratory. They were used soon after capture or maintained in the aquaria of the laboratory. Cerebral ganglia with the apposed juxtaganglionic tissue, and pedal ganglia were fixed in either Stieve's solution or the Bouin-Hollande fluid. Paraffin sections were stained with hematoxylin and eosin, Heidenhain's azan, paraldehyde fuchsin, alcian blue, or Masson's trichrome.

For ultrastructural studies the cerebral and pedal ganglia were fixed in veronal acetate-buffered osmium tetroxide with added sucrose (300mg/ml) or paraformaldehyde/glutaraldehyde buffered with cacodylate to pH 7.5 (KARNOVSKY, 1965). The sections were stained with uranyl acetate and lead citrate prior to study in a Siemens Elmskop 1.

The intact cerebral ganglion, the ganglion with most of the juxtaganglionic tissue removed, juxtaganglionic tissue alone, and juxtaganglionic tissue plus the ganglion from which it was removed, were placed in organ culture. Several protein-augmented invertebrate culture media including Streiff's A6 without gelatin (STREIFF & PEYRE, 1963), Sengel's enriched marine invertebrate nutrient medium (ZILLER-SENGEL, 1970) and Streiff's A6 medium with abalone hemolymph substituted for the egg albumin were used, in addition to lyophilized medium 199 plus sea water.

An antibiotic mixture of penicillin (100 units/ml), streptomycin (100 μg/ml) and fungizone (0.25 μg/ml)

(Gibco) was added to all media. Cultures were incubated at 15° C and aerated with 95% O_2 - 5% CO_2. The media were changed every third day, and cultures were maintained for either 3 and 6 days, or 5 and 10 days.

OBSERVATIONS

Cerebral Ganglia

The neuronal perikarya are distributed at the periphery of the cerebral ganglia in the diffuse pattern characteristic of archeogastropods. The cytoplasm of the majority of neurons is uniformly stained by the counterstains of the methods used. A small number (less than 10%) of the neurons are lightly stained peripherally with paraldehyde fuchsin. In addition, there are some structures about the size of neurons, also located in the neuronal zone, that are strongly stained with paraldehyde fuchsin. These structures appear to be clumped masses of "blood cells" in sections that are stained by azan and Masson's trichrome.

The bulk of the ganglion is composed of neuropil. Concentrations of axons containing the possible neurosecretory material are present immediately below the thin layer of neurons. This concentration of paraldehyde fuchsin-stained fibers is especially rich in the dorsal region of the ganglion near the base of the cerebropleural connective (Figures *1* - *3*). Stainable axons in this region were consistently noted in all animals examined and did not appear to vary substantially with seasonal or physiological (spawning) changes. Attempts to trace the stained axons either to stained cell bodies or to neurohemal areas were unsuccessful.

Ultrastructurally, most cerebral neurons contained the usual cytoplasmic organelles along with large membrane-bound inclusions showing various degrees of internal organization (*cf.* Simpson *et al.*, 1966). The nature of the inclusions varied: Some were composed of packed membranes or filaments; others were composed of clumps of strongly osmiophilic material or of homogeneous pale material; frequently a single inclusion showed all 3 features. Other neurons, fewer in number, contained small dense granules similar in appearance to typical elementary neurosecretory granules, in addition to the large inclusions (Figure *4*).

Many axons in the neuropil adjacent to the neuronal layer contained elementary granules as well as vesicles of various sizes (Figure *5*). The paraldehyde fuchsin- and alcian blue-staining region of the neuropil presumably represents this layer of granule-containing axons (Figures *2*, *3*). In addition, there were a few processes bearing neurosecretory-like granules in the loose connective tissue enveloping the cerebral ganglion (Figure *6*). These axon-like processes were too few in number to be detectable with the light microscope.

Explanation of Figures *1* to *4*

Figure *1*: Wholemount of right cerebral ganglion of *Haliotis rufescens*. Circled area is at dorsal side of ganglion, where cerebropleural connective (CPL) joins and where juxtaganglionic tissue is particularly concentrated. Concentrations of this tissue are also found at bases of optic nerve (ON) and tentacular nerve (TN). Also shown are the cerebropedal connective (CPD) and the cerebral commissures (CC). Haematoxylin and eosin. × 10

Figure *2*: Left cerebral ganglion (CG) of *Haliotis rufescens* in frontal section, just dorsal to junction with cerebropleural connective (CPL). Mature animal (major shell diameter 170 mm). Note possible neurosecretory material (NSM). Paraldehyde-fuchsin. × 100

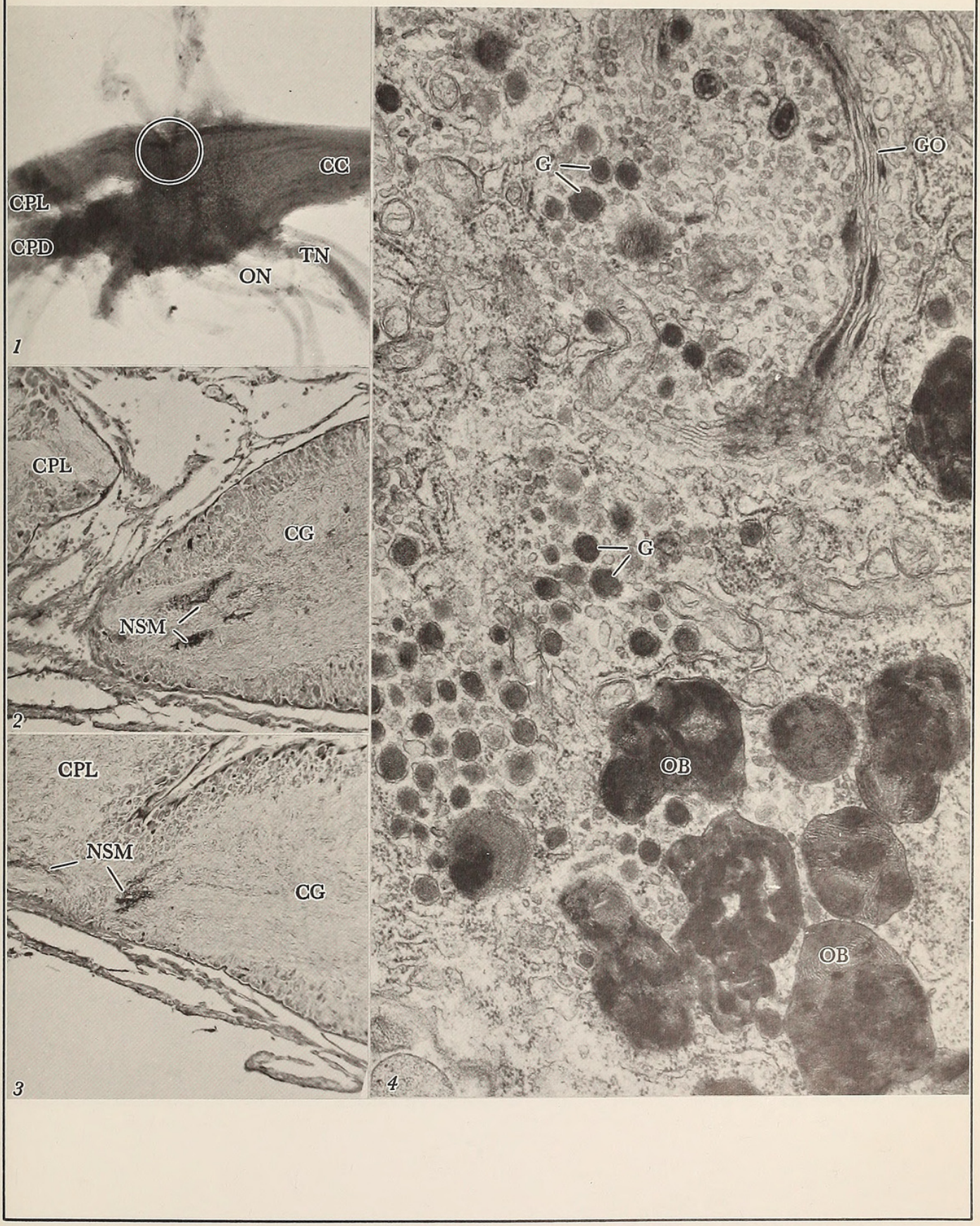
Figure *3*: Same as Figure 2, but more ventral, at junction of cerebropleural connective (CPL) and cerebral ganglion (CG). Possible neurosecretory material (NSM). × 100

Figure *4*: Electron micrograph of portion of neuron from cerebral ganglion containing elementary granules (G) and larger osmiophilic bodies (OB) composed of lamellae, fine fibrils and amorphous structures of varying electron density. Golgi systems (GO) appear very active. × 30 000

Explanation of Figures *5*, *6*

Figure *5*: Electron micrograph of portion of neuropil adjacent to neuronal layer of cerebral ganglion. Note part of one axon with electron-dense granules (G) and irregular vesicles and other axons with small vesicles. Part of another axon contains large granulated vesicles (LGV). Polystyrene spheres = 264 nm. × 30 000

Figure *6*: Electron micrograph of periphery of mitochondria-rich cell of juxtaganglionic tissue with vesiculated osmiophilic bodies (VB) as well as homogeneous inclusions (I). A granule filled process – presumably an axon (A) – lies adjacent to it. × 10 000


CC
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1
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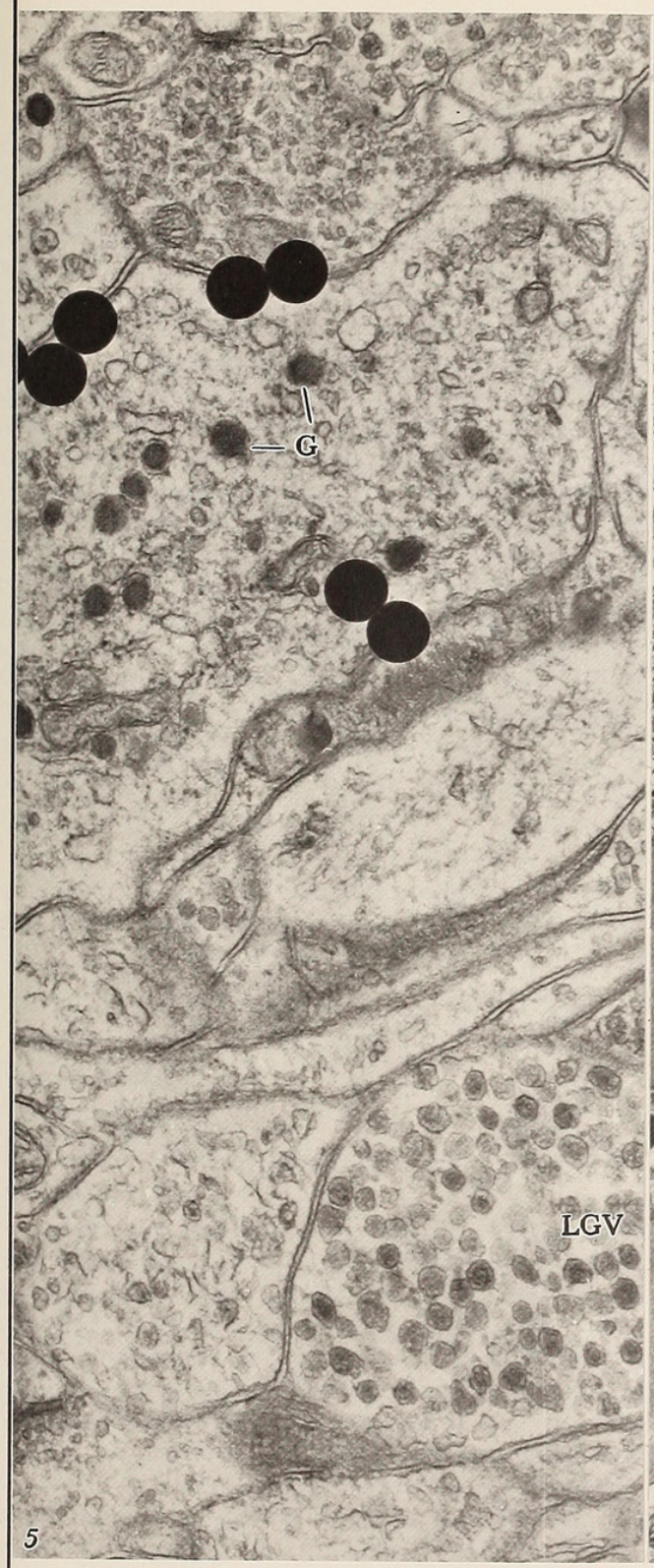
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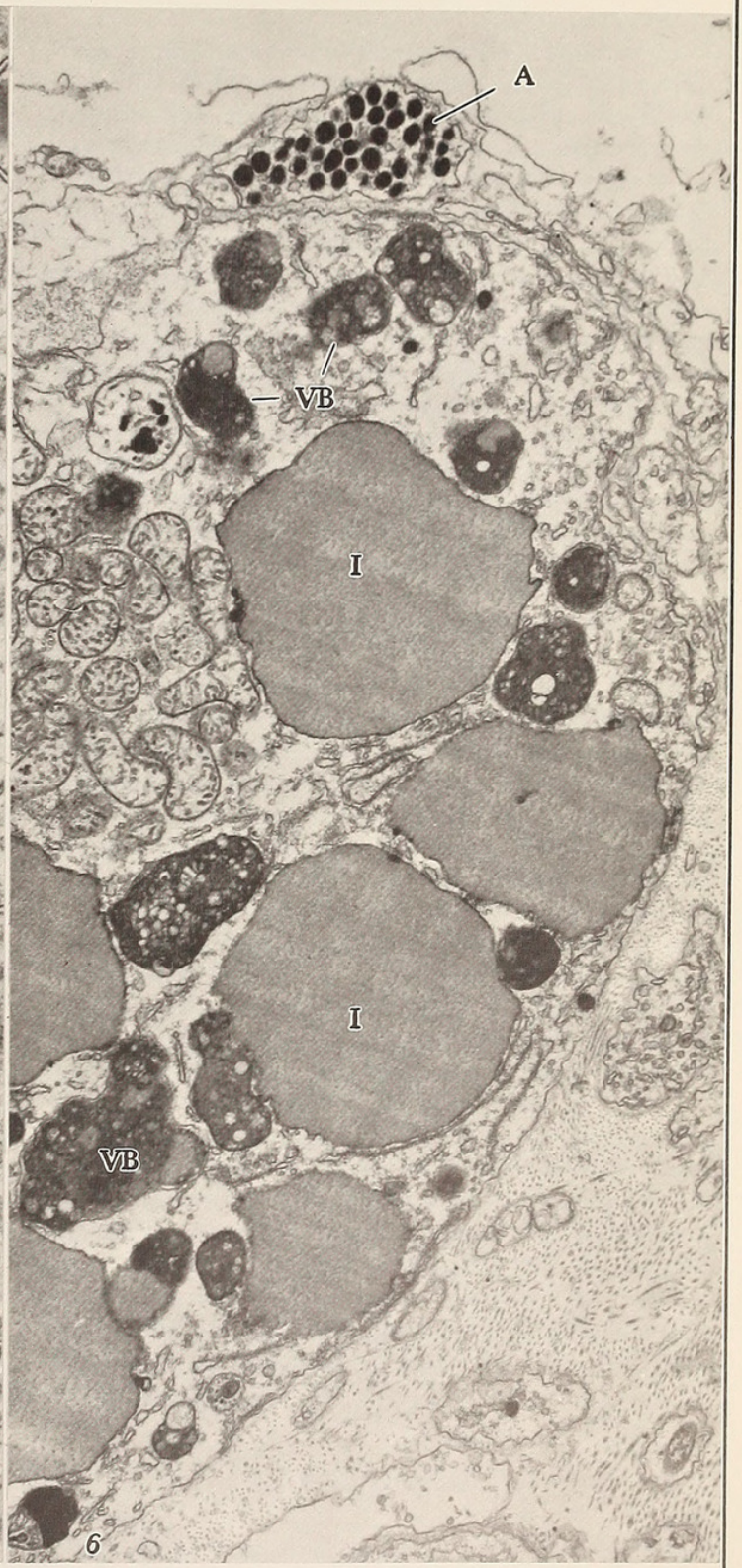
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