(*10*). The heat required to generate such high-Mg melts in the Betts Cove area of Newfoundland could have been provided by an anomalously high concentration of radioactive minerals or some other viable processes operative in the mantle. The erratic occurrence of komatiites in post-Archean rocks may therefore be attributed to local irregularities (steepening) in the otherwise gently sloping smooth geotherms (*26*). These relatively young komatiites tend to diminish the supposed uniqueness of Archean magmatism.

H. D. UPADHYAY
Department of Earth Sciences, Northeastern Illinois University, Chicago 60625

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1. Komatiites are characterized (*3-5*) by the following: high MgO, Ni, and Cr and very low TiO_2 and K_2O contents; low Fe/Mg ratios; and a CaO/Al_2O_3 ratio generally above or close to unity. Occurrence in an extrusive-intrusive environment, the presence of pillow structures, polygonal jointing and brecciation near the flow tops, and, most importantly, the presence of quench ("spinifex" or skeletal) crystals are among their field and textural characteristics. Komatiites have been classified (*3, 6*) into peridotitic, pyroxenitic, and basaltic types that are identified by MgO contents in excess of 20, 12, and 9 percent, respectively; this classification and nomenclature have been adopted here.
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13. These three analyses have been especially chosen for comparison since their MgO and, to a lesser extent, their CaO, Al_2O_3, and SiO_2 contents are of the same order as those of the seven Betts Cove komatiites.
14. The primary silicic material in these and other pillowed samples from Betts Cove are here interpreted to have formed through liquid immiscibility. Microprobe work on the magnesian glass in the globules and the matrix reveals an average MgO content of 25 percent (30 percent in the glassy matrix of sample 70S168).
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27. I thank Drs. N. T. Arndt, G. H. Gale, A. J. Naldrett, E. R. W. Neale, R. W. Nesbitt, M. K. Sood, and D. F. Strong for advice and for critical comments on the manuscript. The chemical analyses were carried out by G. Andrews and D. Press (Memorial University of Newfoundland) and K. Ramlal (University of Manitoba); the microprobe work was done by I. Steele (University of Chicago). This research was supported by grants from the National Research Council of Canada (to Dr. Neale) and from Northeastern Illinois University's Committee on Organized Research.

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Intracellular Calcium: Its Release from Granules During Bursting Activity in Snail Neurons

Abstract. *Pentylenetetrazole induces a bursting activity accompanied by intracellular movement of calcium toward the cell membrane in the snail neuron. The calcium appears to originate from the dense lysosome-like granules in the cytoplasm. Pentylenetetrazole markedly depletes the granules of calcium and alters them ultrastructurally from dense granules to lamella type granules.*

The D neuron in the subesophageal ganglion of the Japanese land snail, *Euhadra peliomphala*, manifests characteristic bursting activity after application of pentylenetetrazole (PTZ) (*1*). This bursting activity strongly resembles the firing pattern of neurons in the mammalian cerebral cortex during seizure discharge induced by PTZ (*2*). The calcium ion has become an important indicator of bursting activity and has been used in the interpretation of data from various electrophysiological experiments, especially voltage clamping (*3-6*). We have demonstrated that intracellular calcium, which is probably bound to a subcellular struc-

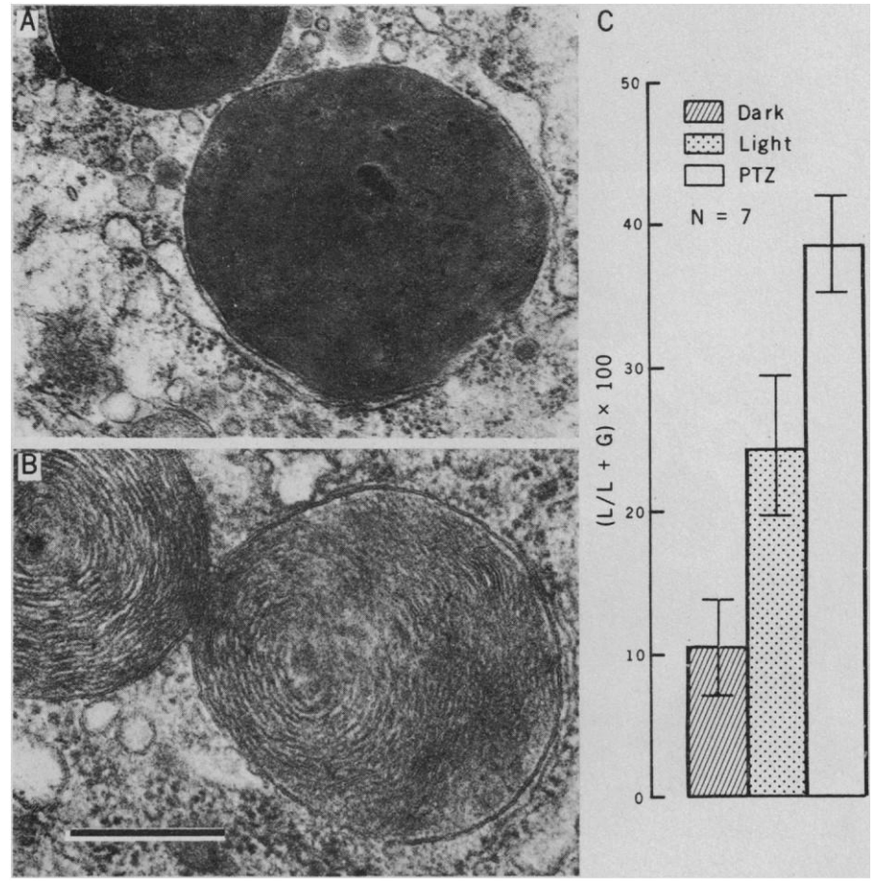

Fig. 1. Electron micrograph of intracellular granules in the snail neuron: (A) Normal neuron. (B) Neuron after 15 minutes of incubation in PTZ-containing Ringer (bar, 0.5 μm). (C) Percentage of lamella type granules (*L*) in dark normal, light normal, and dark, PTZ-incubated neurons; values are the mean ± the standard deviation of seven experiments on each type of neuron.

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ture, moves toward the cell membrane during bursting activity induced by PTZ (*7*). The next question is the source of the calcium which gathers at or near the cell membrane. We report here that, after application of PTZ, calcium is released from lysosome-like granules and ultrastructural changes occur.

We dissected the subesophageal ganglia of the Japanese land snail and carried out the following experimental procedure in a darkroom under indirect illumination from a photographic safety light with a dark red filter (Hanza No. 4). The dissected ganglia were divided into two groups: one group was incubated in snail Ringer solution containing $5 \times 10^{-2}M$ PTZ for 15 minutes at 22°C, and the other group was incubated in normal snail Ringer as a control. Some ganglia were prepared and incubated in normal snail Ringer in daylight with a fluorescent light and compared with specimens prepared in the darkroom. Both groups were fixed with OsO_4 in a darkroom with red light and embedded by a conventional Epon embedding method. The ultrastructural observations were performed with an electron microscope (JEOL 100B) on the ultrathin specimen sectioned with an ultramicrotome (Reichert OM U2).

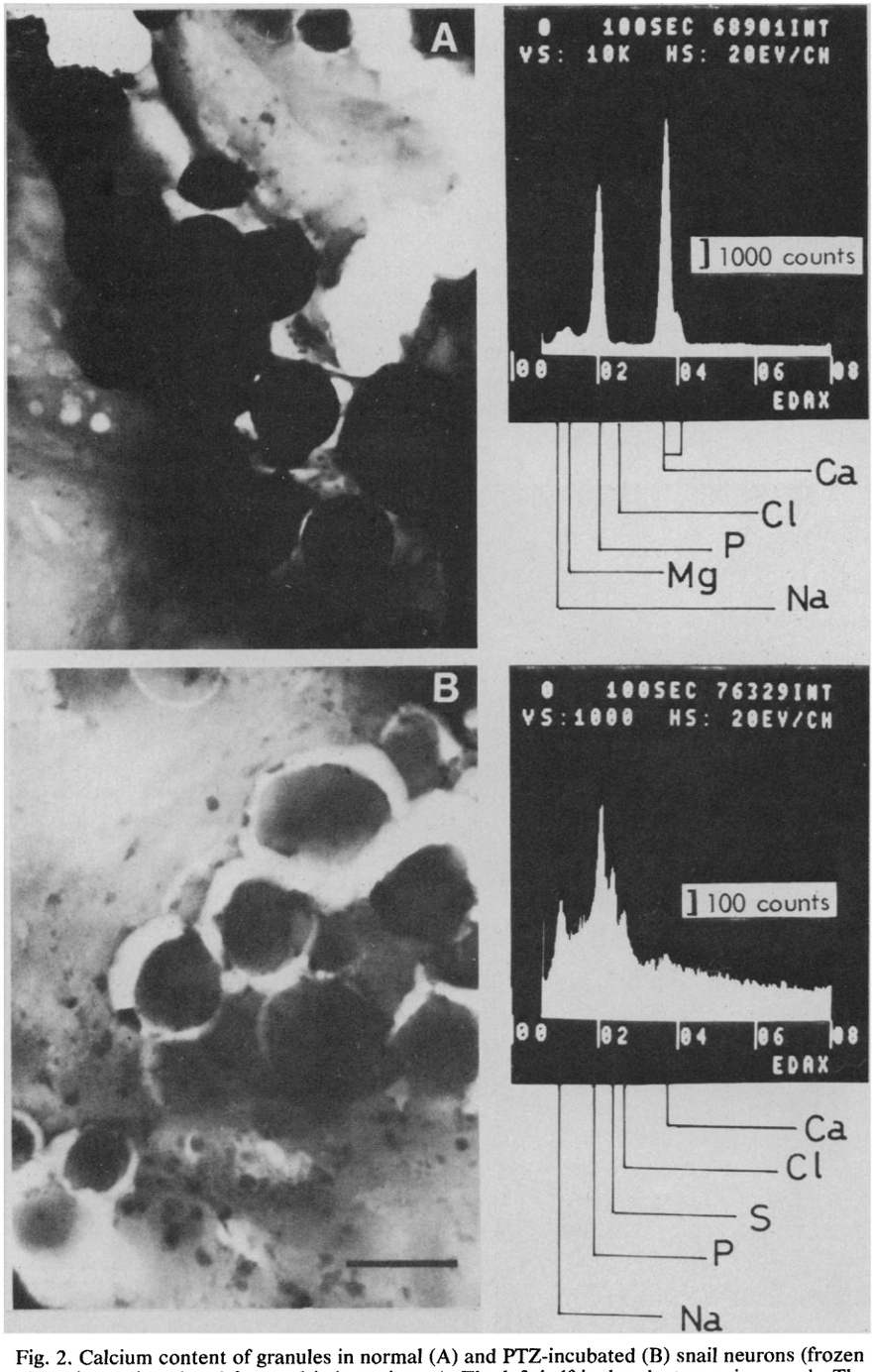

Fig. 2. Calcium content of granules in normal (A) and PTZ-incubated (B) snail neurons (frozen ultrathin-sectioned and freeze-dried specimens). The left half is the electron micrograph. The right half shows the x-ray microprobe analysis of the granules. The abscissa is Kα energy in kiloelectron volts. The right half of (B) shows a gain ten times higher than the right half of (A). Counting time, 200 seconds; bar, 1 μm.

For electron microprobe analysis, we used an energy-dispersive type x-ray detector (Edax 711). The specimens for microanalysis were prepared by the method of frozen and thin sectioning. The ganglia that were prepared under dark red light were frozen by being dipped in a Freon 12 pool surrounded by solid Freon 12 cooled with liquid nitrogen. To facilitate detection of D neurons and control of the specimen temperature, the ganglion was mounted on an aluminum specimen holder precooled with liquid nitrogen and then ultrathin-sectioned at −90° to −100°C; the ganglion was then freeze-dried without passing through a liquid phase by being maintained in an aluminum chamber (containing P_2O_5 and silica gel) attached to the modified cooling chamber of the ultramicrotome for about 3 days under normal pressure initially and then in vacuum. The freeze-dried specimen was placed on a handmade carbon mesh, coated with carbon, and observed with an electron microscope with an electron microprobe apparatus.

In the D neuron of *Euhadra*, there are many lysosome-like granules which have diameters of about 0.5 to 1.5 μm and are packed with dense globules closely resembling the intracellular granules of the *Aplysia* neuron (*8*) (Fig. 1A). These appear to be the kind of granules that were called lipochondria by Chalazonitis (*8*), Henkart (*9*), and Brown *et al.* (*10*). In cells that had been incubated in PTZ-containing Ringer, most of the pigmented granules were converted into the lamella-like structure (Fig. 1B).

We measured the percentage of the ratio of lamella type granules to the total number of granules in dark normal, light normal (about 1000-lux illumination), and dark, PTZ-incubated D neurons. The rate at which the structure was changing was measured as follows. Ultrathin sections of the equatorial slice and slices from approximately midway between the poles and the equatorial region were made for each D neuron; the number of dense granules and lamella type granules were counted for each slice. Granules that were partly of the lamella type were counted as lamella types. Figure 1C shows the result expressed as a percentage of the lamella type to the total number of granules ($L/L + G \times 100$) obtained. The results show the remarkable

increase of lamella type granules in PTZ-incubated neurons.

Figure 2 shows the results of the calcium analysis carried out with the energy-dispersive type electron probe microanalyzer. The left parts of Fig. 2, A and B, show the electron micrographs of freeze-dried thin sections of normal and PTZ-treated cells, respectively. Because of freeze-drying and the absence of staining, the electron microscopic image was not good, but the granules were recognizable. The PTZ-treated granules were less dense than normal granules (Fig. 2B). The accelerating voltage was 60 kV, and the analysis was performed on several granules in each specimen. At the same time, several surrounding spots where there were no visible intracellular organelles were analyzed in the same manner, and no detectable calcium was found. The normal granules have a considerable amount of calcium (right part of Fig. 2A), and the PTZ-treated granule no longer had detectable calcium (right part of Fig. 2B); these results suggest that PTZ released the calcium previously bound to the granules. Henkart (*9*) and Brown *et al.* (*10*) demonstrated that the pigmented granules of the *Aplysia* neuron release calcium and are converted from dense granules into lamella type granules after illumination. We also compared the ganglia illuminated to those prepared under dark conditions. The preparation was performed in daylight under a fluorescent lamp (Toshiba FLR-40 SW tube) positioned about 0.5 m from the ganglia. This light supplied about 1000 lux over a wavelength range from 400 to 750 nm. The temperature of the solution bathing the ganglia was about 22°C. The results showed that the $L/L + G$ ratio of the illuminated ganglia is increased but far less than after PTZ treatment (middle column of Fig. 1C).

These results demonstrate that PTZ releases the intracellular calcium bound to the dense lysosome-like granules and converts them into lamella type granules. Our PTZ-induced ultrastructural change and release of calcium bear a remarkable resemblance to the light-induced change in the *Aplysia* neuron (*9*, *10*).

The bursting neurons of *Helix pomatia* and *Aplysia* show a voltage-dependent, slow inward current in the steady-state voltage clamp current-voltage curve. This is called the negative slope region or negative resistance characteristics (*3-6*). Electrophysiological analysis of this current has been carried out (*4-6*). Smith *et al.* (*4*) demonstrated that this current is carried by sodium ions, whereas Eckert and Lux (*5*) proposed that it is carried by calcium ions. Johnson (*6*) proposed the possibility of a responsive carrier of both sodium and calcium ions. We carried out additional experiments on the preparation of a contour map of sodium ions in normal and PTZ-treated neurons by the method described elsewhere (*7*). No clear change in the sodium distribution pattern was found between the normal and PTZ-treated neurons. Several additional experiments with a nondispersive type electron probe microanalyzer in the area where there were no organelles showed no definitive change in sodium. As previously demonstrated (*7*), the intracellular calcium scattered in the cytoplasm is concentrated in the cell membrane by PTZ treatment. This newly concentrated calcium in the cell membrane showed a different type of chemical shift of the x-ray wavelength, which means a new binding state of calcium occurs within the membrane (*11*). On the basis of the present findings together with earlier data (*7*), we believe that the bursting activity caused by PTZ can be explained as follows: PTZ acts on the intracellular granules and releases the bound calcium; the released calcium binds to the cell membrane structure and modifies the ionic channels and induces the bursting activity. The determination of the identity of the carrier ions responsible for the negative resistance characteristics of bursting activity will require further research. In any case, the calcium release from intracellular granules and the movement of the released calcium toward the cell membrane is the essential step required to induce the bursting activity of neurons.

EIICHI SUGAYA
MINORU ONOZUKA
Department of Physiology, Kanagawa Dental College, Yokosuka, Japan

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Ion Shower Milling: Its Application to Cell Membrane Removal

Abstract. *A thickness of about 100 angstroms of the cell membrane of an isolated single freeze-dried neuron of the snail can be etched off with an ion shower milling machine. The calcium content of the cell membrane area was more than one-fifth of the whole cell. The calcium content of the cell membrane area increased during pentylenetetrazole-induced bursting activity.*

In the nerve cell, the combination of ions and the cell membrane structure plays an important role. The ions bound to the membrane, however, cannot be measured separately, and it is desirable to measure various substances in the cell membrane and cytoplasm separately. For this purpose, only fractionation by the density gradient method has been used.

The ion shower milling machine has been used to etch thicknesses of several hundreds of angstroms off the surfaces of metals, glass, and organic polymers, and this machine has been of great value in the etching of transistors and integrated circuits. The ion shower milling machine was first applied to biological specimens by Lewis *et al.*, and they found a submembranous structural difference between normal erythrocytes and those that are characteristic of sickle cell anemia (*1*).

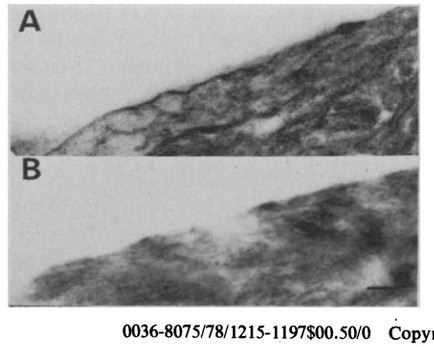

Fig. 1. Electron micrograph of a freeze-dried cell surface of the snail neuron (A) before etching of the cell surface and (B) after removal of the cell membrane area by the ion shower milling machine. Scale, 0.1 μm.

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