which has a maximum at 520 nm (*6*). They agree well with the secondary peak at 530 nm in the spectral sensitivity of the median ocellus of *Limulus*. This peak is suggested to result from a pigment distinct from the one responsible for the main peak at 360 nm (*7*). Recordings of single cells show that the lateral eye contains two cell types —alpha cells with maximum sensitivity at 525 nm and beta cells with broad sensitivity from 550 nm to 350 nm (*8*). The photopigment of the alpha cells may be the same as the one reported here for the photoreceptor cells of the lateral olfactory nerve.

GEORGE C. MURRAY
Department of Biophysics,
Johns Hopkins University,
Baltimore, Maryland 21218

References and Notes

1. R. Millecchia, J. Bradbury, A. Mauro, *Science*, this issue.
2. R. Demoll, *Die Sinnesorgane der Arthropoden* (Friedrich Vieweg and Sohn, Braunschweig, 1917); B. Hanstrom, *Lunds Univ. Arsskr. Avd. 2*, **5**, 1 (1926).
3. Program abstract titled "Computation of spectra of visual pigments" in Johns Hopkins University Applied Physics Laboratory Computing Center's Annual Report.
4. W. B. Marks, *J. Physiol. (London)* **178**, 14 (1965).
5. H. J. A. Dartnall, in *The Eye*, H. Davson, Ed. (Academic Press, New York, 1962), vol. 2, p. 378.
6. R. Hubbard and G. Wald, *Nature* **186**, 212 (1960).
7. G. Wald and J. M. Krainin, *Proc. Nat. Acad. Sci. U.S.* **50** 6, 1011 (1963).
8. G. S. Wasserman, *Physiologist* **9**, 314 (1966).
9. I thank A. Mauro for bringing these cells to my attention and for cooperating in these experiments, and R. Millecchia and J. Bradbury for assisting in the preparation of the specimens. Research supported by NSF grant G18886 and NIH grant NB-0358 to E. F. MacNichol, Jr., whom I thank for aid and technical assistance.

2 September 1966

Periventricular Cerebral Impedance after Intraventricular Injection of Calcium

Abstract. *Injection of small volumes of calcium solution into the lateral ventricle of the cat was followed by large electrical-impedance changes in gray matter bounding the ventricle, including the caudate nucleus and hippocampus. These changes lasted more than 24 hours and were accompanied by epileptiform electroencephalographic activity. Biweekly injections led to status epilepticus. Injections of similar amounts of magnesium ions were without comparable effects. Possible interactions between calcium ions and intercellular macromolecular material are discussed as a basis for certain impedance shifts in cerebral tissue.*

Impedance-measuring currents applied to cerebral tissue appear to pass mainly through intercellular fluid (*1*) and perhaps also through neuroglial cells. The role of these cells as a substantial pathway for current remains uncertain, but low resistance of the neuroglial membrane has been reported in tissue culture (*2*). Neuronal membrane resistance has been repeatedly estimated at 4 to 7000 ohm cm^2 in the mammal, at least several orders of magnitude greater than resistance of intercellular fluid (*3*).

Impedance changes in cerebral tissue have been detected in the course of alerting, orienting, and discriminative responses (*4*). Nevertheless, the origin of impedance changes accompanying physiological responses has remained obscure. Electron micrographs prepared with glycol solutions (*5*) showed a substantial content of macromolecules, possibly mucopolysaccharides, in cerebral extracellular fluid. This suggested to us that divalent cations, such as calcium, may be important in regulation of macromolecular configurations at the neuronal surface, thus influencing ionic fluxes that determine neuronal excitability and also modulate conductance characteristics in perineuronal fluid.

Impedance measurements were made in six cats with coaxial electrodes chronically implanted bilaterally in the caudate nucleus, dorsal hippocampus, and amygdala. This technique employed a small measuring signal with an amplitude of 20 μv and a typical current density of 10^{-13} amp per square micron of electrode surface at 1.0 khertz and with the tissue in one leg of a Wheatstone bridge. More than 90 percent of the current passed within a volume of 1.0 mm^3 surrounding the electrode tip. With this method, the residual bridge signal was amplified and sampled through two gated pulse "windows" placed in in-phase and quadrature (90° displaced) positions relative to the 1.0-khertz sine-wave test signal (*4*). The integrated output of signals admitted through these windows provided a measure of relative resistive and reactive components of tissue impedance. This coherent detection method is extremely sensitive to small phase-locked components of amplified output of the bridge. Our previous studies indicated that impedance responses accompanying physiological stimuli do not arise in simple relation to such factors as cerebral blood pressure, cerebral blood flow, or tissue temperature, but appear to relate directly to physiological phenomena requiring the presence of normal neural elements (*4*, *6*).

After a postoperative interval of 30 days for impedance base line stabilization, injections of calcium chloride solution (40 or 60 μeq in 0.1 ml) were made through an intraventricular cannula, preceded by injections of an equal volume of normal saline (Fig. 1). No changes followed the saline injections. A sharp decline occurred in both resistive and reactive components, beginning 15 to 30 minutes after injection of 40 to 180 μeq of calcium solution, in the structures which bound the lateral ventricle.

In some cases impedance readings were shifted from base line values by as much as 25 percent for periods that exceeded 36 hours. Maximum shifts occurred within 2 hours of injection, with slow return to base line thereafter. Changes were largest and earliest in structures closest to the tip of the cannula and were delayed 30 to 50 minutes in reaching peak values in symmetrically placed leads in the opposite half of the brain. Injection of calcium into the ventricle was regularly followed by a topographically determined sequence of impedance changes, consistent with diffusion from the injection site through the cerebrospinal fluid, but direct injection of calcium solutions in doses up to 120 μeq into periventricular structures was without comparable effects, except at electrodes immediately adjacent to the injection site.

Onset of impedance shifts in hippocampus and amygdala was accompanied by seizure-like discharges. However, impedance shifts were prolonged for many hours beyond the cessation of gross electroencephalographic abnormalities. In animals given 40 to 100 μeq of calcium at intervals of 2 weeks, status epilepticus resulted after three or four injections, with generalized convulsions leading to death.

Although these findings strongly suggest effects attributable to calcium, other factors require consideration. Changes due to altered volume of cerebrospinal

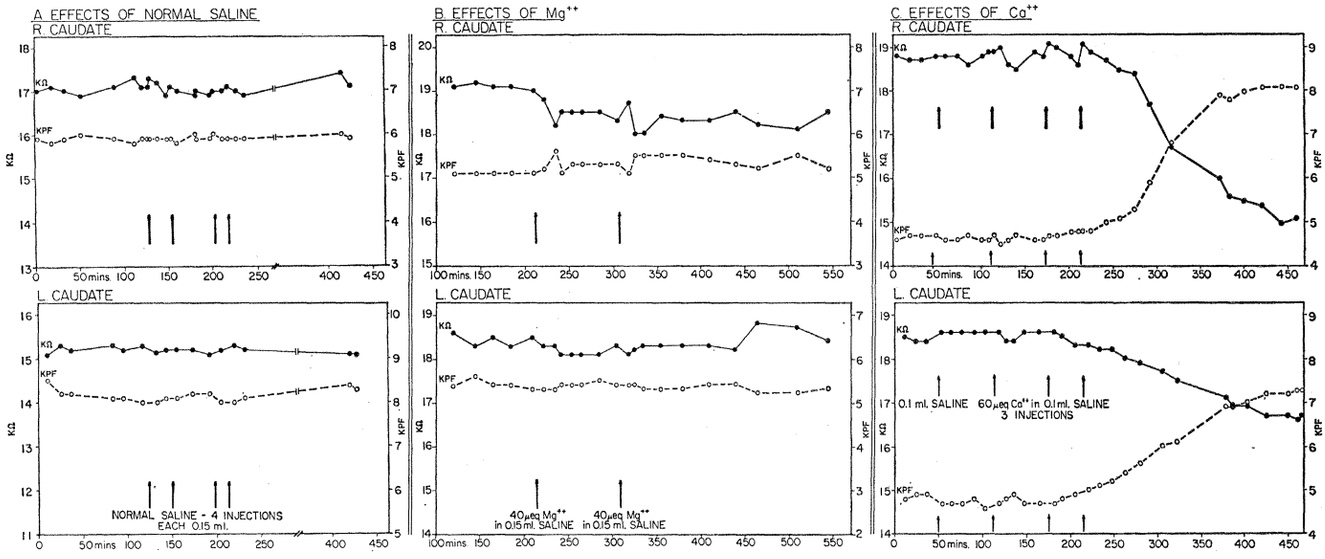

Fig. 1. Periventricular impedance after right ventricular injection. Impedance in focal volumes (approximately 1.0 mm³) of left and right caudate nuclei following injections into the lateral ventricle of normal saline (0.15 ml), magnesium chloride (40 μeq in 0.1 ml), and calcium chloride (60 μeq in 0.1 ml). Major impedance changes occurred only with calcium solutions.

fluid were excluded by injections of comparable volumes of normal saline. On the other hand, mere osmotic changes from hypertonic solutions might produce impedance shifts, and hypertonic sodium chloride has been shown to produce seizures (*7*). If one assumes a lateral ventricular volume of 1.0 ml, 40 μeq of calcium uniformly distributed through this ventricular space would involve a concentration increment of 20 times. At normal rates of secretion of cerebrospinal fluid, this would obtain for many minutes. Concentration gradients from cerebrospinal fluid to electrode sites within brain substance would undoubtedly diminish the magnitude of shifts occurring at the surfaces of juxtaventricular nerve cells, but significant modifications would still be anticipated.

Intraventricular injections of magnesium chloride were made identical in molarities and volumes with those used for calcium. From 40 to 80 μeq of magnesium chloride evoked only small perturbations in impedance base lines in hippocampus and caudate nucleus on the same side as the injection, with a trend toward decreased impedance amounting to only 2 or 3 percent of base line values. At electrodes more remote from the injection site, including ipsilateral amygdala and in all contralateral placements, no changes occurred. Brief epileptiform discharges in the hippocampal site adjacent to the ventricular cannula were noted but persisted for much shorter periods than with calcium.

These findings with magnesium clearly indicate that impedance charges induced by calcium do not arise simply from a nonspecific increase in concentration of divalent cations or anions, such as chloride. Rather, considerable specificity appears to attach to the mode of action of calcium, similar to its specificity of attachment to certain chelating agents and other molecules (*8*).

There is recent evidence that calcium has a vital role in electrically activated synapses (*9*). Susceptibility of macromolecular polycarboxylic acids to precipitation by alkaline-earth cations has been emphasized. Remarkable shrinkage in volume is induced by exchanging part of the sodium counterions at the membrane surface with calcium ions (*10*). Studies with cultured neurons and dissected fragments of neuronal membrane showed electrokinetic effects in the presence of a focal electromotive force, in ways indicative of fixed negative membrane charges and apparently related to adherent macromolecular layers (*11*). Their direct role in determining differential entry of sodium and potassium to positions close to the plasma membrane has been proposed (*12*), and evidence has been advanced that levels of perineuronal calcium can modify membrane potentials in isolated nerve fibers (*13*).

Evidence presented here is consistent with the view that cerebral impedance changes accompanying physiological responses may arise in perineuronal fluid with a substantial macromolecular content and that calcium ions may modulate perineuronal conductivity, as well as fluxes of sodium and potassium across the neuronal membrane. For these reasons, the disclosure of impedance changes in cerebral tissue, in the course of alerting, orienting, and discriminative responses, their selective regional distribution, and dependence on levels of learning, has focused attention on the role of perineuronal elements in aspects of transaction and storage of information in brain tissue (*4*).

H. H. Wang, T. J. Tarby
R. T. Kado, W. R. Adey
Brain Research Institute,
University of California,
Los Angeles 90024

References and Notes

1. K. S. Cole, *Cold Spring Harbor Symp Quant. Biol.* **8**, 110 (1940).
2. J. G. Nicholls and S. W. Kuffler, *J. Neurophysiol.* **27**, 645 (1964); P. W. Nicholson, *Exp. Neurol.* **13**, 386 (1966); W. Hild and I. Tasaki, *J. Neurophysiol.* **25**, 277 (1962).
3. J. S. Coombs, D. R. Curtis, J. C. Eccles, *J. Physiol. London* **145**, 505 (1959).
4. W. R. Adey, R. T. Kado, J. Didio, *Exp. Neurol.* **6**, 47 (1962); W. R. Adey, R. T. Kado, J. Didio, W. J. Schindler, *ibid.* **7**, 259 (1963); W. R. Adey, R. T. Kado, D. O. Walter, *ibid.* **11**, 190 (1965); W. R. Adey, R. T. Kado, J. T. McIlwain, D. O. Walter, *ibid.* **15**, 490 (1966).
5. D. C. Pease, *Anat. Rec.* **154**, 400 (1966).
6. R. Porter, R. T. Kado, W. R. Adey, *Neurology* **14**, 1002 (1964).
7. G. H. Glaser, *Epilepsia* **5**, 97 (1964).
8. H. K. Kashiwa and W. B. Atkinson, *J. Histochem. Cytochem.* **11**, 258 (1964); *Handbook of Chemistry and Physics*, C. D. Hodgman, R. C. Weast, R. S. Shankland, S. M. Selby, Eds. (Chemical Rubber Publishing Co., Cleveland, 1962), pp. 1476–1491; F. H. Epstein and R. Whittam, *Biochem. J.* **99**, 232 (1966).
9. R. D. Penn and W. R. Loewenstein, *Science* **151**, 88 (1966).

10. A. Katchalsky, in *Connective Tissue: Intercellular Macromolecules* (Little, Brown, Boston, 1964), p. 9.
11. R. Elul, *Nature* **210**, 1127 (1966).
12. H. S. Bennett, *J. Histochem. Cytochem.* **11**, 14 (1963).
13. S. Nishi, H. Soeda, K. Koketsu, *J. Neurophysiol.* **28**, 457 (1965).
14. Assisted by NIH grant MH-03708 and U.S. Air Force Office of Scientific Research contract AF 49 (638)-1387.

16 September 1966

Electron Microscopy of Living Insects

Abstract. *Electron micrographs of living specimens of the various developmental stages of the insect* Tribolium confusum *have been obtained with a scanning electron microscope. In most cases the specimens resumed their normal activity after being examined with the electron microscope and underwent metamorphosis into the next stage.*

Successful electron microscopy of living material must overcome the effects of the vacuum and of the electron beam on the specimen. Most studies have concentrated on mitigating the effect of vacuum (*1*). We report some results obtained using a scanning electron microscope (*2*), in which the electron current is very much lower than that in the conventional electron microscope (*3*).

Table 1. Survival of *Tribolium* after exposure to a pressure of 10^{-3} torr for 30 minutes.

Developmental stage	Specimens (No.)	Survived (No.)
Eggs	78	56*
Larvae	30	30
Pupae	30	30
Adults	30	30

* Represents the approximate hatch rate of control eggs.

Table 2. Survival of *Tribolium* after exposure in the scanning electron microscope.

Developmental stage	Specimens (No.)	Survived (No.)
Eggs	6	3*
Larvae	2	2
Pupae	4	4
Adults	2	1†

* Represents the approximate hatch rate of control eggs. † The adult that died suffered prolonged (50 minutes) exposure to the electron beam. Leg motion was observed in this animal after exposure. Although exposure to scanning electron microscopy did not prevent the insects from passing through successive stages of development, latest observations on these animals two months after exposure suggest that adult lifetime is significantly shortened.

The initial search was for specimens capable of surviving a vacuum; a previous report that a beetle had apparently survived being placed in a vacuum did not identify the species (*4*). The criterion for "survival" was that the specimen be able to pass into the next stage of development with normal appearance and activity.

We used *Tribolium confusum*, a small beetle that normally lives in a dry environment and whose behavior and appearance are well documented. Radiobiological studies of this particular insect have been carried out for some years at this laboratory (*5*). Samples of the eggs, larvae, pupae, and adults were kept for 30 minutes in a chamber evacuated to a pressure of 10^{-3} torr (Table 1). All developmental stages of *Tribolium* can survive this vacuum.

Samples of eggs, larvae, pupae, and adults of *Tribolium* were viewed with the scanning electron microscope. The periods of exposure to the vacuum varied from 2 minutes to 1 hour; the periods of exposure to the electron beam were of the same order. The electron current varied from 2×10^{-11} to 2×10^{-10} amp, and the electron energy was 25 kev. Living *Tribolium* in all four stages can be observed in the scanning electron microscope (Table 2).

Although the highest magnification shown in the micrographs is only 670 times, there is no reason why the full resolution of the scanning electron microscope should not be used if necessary—that is, a useful magnification of 20,000 times (*3*). In our experiments, the specimen was placed farther from the final lens so that an area large enough to show the whole insect could be scanned; this resulted in poorer resolution. Even so, the depth of focus shown in these micrographs is greater than that which could be attained in a light microscope.

Consider the density of radiation at the surface of a specimen when irradiated by a stationary electron beam with a current of 10^{-11} amp, energy of 25 kev, and a small diameter. The range in carbon of electrons having this energy is about 10^{-3} cm (*6*), and x-ray production is negligible. One can calculate the energy dissipated per unit volume assuming that the energy is dissipated uniformly in a hemisphere with a radius of 10^{-3} cm (that is, with a volume of 2×10^{-9} cm^3); hence the power dissipated is about 100 watts cm^{-3}. This represents a dose rate of 10^7 rad/sec or about 1 percent of the dose estimated for a specimen in the standard, transmission, electron microscope (*7*).

If the beam scans an area of 1 mm^2 (corresponding to a magnification of 100 times), then the power per cubic centimeter is reduced to

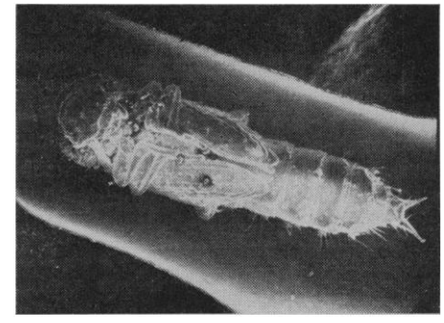

Fig. 1. Scanning electron micrograph of entire living pupa of *Tribolium confusum* (× 15). This picture demonstrates the very large field of view which can be studied with the scanning electron microscope.

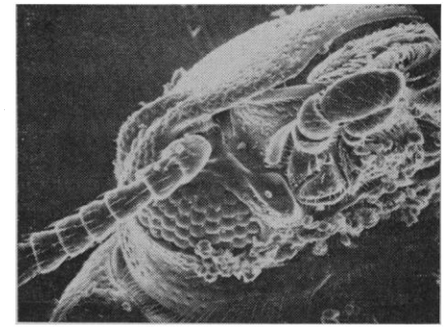

Fig. 2. Scanning electron micrograph of the head of a living adult *Tribolium confusum* (× 109). One antenna, one eye, mouth parts, and many other details are clearly visible owing to the large depth of field.

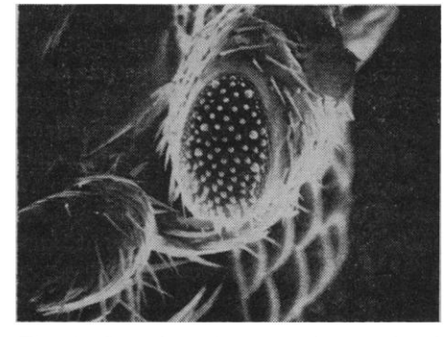

Fig. 3. Scanning electron micrograph of the end of the mandible of a living adult *Tribolium confusum* (× 436). The "papillae" are clearly visible; the eye is partly visible in the background.