acorns in 1977, and it also fruited at a later date.

These data indicate that in years when the oaks produce many acorns, some summer groups on territories without extensive storage facilities are able to store enough acorns to provide food during the winter. These birds are then able to remain resident. Juveniles (and possibly additional adults) may stay on these territories and participate in the utilization and defense of the acorn stores. This in turn may lead to the establishment of stable social groups. In other years, when the mast crop is poor, the limited stores are quickly consumed and the birds are forced to migrate. Thus the acorn woodpecker in this area appears to be able to shift between two different strategies, depending upon the local abundance of a single food resource.

The plasticity of the birds at the Research Ranch may be an evolutionary adaptation to the marginal characteristics of the habitat in this area. During most years the oaks on many territories do not produce sufficient acorns to permit resident groups to become permanently established. During the summer, however, acorn woodpeckers utilize other resources, primarily insects, in addition to acorns (*14*). The Research Ranch is suitable habitat for this species during reproduction, and these birds have the option of moving elsewhere when mast crops are poor (*15*). By migrating during the winter, the woodpeckers can occupy breeding habitat that otherwise would be unsuitable. Selection should favor birds that store acorns and remain resident when conditions permit, because stores are a reliable source of food during the winter and the risks of migration are avoided. Since groups appear to be more efficient than individuals at harvesting and defending mast stores (*12*), selection should also favor individuals that are capable of shifting their behavior to the highly cooperative social organization characteristic of this species in other parts of its range.

The extent to which animals are "locked" into a particular type of social organization by genetic predispositions has attracted considerable interest and controversy (*16*). The results of this study point to the potential dangers of categorizing species or even subspecies in terms of a single social system. The acorn woodpecker has been studied extensively since the 1920's (*17*), but to our knowledge no real suggestion of social plasticity emerged. We have found that acorn woodpeckers at the Research Ranch can follow two entirely different and almost opposite strategies. In one strategy, the birds do not invest time or energy to make acorn storage trees, they migrate during the winter, and they exhibit an essentially asocial type of organization in which the only group is a temporary reproductive pair. The other strategy is characterized by cooperative behavior and by long-term bonds among individuals. Groups construct large storage facilities, collectively utilize and defend the acorn stores, are permanent residents, and breed communally. We have found that both patterns of behavior can occur within the same population and that individual woodpeckers may be able to shift between these strategies in response to different ecological conditions.

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8. H. Swarth [*Pac. Coast Avifauna* **4**, 14 (1904)], in a study of the avifauna of the Huachuca Mountains, said of the acorn woodpecker, "I saw but two or three during February and the early part of March, about the middle of March they began to arrive in numbers, and by April 1 were most abundant."
9. Many of these behavioral characteristics are illustrated by the history during 1976 of the confluence group on the main study area. This group contained an adult male and adult female and fledged three young on 24 August (all birds were banded). One of the juveniles disappeared on 15 September. The rest of the group remained on the territory until 6 October, when the adult male left. A second juvenile departed approximately 16 October. The remaining juvenile and the female did not disappear until more than a week later, on 23 October. A transient adult male temporarily joined these birds on the territory before they left. This male remained on this territory by himself for a week after the female and juvenile departed.
10. These species include the red-headed woodpecker, *Melanerpes erythrocephalus* [L. Kilham, *Auk* **94**, 231 (1977)] and the Lewis's woodpecker *Melanerpes lewis* [C. Bock, *Univ. Calif. Berkeley Publ. Zool.* **92**, 1 (1971)].
11. Resident groups with storage trees were located in Carr Canyon in the Huachuca Mountains and Madera Canyon in the Santa Rita Mountains, west of the study area.
12. The importance of storage facilities for the creation and defense of acorn stores and the relationship between storage behavior and social organization in the acorn woodpecker will be discussed [P. Stacey and C. Bock (in preparation)]; see also MacRoberts and MacRoberts (*1*).
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Endoplasmic Reticulum Sequesters Calcium in the Squid Giant Axon

Abstract. *Axons were loaded with calcium, rapidly frozen, and freeze-substituted. The endoplasmic reticulum, in addition to mitochondria, contained calcium deposits, as indicated by electron probe x-ray microanalysis. Oxalate injected into living axons helped to preserve calcium-containing deposits during preparation for microscopy. It is concluded that the endoplasmic reticulum is a calcium-sequestering compartment in the squid giant axon.*

A variety of cell functions are now known to be regulated by cytoplasmic calcium. The best-known example is control of muscle contraction (*1*); others include actin-myosin interactions leading to motility in nonmuscle cells (*2*), secretion of neurotransmitters and hormones (*3*), control of membrane permeability to other ions (*4, 5*), and processes dependent on polymerization or function of microtubules, or both (*6*). For all of these functions to be under precise control in appropriate regions of each cell and responsive to appropriate stimuli, control of the cytoplasmic free calcium concentration is of critical importance. Studies in a variety of systems have shown that calcium is controlled by a number of co-

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operating mechanisms (*7*). Extrusion through the surface membrane and sequestration in intracellular compartments are two principal routes of removal of calcium from the cytoplasm. In most cells mitochondria have been thought to be the primary intracellular compartment involved in calcium sequestration (*8*), but in muscle cells, in addition to the mitochondria, a specialized form of endoplasmic reticulum (ER) known as sarcoplasmic reticulum (SR) also takes up and stores calcium. This was demonstrated morphologically by Costantin *et al.* (*9*), using calcium-loaded skinned muscle fibers treated with oxalate to precipitate calcium. This observation has been confirmed by electron probe x-ray analysis of freeze-dried sections of muscle (*10*), but it remains difficult to measure small amounts of diffusible ions in small structures in cells lacking the regular arrangement of striated muscle. Application of oxalate from outside intact cells and other methods devised thus far for localization of calcium-accumulating structures in cells other than muscle have not generally proved satisfactory (*11*).

Much has been learned about the physiology of calcium handling in the squid giant axon (*12*). We used this preparation in combination with rapid freezing and freeze-substitution to identify calcium-accumulating structures. We report here that, in addition to mitochondria, the ER is an intracellular compartment capable of sequestering calcium.

Squid axons were loaded with calcium by stimulation at 30 Hz for 35 to 40 minutes in artificial seawater (ASW) containing 112 m*M* calcium (*13, 14*). The total calcium content produced by this treatment was calculated to be about 1 mmole per kilogram of axoplasm (*15*). Loaded axons were immediately transferred to calcium-free ASW in which all the sodium was replaced by choline. This solution minimizes calcium efflux across the surface membrane (*15*) and largely eliminates further calcium influx. Thus, virtually all the calcium that entered during stimulation should have been sequestered by compartments within the axon. About 20 minutes after the end of stimulation, the axons were injected with an isosmotic solution containing oxalate (*16, 17*). The oxalate was expected to penetrate to sites of calcium sequestration and form insoluble calcium oxalate precipitates (*18, 19*). Three sets of control axons were prepared: (i) with calcium loading but without oxalate injection, (ii) with oxalate injection but without calcium loading, and (iii) with neither

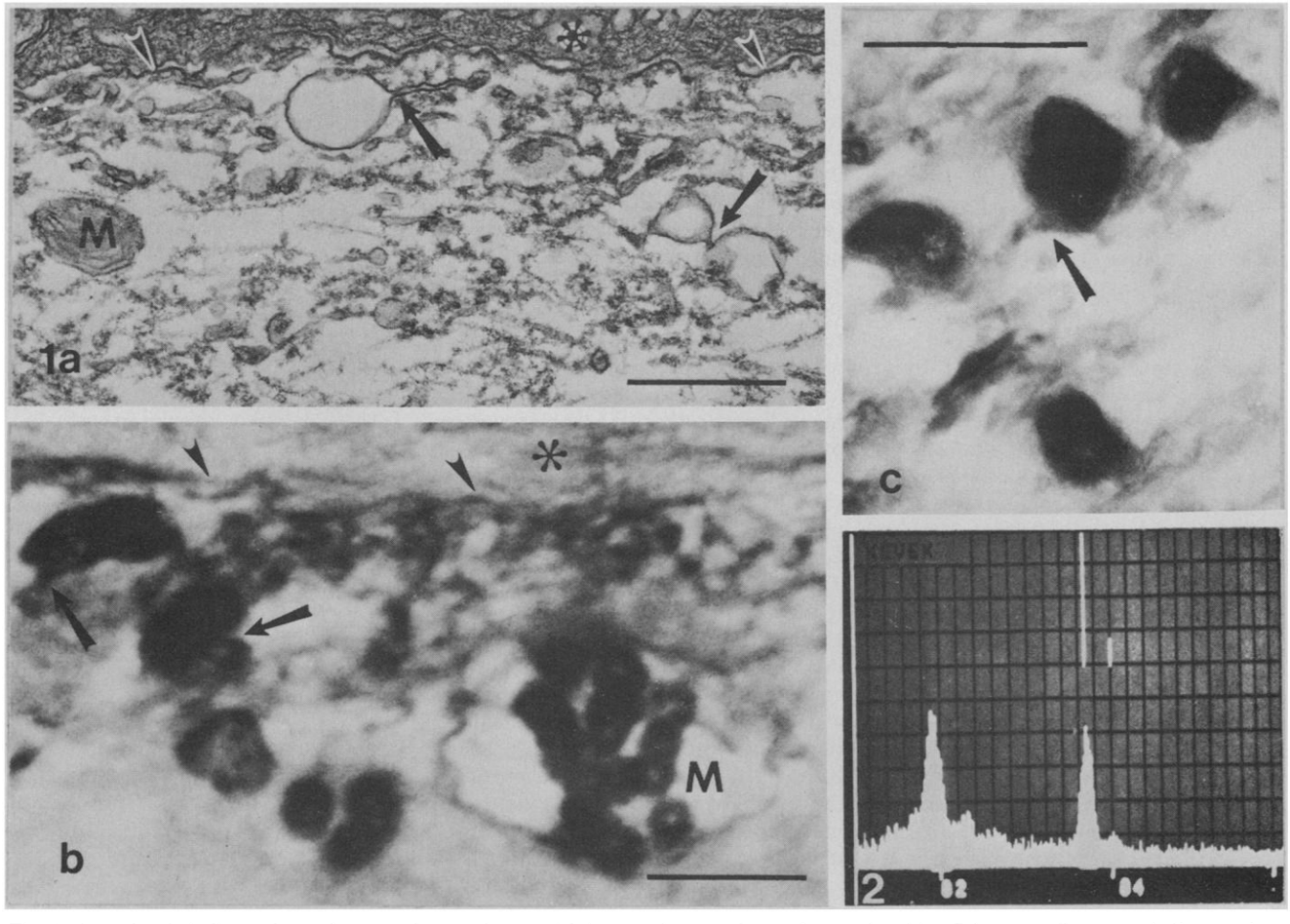

Fig. 1. Arrowheads indicate the surface membrane; the asterisk is over the cytoplasm of an ensheathing Schwann cell; arrows indicate points where large cisterns of the ER are continuous with smaller-diameter tubular regions; *M* indicates mitochondria. Scale bars, 0.5 μm. (a) Transmission electron micrograph of a small region of the surface of a conventionally prepared (*24*) thin section of a squid giant axon (×45,000). (b) Scanning transmission electron micrograph of an unstained section (about 0.25 μm thick) of an axon loaded with calcium and injected with oxalate. This thick section demonstrates the distribution of the ER, which is filled with an electron-opaque deposit (×45,000). (c) Higher-magnification transmission micrograph of a 0.25-μm-thick section taken at an accelerating voltage of 120 kV. The deposit is confined to cisterns and tubules of ER (×63,000). In (b) and (c) the contrast is due only to osmium and to the electron-opaque deposits. Fig. 2. Portion of the x-ray energy spectrum obtained in 100 seconds from the electron-opaque deposit contained in a small tubular region of ER. The long and short, bright vertical lines in the upper portion of the grid indicate the positions of the calcium K_α and K_β emission peaks. The large peak to the left is primarily from osmium in the sample. The full vertical scale is 512 counts.

calcium loading nor oxalate injection. All axons were rapidly frozen (*20*) and brought to room temperature in a solution of 1 g OsO_4 in 10 ml of acetone, starting from liquid nitrogen temperature. As the acetone thawed it replaced the ice in the tissue (*21*) so that the tissue was not exposed to an aqueous fixative that could redistribute the calcium precipitate. The axons were embedded in Araldite, and sections of various thicknesses were cut (*22*) and examined, unstained, in transmission or scanning transmission electron microscopes (*23*). Electron-opaque deposits were subjected to energy-dispersive x-ray analysis.

Figure 1a illustrates the structure of a conventionally prepared (*24*) squid giant axon. The ER comprises a system of membrane-bound tubules which, at intervals, expand into larger-diameter cisterns. Although the expanded cisterns often appear as isolated vesicles in thin sections, serial sections or thicker sections usually reveal their connection to a smaller-diameter reticulum and to each other. In such conventionally fixed fresh squid axons (*Loligo peallei*) the ER occupies 4 to 5 percent of the volume of the axoplasm and the mitochondria occupy about 1 percent (*25*). Figure 1b is a scanning transmission electron micrograph of an area of the periphery of a calcium-loaded, oxalate-injected axon. Electron-opaque deposits containing calcium were found in mitochondria, but the ER also contained an electron-opaque deposit. Figure 2 is an x-ray energy spectrum obtained when ER containing an opaque deposit was irradiated with the electron beam. Emission peaks characteristic of calcium were obtained from areas in both mitochondria and large- or small-diameter ER that contained deposits. Irradiation of areas of nearby cytoplasm without visible dense deposits produced a very low but detectable calcium peak. In axons that were loaded with calcium but not injected with oxalate, the ER and mitochondria also contained a dense deposit (*26*), which was identified as calcium by x-ray analysis. These sections also contained a number of holes, which we interpret as sites from which calcium was lost during preparation of the sections. The ER of axons that were injected with oxalate without calcium loading contained small amounts of opaque deposits containing calcium, as indicated by x-ray analysis. The mitochondria, however, did not contain conspicuous calcium deposits in axons that were not specifically calcium-loaded.

Our interpretation of these results is that the ER, as well as mitochondria, of the squid axon accumulates calcium during loading. Oxalate injection is not required for calcium uptake by the ER, but when present, oxalate aids in preserving the calcium deposits during preparation for microscopy (*27*). In axons that were injected with oxalate but not calcium-loaded, the ER also contained small identifiable calcium deposits. Thus, the ER also sequesters calcium under more physiological conditions.

The possibility that intracellular compartments other than mitochondria might be involved in calcium regulation in nonmuscle cells is suggested by several other lines of evidence. First, recent physiological studies in the squid axon indicate that about two-thirds of a calcium load imposed by stimulation or by soaking in sodium-free solution is buffered by a system insensitive to mitochondrial poisons. In fact, calcium uptake by mitochondria is rather slow compared to the uptake by nonmitochondrial buffer at the resting free-calcium concentration of about 30 n*M*. Mitochondrial calcium uptake in situ reaches rates comparable to those in vitro only when the free calcium is in the range of 10 to 50 μM [(*15*); compare with data of Blaustein *et al.* (*19*)]. (In the experiments reported here we did not attempt to measure the amounts of calcium in the ER or mitochondria, but the amount in the ER may be several times greater since the ER comprises four to five times more of the normal axon volume than the mitochondria.) Second, particulate subcellular fractions of neural tissue and other nonmuscle cells are capable of accumulating calcium by an adenosine triphosphate–dependent mechanism similar to that of isolated SR vesicles from muscle (*18, 19*). Third, in several cell types the ER swells under conditions that produce calcium loading. It has been suggested that this is due to osmotic fluxes of water following calcium uptake into ER (*5, 28*). Our results strongly suggest that at least a portion of the nonmitochondrial calcium buffer in the squid axon, and probably the source of microsomal calcium-accumulating subcellular fractions of other nonmuscle cells, is the ER.

Since the capacity of intracellular calcium-accumulating systems must be finite, extrusion through the surface membrane must be responsible for long-term maintenance of low intracellular free calcium. The ability of the ER to accumulate calcium, however, provides the axon (and possibly other nonmuscle cells) with a spatially distributed system for regulation of the calcium level throughout the cytoplasm. Against this background of a low calcium concentration, then, controlled increases of calcium in local regions of the cell may be used to modulate local events.

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cium content of fresh, unstimulated axons (about 70 μmole/kg). Parallel experiments, using arsenazo III or antipyrylazo III as monitors of free calcium, indicated that, with the stimulus parameters used, mean free calcium in the axoplasm does not rise above 2 to 3 μ*M* during loading, although the concentration of free calcium immediately beneath the axolemma may be several times higher [F. J. Brinley, Jr., T. Tiffert, A. Scarpa, L. J. Mullins, *J. Gen. Physiol.* **70**, 355 (1977)].
16. The composition of the injection solution was (m*M*): potassium oxalate, 150; potassium isethionate, 292; K-TES buffer, 5; *p*H 7.3. The solution was diluted about tenfold by diffusion throughout the volume of the axon.
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23. Transmission micrographs were taken with a Philips 400 electron microscope, and scanning transmission micrographs and x-ray analyses were done on a Hitachi H-500 electron microscope equipped with a Kevex energy-dispersive x-ray spectrometer.
24. The axon was prepared as described for *Aplysia* neurons [M. Henkart, *Science* **188**, 155 (1975)].
25. Measurements were made by cutting out and weighing the various structures in electron micrographs.
26. In calcium-loaded axons not injected with oxalate the dense deposit did not fill the lumen of the ER cisterns as uniformly.
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Tricyclic Antidepressants: Long-Term Treatment Increases Responsivity of Rat Forebrain Neurons to Serotonin

Abstract. *Long-term treatment of rats with clinically effective tricyclic antidepressant drugs induced a selective increase in the inhibitory response of forebrain neurons to serotonin applied by microiontophoresis. Long-term administration of some related drugs which lack antidepressant efficacy failed to induce such a change. The enhanced response to serotonin induced by the clinically active tricyclic drugs took 1 to 2 weeks to develop, a time course which correlates with the delayed onset of therapeutic effects in humans.*

Glowinski and Axelrod (*1*) demonstrated in 1964 that the tricyclic antidepressant (TCA) drug imipramine blocks norepinephrine uptake into synaptic terminals in the central nervous system. Subsequently, other TCA drugs have been shown to be potent blocking agents of norepinephrine or serotonin. Since reuptake is believed to be a major mechanism for termination of neurotransmitter action in monoaminergic systems, it has often been assumed that this pharmacological property accounts for the clinical efficacy of these drugs. However, uptake inhibition can take place within minutes (*2*), whereas the clinical response requires a minimum of 8 days (*3*). Moreover, some clinically active TCA drugs such as iprindole (*4, 5*) are not efficient blocking agents of amine uptake. Conversely, some drugs such as cocaine (*6*) and FG-4963 (*7*) are potent blocking agents of amine uptake but are not effective antidepressants. Furthermore, there is little correlation between clinical response and degree of amine uptake blockade in humans (*8*). Finally, despite their rather similar intrinsic antidepressant effects, tertiary and secondary amine TCA drugs have differential effects on psychomotor activity (*9*) and on amine uptake [for example, tertiary amine TCA drugs preferentially block serotonin uptake, whereas secondary TCA drugs preferentially block norepinephrine uptake (*2, 10*)].

In short-term, single-cell recording studies, Bradshaw *et al.* (*11*) reported that TCA drugs applied iontophoretically could rapidly modify the response of cortical neurons to serotonin and norepinephrine; however, these effects were not specific for the monoamines. Short-term binding studies have shown that some TCA drugs interfere with the binding of ^{3}H-labeled lysergic acid diethylamide (LSD) to serotonin receptors (*12*). However, the effect of long-term systemic administration of TCA drugs on serotonin receptors has, to our knowledge, never been studied. We report here that the responsiveness of postsynaptic neurons in rat forebrain to serotonin, but not norepinephrine, is increased by the long-term administration of TCA drugs.

Male albino rats (Charles River, 200 to 250 g) were given daily intraperitoneal injections of the hydrochloride salts of iprindole (2.5 mg/kg; Wyeth), desipramine (5 mg/kg; Ciba-Geigy), imipramine (5 mg/kg; Ciba-Geigy), chlorimipramine HCl (5 mg/kg; Ciba-Geigy), amitriptyline (5 mg/kg; Merck Sharp & Dohme), FG-4963 (5 mg/kg; Ferrosan), fluoxetine (10 mg/kg; Lilly), or chlorpromazine (10 mg/kg; Smith Kline & French). All these doses fall within the range of daily doses used clinically (*3*). The drugs were administered for various lengths of time ranging from 1 to 14 days.

A *cerveau isolé* unanesthetized preparation was used for iontophoretic experiments in both untreated controls and drug-treated rats. The experiments were performed 24 hours after the last drug injection. Single-cell recording and iontophoresis were carried out in two representative forebrain areas: the ventral nucleus of the lateral geniculate body (VLG), which receives a dense input of serotonin-containing nerves (*13*), and the CA_3 region of the dorsal hippocampus (pyramidal cells), which is innervated by both ascending serotonin (*14*) and norepinephrine projections (*15*).

A conventional iontophoretic technique was used (*16*). The following substances were tested: serotonin creatinine sulfate, 0.04*M*, *p*H 3.6 (Regis); γ-aminobutyric acid (GABA), 0.05*M* in 0.05*M* NaCl, *p*H 4 (Calbiochem); norepinephrine bitartrate, 0.1*M*, *p*H 4 (Regis); and LSD bitartrate 0.001*M* in 0.1*M* NaCl, *p*H 4 (PHS-NIDA). The LSD was tested only in the VLG and norepinephrine only in the hippocampus. One side channel containing a 4*M* NaCl solution was used as a current-balancing channel. The central channel, used for recording unitary activity, contained a 2*M* NaCl solution saturated with Fast Green. Fast Green was deposited at the bottom of each electrode track and enabled us to verify histologically the locations of the recording sites (*17*). The time of onset of the effect of iontophoretically applied substances is the most sensitive index of receptor responsiveness (*18*). Accordingly, the sensitivity of the neurons to iontophoretic application of these substances was estimated from the charge (C_{50}) required to obtain a 50 percent decrease in the rate of spontaneous firing (*19*).

Twenty-four hours after a 14-day treatment with TCA drugs both VLG

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