Table 1. Occurrence of isolated veins in endemic Hawaiian *Euphorbia*.

| Species and varieties | Abundance of isolated veins | Distribution of species (including varieties) |
|---|---|---|
| *E. rockii* | Abundant | N.E. Oahu |
| *E. forbesii* | Abundant | N.W. Oahu |
| *E. clusiaefolia* | Common | E. Oahu |
| *E. arnottiana* | Common | S.E. Oahu |
| *v. integrifolia** | Occasional | W. Maui |
| *E. remyi* | Occasional | Kauai |
| *E. hillebrandii* | Rare-occasional | Oahu, W. Maui |
| *E. celastroides* | Rare | All main islands |
| *E. halemanui** | Rare | N.W. Kauai |
| *E. atrococca* | Rare | Kauai |
| *E. degeneri* | Rare | All main islands |
| *E. olowaluana* | Rare | W. Maui, Hawaii |
| *E. multiformis* | Rare | All main islands |
| *E. kuwaleana** | None | W. Oahu |
| *E. skottsbergii* | Rare | W. Oahu |
| *E. deppeana*† | | Oahu |

* Three leaves from a single specimen available for survey. † No leaves available for study.

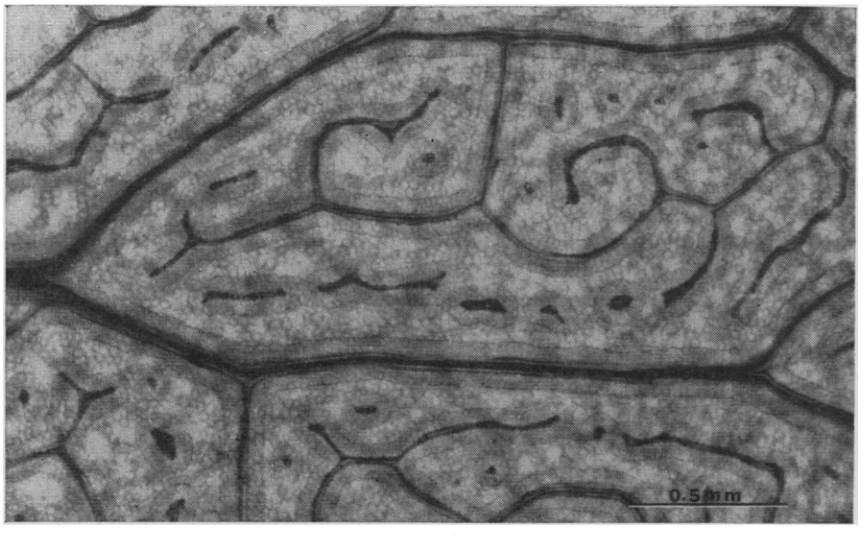


Fig. 1. Portion of a leaf of *Euphorbia rockii* cleared with 2.5 percent NaOH and stained with safranin according to the method of Foster and Arnott. A branched secondary vein and the minor venation including the isolated veinlets of an areole are shown. Specimen is from an elevation of 2000 feet (608 m), Punaluu, Koolau Mts., Oahu (Herbst No. 1117, HAW).

leaving isolated groups of tracheids as idioblastic structures in the leaf mesophyll. Subsequently a sheath of large parenchyma cells develops, as is usual in the subgenus *Chamaesyce*, and completely surrounds and further isolates the pieces of veinlet (Fig. 1). The resultant idioblastic veinlet may consist of a single tracheid, a cluster of tracheids, or, less commonly, a short, branched section of reticulum. Studies of paradermal sections reveal that the isolated veins consist solely of tracheids with no associated xylary parenchyma or phloem cells.

The ends of the normal minor veins also consist of tracheids only. Since the xylem in leaves of most angiosperms frequently—but perhaps not as commonly as in these species—differentiates disjunctly, and since the isolated veins generally form a distinct but broken line, they presumably are the result of the failure of certain procambial cells to differentiate.

The native Hawaiian species of *Euphorbia* are believed to have evolved from a single introduction, perhaps from the same ancestral stock as several common Pacific strand species (*5*). Through the use of cleared leaves, I surveyed 112 taxa of *Euphorbia*. Isolated veins were found, if only rarely, in the majority of Pacific members of the subgenus *Chamaesyce* I was able to survey adequately; none was found in other subgenera of the genus. However, future studies may reveal this condition to be more common than this survey indicates. The isolated veins were found in various degrees of abundance in six large-leaved species which grow primarily in mesic to boggy areas on the islands of Oahu and Kauai (Table 1). Conceivably the disjunct tracheary strands could be an adaptation to an increased laminar volume or a response to a wetter environment.

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## Synaptic Facilitation: Long-Term Neuromuscular Facilitation in Crustaceans

Abstract. *Continuous stimulation at frequencies equal to or greater than 5 hertz for 20 to 30 minutes results in a two- to fivefold increase in the amplitudes of excitatory postsynaptic potential recorded from certain stretcher and opener muscles of decapod crustaceans. This long-term facilitation appears to result from an accumulation of sodium ions within the nerve terminals. It persists for at least 1 hour after stimulation has stopped.*

The efficacy of synaptic transmission is increased by repeated nerve impulse activity at many synapses. This property is termed facilitation; it results from an enhanced probability of transmitter release following the arrival of an

impulse at the nerve terminal. The number of quantal "units" of transmitter substance liberated by succeeding impulses increases, yielding successively larger postsynaptic potentials (*1*).

The processes underlying the increased probability of transmitter release persist for relatively short periods (seconds) at neuromuscular junctions (*2*). Recently, we have found that certain crustacean neuromuscular synapses exhibit not only the usual "short-term" facilitation, but also another type, which requires much longer to develop and which persists for a considerable period of time after impulse activity has ceased. This "long-term" facilitation is seen only after many minutes of constant stimulation at frequencies equal to or greater than five per second (5 hz). It appears to be triggered by an accumulation of sodium ions within the presynaptic nerve terminals.

We have observed long-term facilitation in the claw opener muscle of the crayfish *Procambarus clarkii* (Girard) and in the leg stretcher muscles of the Bermuda crabs *Grapsus grapsus* (Linn.) and *Gecarcinus lateralis* (Frem.). Although long-term facilitation was somewhat more pronounced in the first two species (for example, compare A and B with C and D in Fig. 1), we used the stretcher muscle of *Gecarcinus lateralis* in the majority of our experiments, since this preparation was the hardiest and could best withstand the experimental procedures.

The opener and stretcher muscles of crustacean legs are innervated by a single excitatory axon, but their muscle fibers display widely varying physiological properties (*3*). Some fibers show relatively large excitatory postsynaptic potentials (EPSP's) which undergo little or no short-term facilitation, whereas others show rather small EPSP's which may increase up to ten times in amplitude when the frequency of stimulation is raised from 1 to 10 hz. We found that the former fibers show much less long-term facilitation than the latter ones. Therefore, we used only fibers that showed marked short-term facilitation in the following experiments.

Conventional intracellular microelectrode recording techniques were employed to monitor EPSP amplitudes from single muscle fibers under various experimental conditions. Preparation of the stretcher muscle and its excitatory axon, stimulation procedures, and physiological bathing solution were standard for this laboratory (*4*). Physiological solutions of lowered NaCl concentration were kept isotonic with added sucrose. Muscles treated with these solutions were equilibrated for 30 minutes before stimulation. Some muscles were bathed continuously, starting 20 minutes before stimulation, in a solution of $10^{-4}M$ ouabain made up in the physiological solution. For measurement of the input resistance of the muscle fiber membrane (*5*), a current pulse was passed into the fiber through a second microelectrode adjacent to the recording electrode to hyperpolarize the membrane by about 5 mv, and the current was monitored across a 10-kilohm series resistor.

The EPSP's evoked by constant stimulation at 10 hz grew rapidly in amplitude in the first few seconds and then remained at a relatively uniform mean amplitude for several minutes. This initial growth constituted short-term facilitation. After about 10 minutes the EPSP amplitude began to increase slowly once again, until by 30 minutes it was often two to four times greater than during the first 5 minutes of stimulation (Fig. 1, A–D). Most of the increase in EPSP size was apparent within the first 30 to 60 minutes, although a small additional increase sometimes occurred thereafter.

We attempted to determine if changes in the properties of the postsynaptic membrane contributed to the change in size of the EPSP's. Since the amplitude of an EPSP is influenced in part by the input resistance of the muscle fiber (*5*), measurements of this parameter were made at the start of an experiment and at various times during

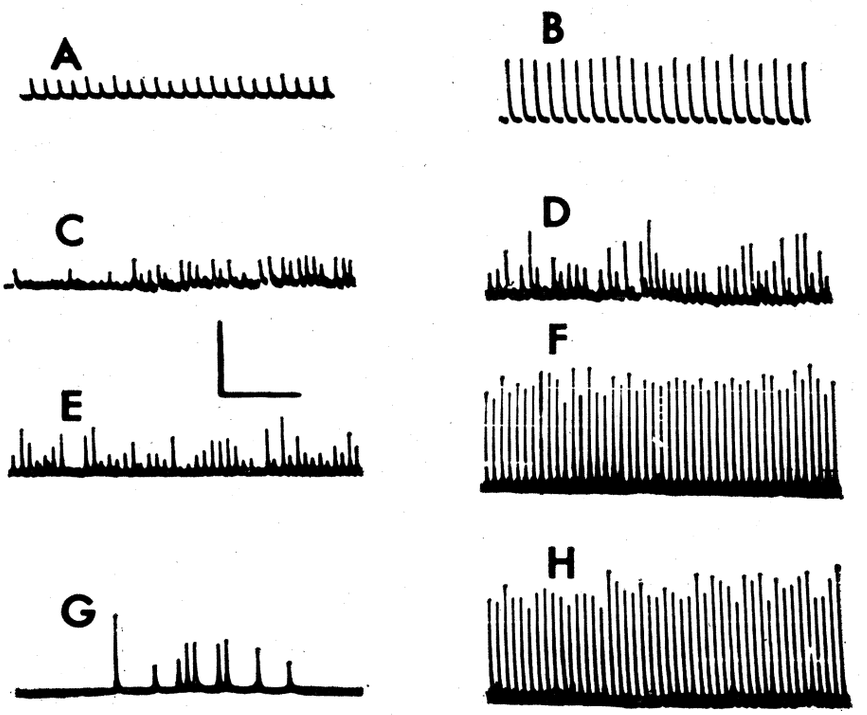


Fig. 1. (A) Excitatory postsynaptic potentials (EPSP's) recorded from the stretcher muscle of *Grapsus grapsus* after 5 minutes of constant stimulation at 5 hz; (B) EPSP's from the same cell as in A, but after 40 minutes of stimulation; (C) EPSP's recorded from the stretcher muscle of *Gecarcinus lateralis* after 2 minutes of constant stimulation at 10 hz. Note the variation in EPSP amplitudes and transmission failures which are common for greatly facilitating terminals of low transmitter output in this muscle. (D) Same as C, but after 30 minutes of stimulation. Note the larger mean EPSP amplitude and the smaller number of transmission failures. (E) EPSP's after 2 minutes of stimulation of the *G. lateralis* stretcher muscle at 10 hz during ouabain treatment (different preparation). (F) Same as E, but after 20 minutes of stimulation. The gain is one-tenth of that in E. (G) Same as F, but after 25 minutes of stimulation. Note the decrease in EPSP amplitude and widespread occurrence of transmission failures. (H) Same cell as G, showing the resumption in transmission after a short period (few seconds) without stimulation. Vertical calibration: 4 mv in A and B; 1 mv in C, D, and E; 10 mv in F; 2 mv in G; and 5 mv in H. Horizontal calibration: 1 second in all records.

stimulation. A marked increase in resistance would have had to occur to produce the enhanced EPSP's. We observed either no change or else a small decrease in input resistance accompanying long-term facilitation. Therefore, the processes underlying the increase in EPSP amplitudes most likely occur presynaptically and truly represent facilitation. Another observation supporting this conclusion is the decreased number of failures of synaptic transmission apparent after a period of constant stimulation (Fig. 1, C and D).

We studied the effects of different frequencies of stimulation (2, 5, 8, 10, and 20 for 2 hours) on long-term facilitation. At 2 hz, no measurable increase in EPSP amplitude occurred during 2 hours of stimulation. All higher frequencies produced two- to fourfold increases in EPSP amplitudes. Although there was no clear dependence of the magnitude of the increase on frequency, the time course of long-term facilitation was dependent on frequency: the maximum increase was seen sooner at higher frequencies. Furthermore, for long-term facilitation to be obtained, the nerve had to be stimulated continuously. When the preparation was stimulated at 10 hz once every 10 minutes for 3 minutes, there was no net change in EPSP amplitude throughout a 2-hour experiment.

Birks and Cohen (*6*) found that a frog neuromuscular preparation treated with ouabain showed a progressive increase in EPSP amplitude. According to them, ouabain inhibited the sodium pump in the motor nerve, causing sodium ions to accumulate in the nerve endings. The accumulation of sodium ions led to an increase in the concentration of free calcium ions due to a competitive interaction between these ions for sites in the nerve terminal membrane. The increased amount of free calcium produced an increase in EPSP amplitude, because the amount of transmitter released is dependent on the availability of calcium ions in the nerve endings (*2*).

From our observations it seemed likely that the internal concentration of sodium ions in the nerve terminals may have increased during the prolonged periods of stimulation. Sodium ions would accumulate until an equilibrium was reached between the rate of sodium influx during nerve impulse activity and the rate of sodium efflux due to the sodium pump. On the basis of Birks and Cohen's findings, such an increase in internal sodium ions could account for the occurrence of long-term facilitation.

Support for this hypothesis was obtained in experiments involving ouabain and reduced external concentrations of NaCl. Addition of ouabain to the normal bathing solution produced a striking increase in the rate and magnitude of the long-term facilitation seen at 10 hz. In the presence of ouabain the amplitude of the EPSP's was increased 10 to 40 times within a 20- to 30-minute period of stimulation (Fig. 1, E and F). Shortly after the maximum increase occurred, the EPSP's began to fluctuate greatly in amplitude and transmission failures were common (Fig. 1G). Soon after, transmission failed completely, apparently because of a blockade of the nerve terminals. Brief periods of rest (no stimulation for several seconds) temporarily restored transmission. The EPSP's seen at this point were larger than those at the beginning of the period of constant stimulation (Fig. 1H), indicating a persistent increase in probability of transmitter release. Furthermore, electron microscopic examination of the opener muscles of the crayfish claw after ouabain treatment and constant stimulation showed no depletion of synaptic vesicles. Ouabain was effective only if coupled with nerve stimulation, for after the muscle was bathed in ouabain for 50 minutes without stimulation and then given a test stimulation at 10 hz, there was little increase in EPSP amplitude.

Lowering the concentration of NaCl to one-third of that normally present in the bathing solution completely eliminated the occurrence of long-term facilitation. In this case, stimulation at 10 hz for 90 minutes usually produced a slight decline in EPSP amplitudes. The absence of long-term facilitation in this experiment could have resulted from reduced entry of sodium ions into the nerve terminals during spike activity.

Long-term facilitation persists for at least 1 hour after stimulation has ceased. Test trains given 1 hour after a 90-minute period of stimulation at either 10 or 20 hz revealed that the EPSP's were one and a half to two times larger than those recorded during the first 5 minutes of the period of constant stimulation. This long-lasting effect might be due to slow removal of sodium ions from the nerve terminals. Alternatively, the prolonged period of stimulation may have triggered long-lasting changes in the metabolic activities of the nerve terminals.

Crustacean neuromuscular systems are often thought of as models for vertebrate central synaptic processes, for a great diversity of synaptic properties occur in these crustacean systems. For example, Bruner and Kennedy (*7*) recently found that the fast flexor neuromuscular system of the crayfish abdomen, which is quite unlike the ones we studied here, shows a great deal of plasticity in its behavior. Transmission is depressed by very low (one per minute) and high (above ten per second) frequencies of stimulation, whereas it is enhanced at intermediate (one or two per second) levels of activity. The occurrence of long-term facilitation in the neuromuscular systems we studied not only provides a method of recruitment of additional postsynaptic elements during prolonged activity, but perhaps more significantly provides a way of producing a relatively long-lasting increase in synaptic efficacy. This long-term effect could have important implications in such central processes as memory formation and learning.

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