lay). This set has undergone 50 transfers during a 6-mo period. After the 16th transfer a subline was started in antibiotic-free medium. It has undergone 34 transfers. No antibiotics were employed in set II cultures; this was to rule out possible effects of these agents in preventing detection of certain bacterial contaminants. Set II cultures were discontinued after the 15th transfer, when tests showed clearly that axenic cultivation had again been achieved.

Repeated sterility checks failed to uncover any evidence of cryptic microbial contamination of the amoebic cultures. Gross contamination occurred in approximately 2 percent of some 800 tubes used and was traceable in each instance to airborne contamination which occurred during subculturing. Tests with STB medium demonstrated that *Crithidia* introduced with the original inoculum of amoebas died out prior to the third transfer.

The effect of heat on growth-promoting factors present in embryo extract was investigated. A subline from set I cultures was started in medium containing $CEEH_{25}$. Yields from this subline (still in existence after 29 transfers) were equal to those obtained with unheated extract. These results were unexpected in light of earlier studies (*2, 8*) which showed that even milder heat treatment destroyed growth-promoting activity. The highly supplemented nature of the diphasic medium may explain, in part, the results obtained. Baernstein *et al.* (*8*), in working with amoebas grown in microcultures, discovered that enriching their medium with vitamins, amino acids, and ribonucleic acid partially restored factors that had been lost through heating the extracts (*9*).

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20 April 1961

Intracellular Study of Recurrent Facilitation

Abstract. Recurrent facilitation in the cat's spinal cord has been studied in deep peroneal and quadriceps motoneurons with the use of intracellular recording. The presence of facilitation was indicated by several criteria, among them increased firing index of the cells or decreased latency of firing. In many, but not all, facilitated cells the conditioning volley caused a small visible depolarization. Subthreshold synaptic potentials were frequently increased in magnitude by the conditioning volley, which also increased the effectiveness of a stimulus applied through the microelectrode. Facilitation was found in a large percentage of the motoneurons investigated and was clearly able to bring about pronounced changes in the excitability and firing behavior of these cells.

Previous experiments in this laboratory have shown that recurrent inhibition and recurrent facilitation are distributed in an organized manner in the cat spinal cord (*1*), and subsequent work has suggested that the facilitation may in fact be a disinhibition which enhances reflex discharge by decreasing background inhibitory activity (*2*). The purpose of the present investigation has been to record, in individual motoneurons, the intracellular changes which accompany recurrent facilitation of reflex discharge, in order to study the distribution of this phenomenon among motoneurons and to obtain further evidence on the synaptic mechanism of recurrent facilitation.

All experiments have been performed on cats whose spinal cords had been severed in the upper cervical region, and the preparation was the same as previously described (*1*, *3*). So far, studies have been made of motoneurons of the deep peroneal group and of quadriceps whose action is usually facilitated by antidromic stimulation of the nerve to gastrocnemius-soleus and biceps-semitendinosus respectively (*1*). Intracellular recording was by means of glass micropipettes with tip diameters less than 1 μ, filled with 2.7*M* KCl.

The cells that have been studied had action potentials ranging from 50 to 100 mv. Synaptic activity was frequently visible in the absence of electric stimulation and occasional firing was observed in several cells as a result of such activity. Many deep peroneal cells fired repetitively to single dorsal root shocks, in a manner similar to that described for cells whose activity was recorded in ventral root filaments (*4*); it was often possible to obtain as many as four to five responses, the intervals between spikes being as short as 2.5 msec. Such repetitive discharges were superimposed on long and complex synaptic potentials. Many of the penetrated cells had a monosynaptic firing index (*5*) of 100. Such cells were studied either by weakening the strength of the dorsal root volley or by restricting stimulation to a small dorsal rootlet. In this manner it was possible to make a cell respond either with an intermediate monosynaptic firing index or polysynaptically.

Facilitation of single motoneurons was studied only in preparations in which visible recurrent facilitation of the monosynaptic reflex was present. Upon penetration it was first essential to determine whether the cell was facilitated. This was done employing a number of criteria: in many cases a conditioning shock increased the firing index; at times the latency of a polysynaptic discharge was reduced; conditioning has also been seen to increase the number of spikes in the discharge.

Facilitation has been studied in 103 cells, and changes in membrane potential were looked for in a number of these. In many cases the conditioning shock caused small depolarizations, the largest being less than 3 mv. In other facilitated cells depolarizations were much smaller and in some cases they were either absent or vanishingly small. The depolarization shown in Fig. 1 is typical. While it is hard to determine its exact onset, it first becomes clearly evident about 6 msec after the conditioning stimulus and has a time course of approximately 50 msec. These values are compatible with the latency and duration of recurrent facilitation as exemplified in typical facilitation curves (*3*).

As a result of a conditioning volley, subthreshold synaptic potentials in facilitated cells were frequently measurably increased over a considerable part of

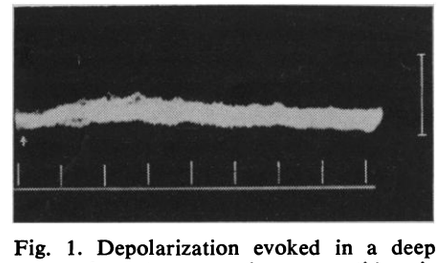

Fig. 1. Depolarization evoked in a deep peroneal motoneuron by an antidromic volley in the nerve to gastrocnemius-soleus. Conditioning stimulus delivered at the arrow. This record was obtained by superimposing approximately 15 faint traces. Time, 10 msec, voltage calibration, 10 mv

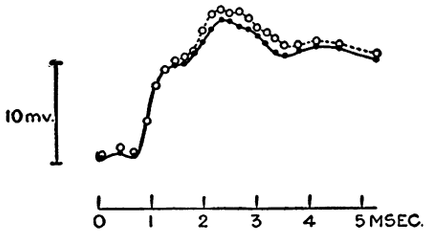

Fig. 2. Effect of conditioning volley in nerve to gastrocnemius-soleus on subthreshold excitatory postsynaptic potential evoked in a deep peroneal motoneuron by stimulation of L6 DR and the upper half of L7 DR. Conditioning-test interval, 20 msec.

their time course. Such an increase in excitatory postsynaptic potential (EPSP) is shown in Fig. 2. The deep peroneal motoneuron concerned initially had a monosynaptic firing index of 0, and a polysynaptic firing index of 100. The firing index of the polysynaptic response, which latter, judging by its latency, was disynaptic, could be modified by recurrent facilitation; at two different levels of orthodromic drive it was increased from 0 to 26 and from 33 to 90. In order to measure the effect of recurrent conditioning on the EPSP, groups of control and conditioned responses were alternated and photographed on moving film. Changes in the subthreshold EPSP were then determined by measuring the amplitude of these conditioned and unconditioned EPSP's at each of 23 different intervals following the stimulus artifact. The second beam of the oscilloscope was used as an arbitrary reference for measurement of EPSP amplitude. The solid line (dots) in Fig. 2 represents the average control EPSP; the dashed line (circles), the average conditioned EPSP. Tracings of conditioned and unconditioned membrane potentials revealed little or no shift in resting potential as a result of conditioning. There was also no difference between the early parts of the two groups of EPSP's. However, most of the later part of the potential was significantly increased by recurrent conditioning ($P < 0.02$). Changes of this type have been seen in a number of motoneurons, and increases in EPSP's have generally been more marked in the later part of the potential.

Intracellular stimulation of motoneurons by means of a bridge circuit similar to that used by Frank and Fuortes (*6*) has shown that an intracellular stimulus delivered through the micropipette is generally made more effective by recurrent conditioning. In the case of the cell whose EPSP is illustrated in Fig. 2, at three different strengths of intracellular stimulation, namely wth current pulses 7.5 msec in duration and ranging from 2.25 to 2.5 $\times 10^{-8}$ A, conditioning increased the firing index from 0 to 19, from 8 to 32, and from 28 to 69. This increase in intracellular firing index is of the same order of magnitude as that of the disynaptic firing index described above.

These observations made by means of intracellular recording are consistent with the hypothesis that recurrent facilitation is due to a reduction of background inhibition. Reduction of tonic inhibitory action and the consequent slight decrease in membrane conductance could lead to both the effects on membrane potential and changes in excitability which have been seen. However, other interpretations of the findings available so far may also be possible and further experiments designed to clarify the meaning of the membrane changes are under way at this time.

Facilitation has been looked for so far in 93 deep peroneal cells and 10 quadriceps motoneurons, and has been found, as evidenced by the criteria listed above, in 59 of the former and 8 of the latter. Eccles, Iggo and Ito (*7*) have found that in cats lightly anesthetized with sodium pentobarbitol, and with the spinal cord sectioned at L2, recurrent facilitation, as judged by the presence of a small depolarization, is recorded very infrequently. And Granit and Rutledge (*8*) have interpreted the findings of these workers as showing that "recurrent facilitating effects were rare and very small." As shown above, the changes in membrane potential that accompany recurrent facilitation are indeed small. However, the fact that facilitation has been found in 63 percent of the deep peroneal neurons and in most of the quadriceps cells that have been studied shows that in the unanesthetized high spinal cat this phenomenon is certainly not rare. Furthermore, while the membrane changes recorded have been small, pronounced changes in firing index have been seen in a number of cells. Clearly, recurrent facilitation is a widespread phenomenon in certain motoneuron nuclei and appears capable of bringing about significant changes in motoneuron firing (*9*).

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20 April 1961

Recovery of the Living Fossil Mollusk, Neopilina, from the Slope of the Cedros Trench, Mexico

Fourteen small monoplacophoran mollusks, relict animals with a discontinuous geologic range between the Paleozoic and the Recent, have been recovered from a biological trawl sample taken 31 December 1960 from the slope of the Cedros Trench, Baja California, Mexico (*1*). Specimens of *Neopilina* previously have been reported from off Costa Rica, off Peru, and off Cape San Lucas, Mexico (*2*). This collection represents the most northern record for the Monoplacophora as well as the largest single collection.

The specimens (Fig. 1) are from the Allan Hancock Foundation station No. 7230–60, located 30 mi off Natividad light, Baja California, Mexico, between 27°52′25″ to 27°51′30″N and 115°44′30″ to 115°45′15″W (dead-reckoning position). The depth varies between 1514 fathoms at the start of the station to 1493 at its completion. The specimens range in size between 1.3 mm and 2.37 mm long.

The minute animals collected have six pairs of diminutive gills and a segmental musculature similar to that described by Lemche and Wingstrand (*3*)

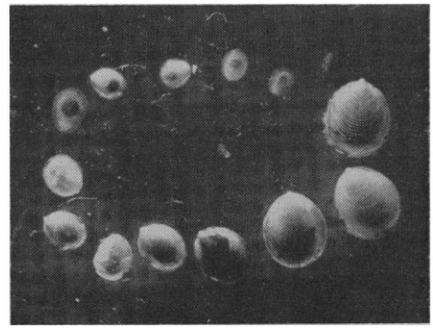

Fig. 1. Specimens of Neopilina.