Habituation: Occurrence at a Neuromuscular Junction

Abstract. *At the neuromuscular junctions between the motor giant axon and fast flexor muscle fibers in crayfish, stimulation at frequencies of one per minute produces a large decline in the amplitude of excitatory junctional potentials. Recovery (dishabituation) can be brought about by increases in stimulus frequency, which trigger a potentiation process; at still higher frequencies, a second form of depression intervenes. The last process appears to be due to depletion of transmitter; the first probably depends instead upon electrical changes in presynaptic terminals. Because of the interactions between the three processes, the junctions display the properties of habituation and dishabituation usually associated with complex central nervous networks.*

Habituation, in behavioral usage, refers to a waning in the response to a repeated stimulus when the stimuli are not followed by any reinforcement (*1*). The phenomenon has been analyzed in single units in the central nervous system (for example, *2*); where appropriate studies have been undertaken with intracellular recording, it has been ascribed to long-lasting diminution of the amplitude of compound excitatory postsynaptic potentials (EPSP's) (*3*). In *Aplysia* giant neurons, the attenuation of unitary EPSP amplitude during habituation suggests that prolonged changes in synaptic efficacy are involved (*4*).

A more exacting criterion is also usually applied to cases of behavioral habituation: it demands that the response be restored when the frequency of the stimulus is changed in either direction from the habituating frequency. Single junctions often show prolonged losses in synaptic efficacy as a result of repetitive stimulation, even at low repetition rates. For example, stimulation of the crayfish motor giant (MG) neuron at frequencies of only 0.1/second markedly reduces the amplitude of evoked excitatory junctional potentials (EJP's), and thus the degree of tension developed, in the flexor muscle fibers it innervates (*5*). Such effects can be interpreted as antifacilitation, that is, as simply a loss of efficacy due, for example, to depletion of stored synaptic transmitter. We now present evidence that the temporal recovery cycles of these junctions depend upon three separate processes, two leading to depression and one to augmentation. Because of this complexity, these peripheral synapses can, in the appropriate range of stimulus frequency, show increased efficacy when the frequency is raised.

To stimulate the MG axon and record from the muscles it innervates, we removed a dorsal strip of cuticle from the isolated abdomen of crayfish (*Procambarus clarkii*). The ventral membrane and the ventral superficial muscle fibers of the third abdominal segment were dissected away, and the exposed main third root was then isolated from the rest of the nervous system by severing the connectives or by putting ligatures close to the third and fourth abdominal ganglia. All intracellular recordings were done with 3*M* KCl-filled floating microelectrodes (*5*) in muscle fibers of the ventral part of the lateral anterior oblique flexor muscle (*6*). In other experiments, the muscle was removed for its entire length, together with the sternite to which its caudal end attaches and a few surrounding muscles. Where the concentrations of $CaCl_2$ or $MgCl_2$ in the bathing solution (*7*) were altered, compensatory changes were made in NaCl concentration to keep the solution isosmotic. Membrane conductance was tested by inserting a second KCl-filled microelectrode and applying hyperpolarizing current pulses through the electrode and a 50 megohm series resistor.

We restricted stimulation to the MG axon by taking advantage of the fact that it and inhibitor axons branch at the first bifurcation of the third root, whereas the other axons do not. The threshold of the MG is usually the lower of these two, and we were able to activate it separately by axon reflex, using another branch of the third root for antidromic stimulation with brief (0.01 to 0.03 msec) electrical pulses applied through bipolar platinum or suction electrodes. In some experiments the MG axon was excited by stimulating a lateral giant fiber in the central nervous system.

When EJP's were evoked by stimulating the MG axon at intervals of 5 minutes or more, no changes in their amplitude could be observed. When the interval was reduced to 1 minute, however, the second response was reduced to 55 to 70 percent of the initial amplitude (Fig. 1A*a*). The depression of EJP amplitude is of remarkably long duration for a neuromuscular preparation: following the three stimuli at 1-minute intervals in Fig. 1A*a*, a pause of more than 5 minutes was necessary to obtain full recovery, and tens of minutes were required after more prolonged stimulation.

The kinetics of the amplitude change depended upon the frequency of stimulation. The diminution of the second EJP at 1/second in Fig. 1A*b* was less pronounced than that in series *a* even though the stimulus frequency was much higher, and the next EJP's actually showed augmentation. After a few stimuli, the initial amplitude was recovered; it then diminished gradually as stimulation was continued. Thirty-two stimuli were necessary to reduce the amplitude to the level of the second stimulus at 1/minute.

These results suggest that separate processes underlie the loss of synaptic efficacy at low frequency and the potentiation shown at higher frequencies. Tests with stimulus trains ranging between 0.2/minute and 5/second showed that the optimal frequency for diminution (process 1) is near 1/minute, whereas the potentiation (process 2) is most marked at frequencies of 1 to 2/second. Stimulation at intermediate frequencies (for example, 0.1/second) thus produces slight augmentation, which often appears only after several stimuli. At frequencies above 1 to 2/second, process 2 is also reduced, though some augmentation can even be observed at 10/second. At any maintained rate of stimulation exceeding 1/minute, however, the amplitude of the axon's EJP's ultimately declines to a very low level.

Aftereffects of the potentiation process are also observed after bouts of repetitive stimulation. For example, in Fig. 1A*c* the rate of decline in amplitude at 1/minute was slower than that observed in Fig. 1A*a*, when the same frequency followed a long rest. Thus the kinetics of the changes in EJP amplitude at a given frequency depend upon the recent history of the junction. Another example of this dependence is shown in Fig. 1A*d*, in which stimulation at 1/second was preceded by a few stimuli at a very low frequency and therefore produced an augmentation of EJP amplitude from the beginning of the series.

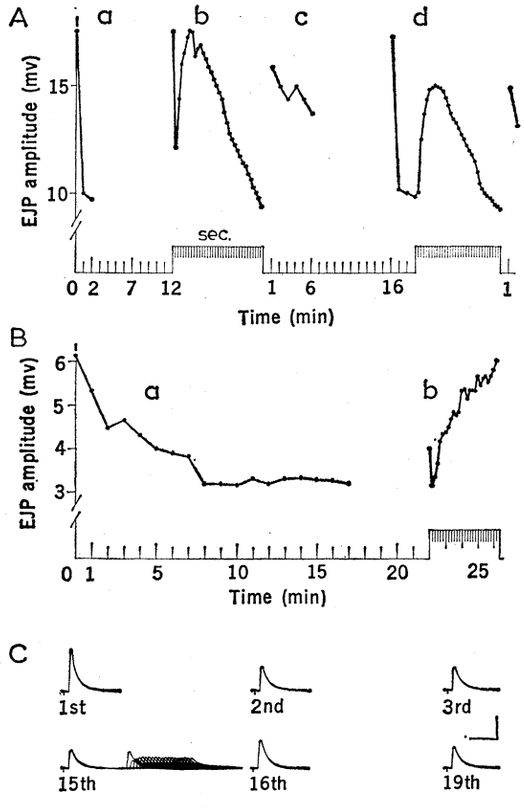
Delivery of high-frequency stimulation after a low-frequency train can speed recovery (Fig. 1, B and C). A

prolonged series of stimuli at 1/minute was applied to MG, reducing the EJP amplitude to about half its initial value. After 5 minutes of rest, the recovery was about 30 percent; but stimulation at 0.1/second (Fig. 1B*b*) produced complete recovery of the original amplitude after about 4 minutes of stimulation. Recovery was also accelerated by brief (3 to 5/second), high-frequency (5 to 10/second) trains (Fig. 1C). A short burst of stimulation at 5/second was directly interposed in a train of stimuli at 0.1/second. During this brief tetanus, the EJP's showed an initial decline, which was followed by slight augmentation and then by a slow diminution in amplitude. A recovery of about 50 percent was observed on the first EJP after the tetanus.

The mechanism underlying process 1 is not yet clear, but some possibilities have been ruled out by additional experiments. First, the decrement is not due to any changes in electrical properties of the postjunctional membrane. Fig. 2B shows conductance measurements made by applying hyperpolarizing pulses across the muscle fiber membrane through a second intracellular microelectrode. Changes in conductance were not found, either during the course of EJP decrement or after brief trains of stimuli that produced potentiation.

The intervention of an inhibitory influence, postulated in the inhibitory buildup theory of habituation (for example, *8*), has also been ruled out. After nine axons in the main third root, including the inhibitor, were severed so as to leave only the MG axon intact, stimulation of the root proximal to the damaged area evoked only the motor giant axon EJP. The rate of decrement in EJP amplitude was nevertheless identical to that found in preparations with intact third roots.

Third, the phenomenon might depend upon the rapid depletion of transmitter and its slow mobilization, as was proposed for EPSP habituation in *Aplysia* (*4*). However, in preparations in which the release of transmitter was reduced by increasing the Mg^{2+} concentration, or diminishing the Ca^{2+} concentration, or both, the rate of decrement was not modified. In the experiment in Fig. 2A, three series of stimuli at 1/minute were applied; these were separated by long pauses to allow full recovery of EJP amplitude while solutions were changed. The amplitude of the initial EJP in a series was changed severalfold without significantly altering the time-course of EJP decrement. Normal rates and extents of decrement were observed even when the initial EJP was reduced below 1 mv. Thus, the diminution of EJP amplitude that occurs during low-frequency stimulation does not depend upon the depletion of transmitter stored in MG axon terminals.



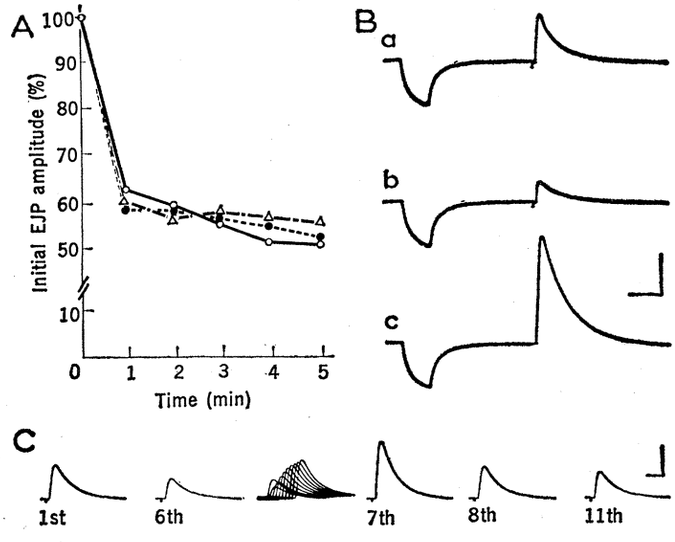


Fig. 1 (left). (A) Changes in motor giant EJP amplitude with repeated stimulation. A*a*, 1/minute stimulation; A*b*, 1/second stimulation, in each case applied after several minutes' rest; A*c*, 1/minute stimulation, applied 1 minute after the end of the train in *b*; A*d*, 1/minute stimulation, preceded by a 10-minute rest and followed by a 1/second train. (B) Recovery with additonal stimulation: B*a*, time course of decrement of motor giant EJP during 1/minute train; B*b*, restoration during a subsequent period of stimulation at 0.1/second. Five minutes elapsed between the two series. (C) Motor giant EJP during stimulation of lateral giant fiber at 10-second intervals; a 4-second burst (5/second) was introduced between the 15th and 16th stimuli of the series, and produced partial restoration. Calibration: 40 mv, 40 msec for each single sweep. Fig. 2 (above). (A) Time course of decrement of motor giant EJP during 1/minute stimulation, in normal van Harreveld's solution (○), in physiological solution containing 23 m*M* $MgCl_2$ (●), and 29 m*M* $MgCl_2$ (△). The amplitudes of the initial potential in each series were: 40 mv, 11 mv, and 1.2 mv, respectively. (B) Constancy of membrane conductance during decrement and restoration (23 m*M* $MgCl_2$). The first response in each trace is a hyperpolarizing current pulse injected through a second microelectrode. The second response is a motor giant EJP, evoked (*a*) at the beginning, (*b*) at the end of a long series of stimuli at 10-second intervals, and (*c*) after a short burst of 5/second stimuli. Calibrations: 5 mv, 10 msec. (C) Recovery of motor giant EJP amplitude in physiological solution containing 23 m*M* $MgCl_2$, at 1/minute stimulation. The 1st, 6th, 7th, 8th, and 11th motor giant EJP are shown. A short burst at 5/second was applied 10 seconds before the 7th stimulus of the series. Calibration: 5 mv, 10 msec for each single sweep. In both figures the ascending parts of some EJP's were retouched.

In preparations with high concentrations of Mg^{2+}, complete recovery of the initial EJP amplitude could be obtained even after a very short, high-frequency train of stimuli (5/second for less than 1 second). Fig. 2C shows that trains of a few seconds' duration produced complete recovery during repetitive stimulation, after the response had shown its initial decline. Such trains also increased the amplitude of subsequent EJP's above the initial level. This is a further indication that the recovery with added stimuli is brought about by a superimposed potentiation process (*4*). It also adds weight to the suggestion that under normal conditions, high-frequency stimulation produces a mixture of potentiation and depression resembling that at other neuromuscular junctions (for example, *9*). This second kind of depression, process 3, is apparently associated with depletion of transmitter, since it is reduced in high concentrations of Mg^{2+}. It appears to be responsible in our preparation for the slow diminution of EJP amplitudes that takes place during the later stages of high-frequency stimulation, and for the lack of complete recovery after such trains.

Our experiments demonstrate three distinct temporal processes at a single neuromuscular junction. Process 1 is a decline in synaptic efficacy at very low frequencies of stimulation which is unrelated to the loss of transmitter, and probably involves labilities in the presynaptic membrane potential or safety factor. Process 2 is a form of potentiation observed at intermediate frequencies, and process 3 is a high-frequency depression probably associated with the depletion of transmitter stores.

These junctions therefore show extremely complex temporal changes in response to conditioning stimuli or to frequency changes. If the proper frequencies are employed (Fig. 1, A*d* and B), postjunctional responses may even be restored when the frequency of stimulation is increased. This is perhaps the most exacting criterion for classifying such phenomena as habituation and dishabituation; our results show that it can be met by a single neuromuscular junction, and does not require the presence of a complex neural network.

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Embossing Arabic Letters and Numbers on New Raised-Line Polyethylene Paper: An Aid for the Blind

Abstract. *A new polyethylene paper may be marked on a hard surface with an ordinary oversize ball-point pen or dull pencil point. Where the paper is marked, a raised-line imprint appears on the same side of the paper as that used for writing. This imprint may be both felt and seen. Newly blinded and partially sighted persons are able to read ordinary Arabic letters and numerals after a few trials.*

Few sighted persons can communicate in Braille with the nonsighted, but mastering Braille is not a simple task, particularly for the elderly, blind individual or the child with multiple disabilities. Most Braille writing slates require the individual to write from the reverse side of the writing sheet. Thus, although the blind learn to read from left to right, they must also learn to write backwards.

Several attempts have been made to develop forward-writing Braille slates, one of which I developed (*1–3*). The initial experiment with this slate resulted in unwanted "ghost" dots on standard Braille paper, but a polyethylene paper (*4*) proved successful in largely eliminating these dots. Important as this step was, it did not resolve the basic problems of finding a way for the blind or visually handicapped to write or print Arabic letters or numbers without learning Braille or some other coding method.

An improved, cheaper polyethylene paper No. 300 (*5*) may lead to a solution of the larger problem, as well as

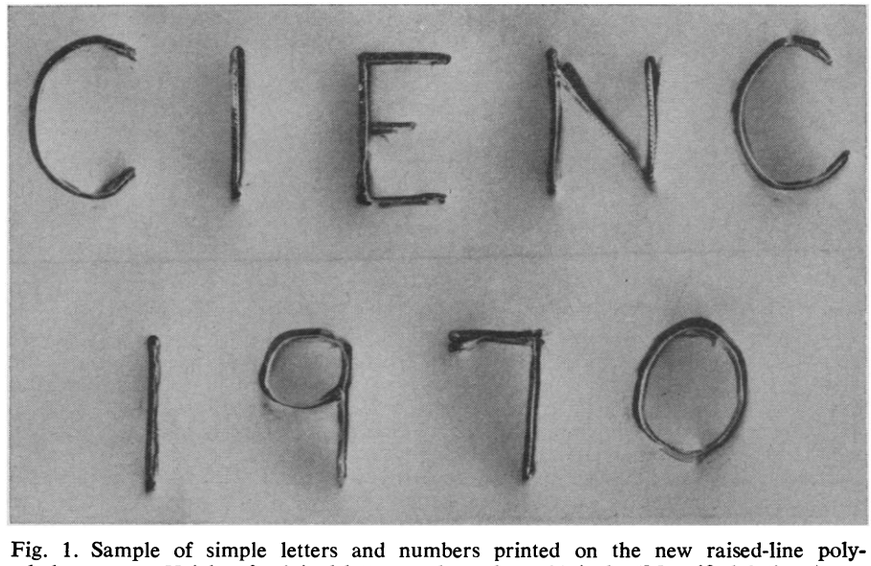

Fig. 1. Sample of simple letters and numbers printed on the new raised-line polyethylene paper. Height of original letters and numbers, ⅜ inch. (Magnified 2 times)