Facilitation is an ability of nerve terminals to release neurotransmitters more efficiently following repetitive stimulation. Facilitation at the crustacean neuromuscular junction is a very robust phenomenon. The number of synaptic vesicles releasing neurotransmitter in response to an action potential increases in proportion to duration of the nerve stimulation and to the frequency of action potentials. The classical explanation for facilitation is the accumulation of Ca^{2+} in the nerve terminal due to repetitive Ca^{2+} entries in response to a train of action potentials (reviewed in Zucker, 1999). In the present study we combined electrical recordings of postsynaptic responses with a computer simulations of neurosecretion to demonstrate the presence of another mechanism for facilitation, namely the increase in the number of vesicles properly activated to undergo the release process, the releasable pool of vesicles

It was shown that the releasable pool of vesicles could be measured employing local applications of hypertonic solution to the synaptic area and recording postsynaptic responses (Rosenmund and Stevens, 1996). We employed this technique to measure the releasable pool of vesicles after eliciting facilitation by trains of action potentials.

Lobster motoneuron was stimulated electriacally via suction electrode. Trains of 30 or 100 action potentials were delivered with the frequency 10 or 20 stimuli per second. Excitatory postsynaptic responses (EPSPs) evoked by action potentials were recorded focally from the dactyl opener synapse using saline filled macropatch electrodes. Hypertonic solution was applied locally to the synaptic area immediately after the train of action potentials for 20 s. We recorded asynchronous vesicles releases (miniature excitatory postsynaptic potentials, mEPSPs) evoked by each hyperosmolarity application. For the control, similar hyperosmolarity applications were employed to the same recording site without eliciting action potentials. The change in the releasable pool of vesicles elicited by a train of action potentials was measured as a difference in the number of mEPSPs recorded after and before the train of action potentials.

The train of action potentials evoked a significant increase in the number of mEPSPs recorded within the first three seconds of a hyperosmolarity application. We interpreted this increase in the asynchronous vesicle release as the increase in the vesicle activation elicited by action potentials (Stevens and Wesseling, 1998). We the releasable pool of vesicles increased in proportion to the duration of the train of stimuli and to the stimulation frequency. This indicates that the releasable pool of vesicles increases during facilitation.

To determine whether the measured increase in the releasable pool of vesicles can account quantitatively for facilitation, we simulated neurosecretory process by the following procedure. Each simulation cycle included three stages: vesicle activation, inactivation and fusion. We postulated that each action potential increases the probabilities of vesicles activation and fusion. The changes in activation and release rates were described by an immediate rise and exponential decay. Thus, neuroscretion process was described by the following parameters: probability of vesicle activation (a_0) and release (p_0) in the absence of stimulation; rate of vesicle inactivation (k_d); growth in a (a) and growth in r (r) evoked by a single action potential; decay in activation probability (t_a) and decay in release probability (t_r) between successive stimuli; rate of release during hyperosmolarity application *(r)*, the size of the total vesicle store *(S)*.

The average number of vesicles in the releasable pool in the absence of the nerve stimulation (R_0) was estimated from the analysis of the kinetics of transmitter release evoked by hyperosmolarity applications under control conditions. The simulation process started from a pool of R_0 vesicles. In each successive cycle, the changes in *a* and *p* were computed. Then the size of releasable pool of vesicles (*R*) and the number of vesicles released (m) were calculated as:

$$R_i=(R_{i-1}(1-p_i)+a_iS)(1- exp(-k_d/f)$$

$$m_i = r_i R_{i-1}$$

where *i* is the order number of an action potential in a train and *f* is a stimulation frequency.

The number of vesicles, *m*, released in each trial, and the total number of vesicles accumulated during a train of action potentials, *R*, were stored. The number of cycles (30 or 100) corresponded to the number of action potentials delivered during the experiment. The outputs, m_i and *R* were compared to the values obtained experimatally. To evaluate experimentally m_i, we divided the amplitude of each EPSP in a train by the amplitude of average mEPSP at the same recording site. *R* corresponded to the size of the releasable pool measured by hyperosmolarity applications after the trans of action potentials.

The experimental and modeling results were in a reasonable agreement. However, the observed increase the releasable pool *R* during facilitation was generally larger than it would be predicted from our model basing on the rate of facilitation in trains. This discrepancy can be explained by a limited number of active zones, which restricts action potential evoked neurosecretion but does not limit continuous release evoked by hyperosmolarity applications. In summary, our results confirm that presynaptic facilitation partially arises from accumulation of vesicles properly activated for release.