INCREASE IN THE RELEASABLE POOL OF SYNAPTIC VESICLES UNDERLIES FACILITATION

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Abstract

Facilitation is the ability of presynaptic terminals to release neurotransmitter more efficiently following repetitive stimulation. We demonstrated that facilitation can be explained by Ca^{2+}-dependent vesicles priming and the increase in the number of synaptic vesicles activated for release. Employing the model with two Ca^{2+} sensors, we computed Ca^{2+} concentration at the sites of priming and release, the size of the releasable pool of vesicles, and the rate of transmitter release during repetitive nerve stimulation. The calculated rates of vesicle release and the increase in the releasable pool during facilitation were in agreement with the results of electrophysiology experiments.

Keywords: Lobster neuromuscular junction, Synaptic plasticity, Neurosecretion, Calcium

1. Introduction

Presynapsic terminals have an ability to increase the amount of neurotransmitter released in response to a single action potential as the nerve is stimulated repetitively, a process termed facilitation. 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. Transmitter release is triggered by Ca^{2+} entry in the nerve terminal. The classical explanation for facilitation suggests accumulation of residual Ca^{2+} in the nerve terminal due to repetitive Ca^{2+} entries in response to a train of action potentials (reviewed in [16]). Basing on statistical analysis of transmitter release, we suggested another mechanism for facilitation [1, 15], namely the increase in the number of vesicles properly activated to undergo the release process, the releasable pool.

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 [10, 11]. We employed this technique to measure accumulation of releasable vesicles during facilitation [3]. Here we present a computational model of Ca^{2+}-dependent priming of synaptic vesicles, which predicts quantitatively accumulation of releasable vesicles and facilitation.

2. Electrophysiology

Recordings and quantal analysis of excitatory postsynaptic responses (EPSPs) from the lobster dactyl opener synapse are described elsewhere [2]. Trains of 100 action potentials, 20 stimuli per second, were elicited to produce facilitation (Fig. 1A). Quantal analysis of each EPSP in a train was performed to determine the number of vesicles released in response to each action potential (quantal content *m*, Fig. 1 B). To elicit the fusion of releasable vesicles accumulated during the stimulation, we applied hypertonic solution to the synaptic area immediately after the end of train (HS2, Fig. 1 A). We

recorded asynchronous vesicles releases (miniature excitatory postsynaptic potentials, mEPSPs) evoked by the hyperosmolarity application. For the control, hyperosmolarity was applied to the same recording site without the stimulation (HS1, Fig 1A). The increase in the releasable pool of vesicles during the train of stimuli was measured as the number of mEPSPs elicited by hypertonicity applied after the train (HS2) minus the number of mEPSPs elicited by the control hypertonicity (HS1). During the train of stimuli, quantal release in response to a stimulus increases and reaches a plateau after approximately 30 pulses. Immediately after the train, HS2 application released 66±26 vesicles more than HS1 (Fig.1 B, solid bar); this indicates that approximately 60-70 releasable vesicles were accumulated during the stimulation.

3. Model

We demonstrated earlier that facilitation can be described quantitatively employing a model with stimulus-dependent vesicle priming and stimulus-independent vesicle inactivation [1, 15]. Here we further developed this model by refining how vesicle priming and release depend upon a stimulus (Fig.2). As proposed in [6, 13], we hypothesize that each docked synaptic vesicles incorporates two types of Ca^{2+} molecular targets: a fast low-affinity sensor mediating vesicle priming (SP) and a slower high-affinity sensor mediating vesicle fusion and release of neurotransmitter (SR). The priming sensor, SP, is situated in the immediate proximity from the Ca^{2+} channels (Fig. 2 A). SP is activated by local Ca^{2+} elevation in response to an action potential. The release sensor, SR is separated from Ca^{2+} channels by a diameter of a single vesicle (Fig. 2 A).

Since SR is a high-affinity sensor, it is sensitive to the residual Ca^{2+} elevation remaining from previous stimuli.

Each action potential initiates vesicle priming with a probability *a*, and a release of primed vesicles with a probability *p* (Fig. 2B). The probability of priming or release is proportional to $[Ca^{2+}]^2$ at the corresponding site; this yields fourth power cooperativity of the whole process in regard to Ca^{2+} [4]. All the primed vesicles constitute the releasable pool. Between the stimuli, vesicles can be lost from the releasable pool, inactivated, with the rate *d* vesicles per second (Fig. 2 B).

4. Computations

Local Ca^{2+} concentration in the vicinity of a Ca^{2+} channel was calculated from the diffusion equation in a sphere:

$$\frac{\partial(rc)}{\partial t} = D\frac{\partial^2}{\partial t^2}(rc) \qquad (1)$$

where *c* is Ca^{2+} concentration, *r* is the distance from the channel, *D* is the diffusion coefficient and *t* is time. The solution of (1) for a point-like source at *t=0* will be [5]:

$$c = c_m \cdot V_m (4pDt)^{-3/2} \exp(-\frac{r^2}{4Dt}) \qquad (2)$$

where c_m is initial Ca^{2+} concentration in a microdomain and V_m is the volume of the microdomain. According to (2), we calculated Ca^{2+} profiles (Fig. 3 A) at the priming site, SP (*r=10 nm*), and at the release site, SR (*r=50 nm*), using the following parameters: $D=200\ \mu m^2/s$ [7], $c_m=100\ \mu M$ [8] and $V_m = 1000\ nm^3$.

Accumulation of residual Ca^{2+} at an active zone after stimulation by *i* pulses at the frequency *f* can be calculated as:

$$c_r^i = c_0 + \frac{c_{md} V_{md}}{V_{az}} n_{ch} \sum_{j=1}^{i} \exp\left(\frac{-b(i-j)}{f}\right) \quad , \qquad (3)$$

where n_{ch} is the number of Ca^{2+} channels at the active zone, V_{az} is the volume of the active zone, c_0 is Ca^{2+} concentration at rest, and b is the rate of Ca^{2+} buffering and extrusion. Accumulation of residual Ca^{2+} (Fig. 3 C) calculated from (3) with the parameters $n_{ch}=50$, c_0=50 nm, b=0.7 nm/s, and $V_{az}=0.2\ \mu m^3$ is in a reasonable agreement with the increase in Ca^{2+} concentration measured by optical methods at the crayfish [12] and lobster [9] neuromuscular junctions.

Priming and release probabilities were calculated as:

$$a_i = k_1 (c_r^i + c_{SP}^{peak})^2 \qquad p_i = k_2 (c_r^i + c_{SR}^{peak})^2 \qquad (4)$$

Since c_{SR}^{peak} , maximal Ca^{2+} concentration at the release sensor, has the same order of magnitude as c_r^i , the residual Ca^{2+} concentration at the active zone (Fig.3), the release probability p_i increases as the nerve is stimulated repetitively (Fig. 4). In contrast, c_{SP}^{peak}, maximal Ca^{2+} concentration at the priming sensor, is about 1000 times higher than c_r^i; therefore accumulation of residual Ca^{2+} and repetitive stimulation have almost no effect on the priming probability a_i (Fig. 4)

The number of releasable vesicles *(R)* accumulated after *i* stimuli can be calculated according to a recurrent equation:

$$R_{i+1} = (R_i (1 - p_i) + a_i S) \exp\left(-\frac{d}{f}\right) \qquad (5)$$

The number of vesicles released in response to i^{th} stimulus will be given by:

$$m_i = R_i p_i \qquad (6)$$

According to (5) and (6), we calculated accumulation of releasable vesicles *R* and quantal release *m* during a train of 100 stimuli, f=20 Hz (Fig. 4). The number of stored vesicles S

was accepted to be 500 to match the number of vesicles observed near the presynaptic membrane in ultrastructure studies [14]. Other parameters ($R_0=1$, $d=0.5\ s^{-1}$, $k1=10^{-7}nM^{-2}$, $k2=10^{-9}nM^{-2}$) were adjusted to reproduce experimental data (Fig. 1).

Our computations predict that quantal release *m* increases during initial 30-50 stimuli and reaches an equilibrium level of approximately three quanta per action potential (Fig. 4), similar to the experimentally observed increase in *m* (Fig. 1 B). The computed equilibrium size of the releasable pool R (65-75 vesicles, Fig. 4) is simular to the measured increase in the releasable pool (66±26 vesicles, Fig. 1).

5. Conclusion.

We developed a model for facilitation, which considers vesicle priming and release as two separate processes mediated by different Ca^{2+} sensors. Experimental and modeling results suggest two mechanisms for facilitation: accumulation of residual Ca^{2+} and accumulation of releasable vesicles due to Ca^{2+}-dependent vesicle priming.

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FIGURE LEGENDS

1. Facilitation and accumulation of releasable vesicles during repetitive stimulation of the nerve. **A.** Experimental protocol. The nerve was stimulated by a train if 100 pulses with the frequency 20 Hz. Hypertonic solution (HS2) was applied to the synaptic area immediately after the end of the train to release all the accumulated vesicles. For the control, hypertonic solution (HS1) was applied before the nerve stimulation. **B.** Facilitation during the train of pulses. The number of vesicles released per an action potential (*m*, open squares) increases and reaches a plateu within the initial 30 stimuli. The number of vesicles accumulated in the end of the train (mEPSPs, HS2-HS1) is marked by a solid bar.

2. The model of vesicle priming and release.

A. A synaptic vesicle has two Ca^{2+} targets: SP- a low-affinity sensor mediating vesicle priming and SR - a high affinity sensor mediating opening the pore and releasing neurotransmitter. **B.** Exchange between the releasable pool of vesicles (R) and vesicle store (S). Vesicle are primed (activated) with the probability *a*, released in response to an action potential with a probability *p*, and inactivated (demobilized) with the rate *d* vesicles per second.

3. Local and residual Ca^{2+} during the nerve stimulation. **A**. Local elevation of Ca^{2+} concentration following Ca^{2+} influx in response to an action potential. Solid line - at SP, the priming sensor; dotted line - at SR, the release sensor. **B.** Increase in the residual Ca^{2+} concentration at an active zone, as the nerve is stimulated repetitively with the frequency 20 Hz.

4. Computed increase in the release probability *p*, releasable pool of vesicles *R*, and the number of neurosecretory quanta *m*.

Figure 1

A


100 APs, 20 Hz
HS1
HS2
20 s
30 min
20 s

B

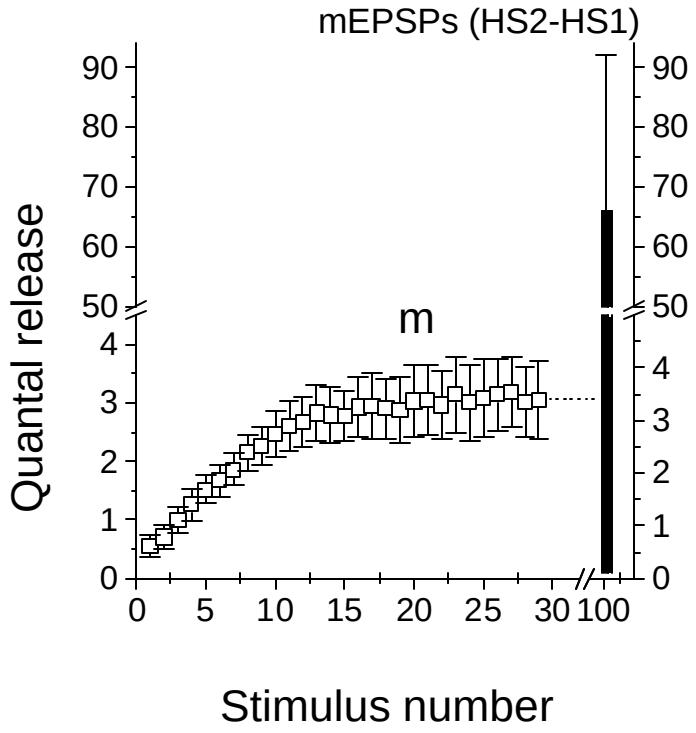

mEPSPs (HS2-HS1)
Quantal release
m
Stimulus number
0
5
10
15
20
25
30
100

Figure 2

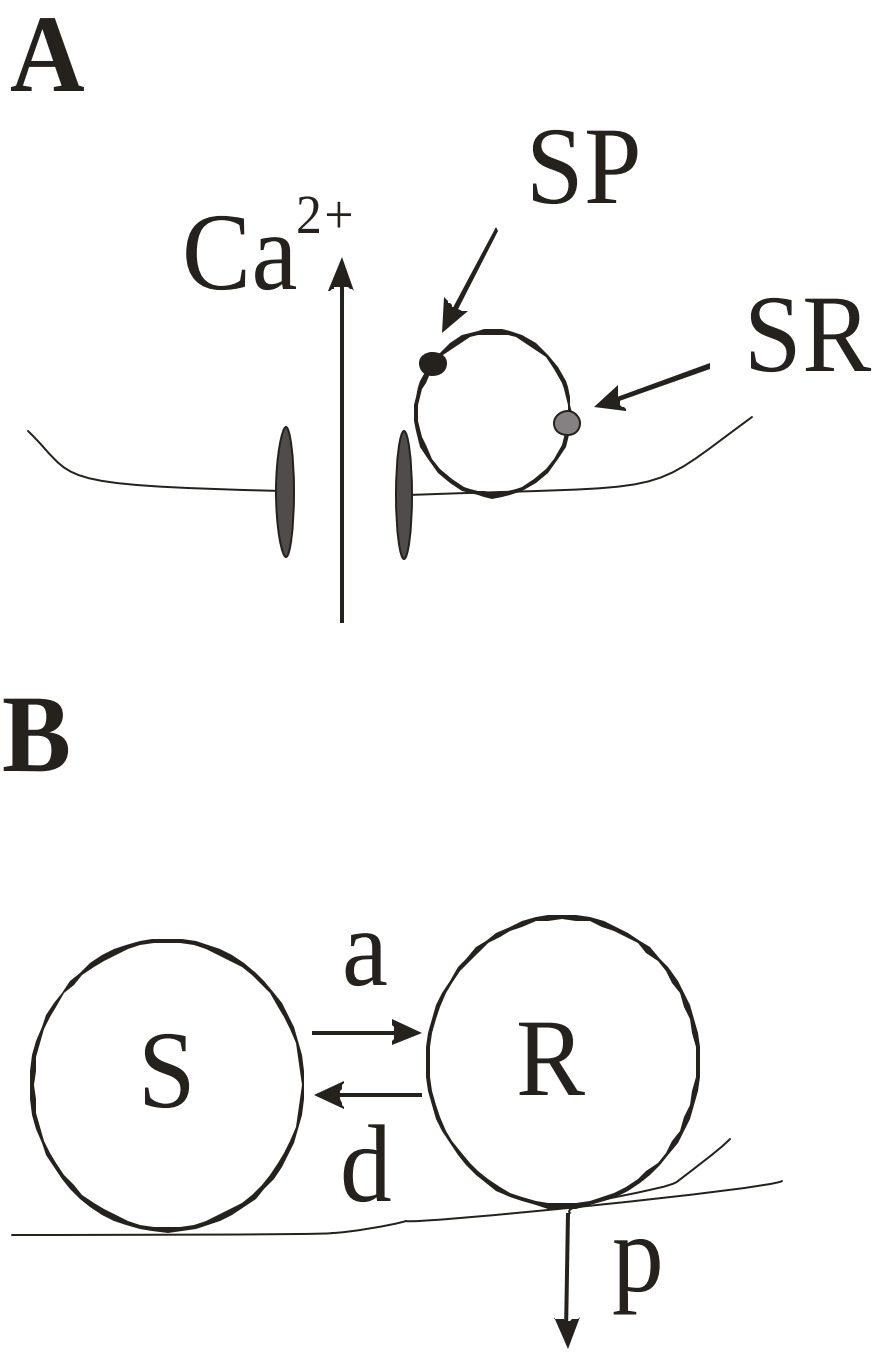


Figure 3

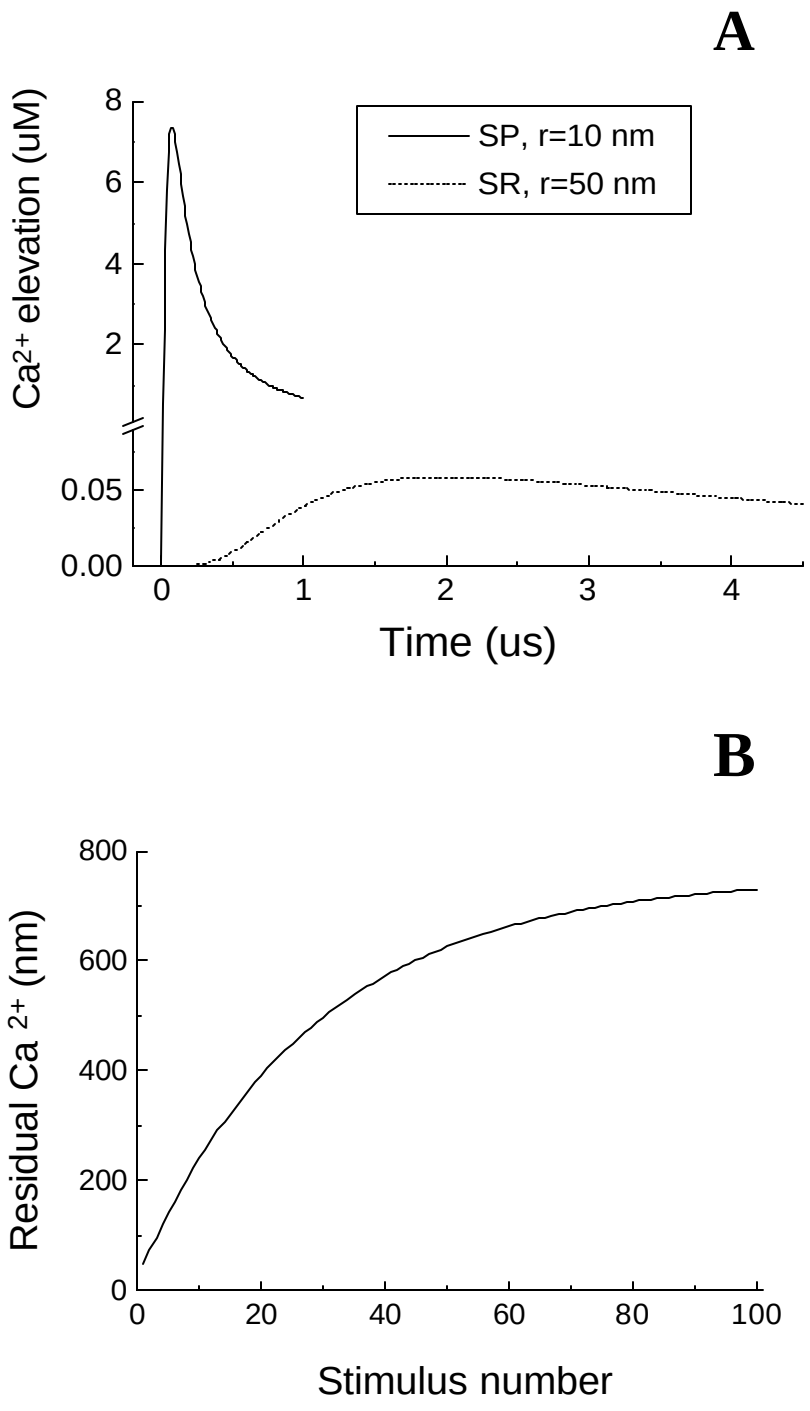
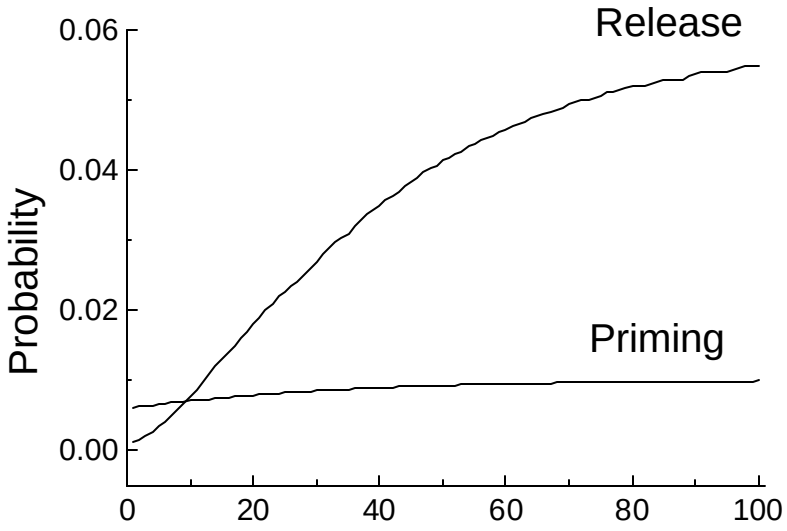
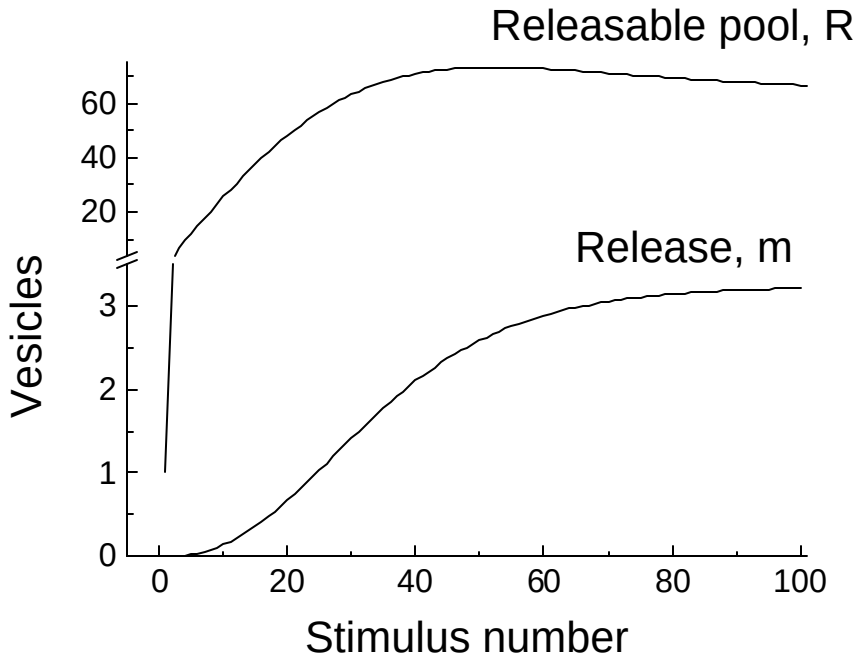

A
SP, r=10 nm
SR, r=50 nm
Ca2+ elevation (uM)
8
6
4
2
0.05
0.00
0
1
2
3
4
Time (us)
B
Residual Ca 2+ (nm)
800
600
400
200
0
0
20
40
60
80
100
Stimulus number

Figure 4


Release
Priming
Probability
0.06
0.04
0.02
0.00
0
20
40
60
80
100


Releasable pool, R
Release, m
Vesicles
60
40
20
3
2
1
0
0
20
40
60
80
100
Stimulus number