DYNAMICS OF NEUROMUSCULAR TRANSMISSION REPRODUCED BY CALCIUM-DEPENDENT SERIAL TRANSITIONS IN THE VESICLE FUSION COMPLEX

Alejandro Martínez-Valencia^{1,3}, Guillermo Ramírez-Santiago² and
Francisco F. De-Miguel³

¹ Posgrado en Ciencias Físicas, Universidad Nacional Autónoma de México,
Universidad Nacional Autónoma de México. C.P. 04510, Ciudad de México.

² Instituto de Matemáticas, Universidad Nacional Autónoma de México, Juriquilla
Querétaro, C.P. 76230, México.

³ Instituto de Fisiología Celular-Neurociencias, Universidad Nacional Autónoma de
México. C.P. 04510, Ciudad de México.

Corresponding Author:
Francisco F. De- Miguel
Instituto de Fisiología Celular-Neurociencias,
Universidad Nacional Autónoma de México,
Circuito Exterior, Ciudad Universitaria,
04510 México D.F., México.
e-mail: ffernand@ifc.unam.mx

Keywords: Transmitter release, quantal release, neuromuscular synapse,
acetylcholine, facilitation, depression, spontaneous release, synapse, fusion
complex, kinetics, calcium.

Abstract:

Neuromuscular transmission, from spontaneous release to facilitation and depression was accurately reproduced by a mechanistic kinetic model of sequential maturation transitions in the molecular fusion complex. The model incorporates three predictions. First, sequential calcium-dependent forward transitions take vesicles from docked to pre-primed to primed states, followed by fusion. Second, pre-priming and priming are reversible. Third, fusion and recycling are unidirectional. The model was fed with experimental data from previous studies while the backward (β) and recycling (ρ) rate constant values were fitted. Classical experiments were successfully reproduced when every forward (α) rate constant had the same value, and both backward rate constants were 50-100 times larger. Such disproportion originated an abruptly decreasing gradient of resting vesicles from docked to primed states. Simulations also predict that: i. Spontaneous release reflects primed to fusion spontaneous transitions. ii. Calcium elevations synchronize the series of forward transitions that lead to fusion. iii Facilitation reflects a transient increase of priming following calcium-dependent transitions. iv. Backward transitions and recycling restore the resting state. v. Depression reflects backward transitions and slow recycling after intense release. Such finely-tuned kinetics offers a mechanism for collective non-linear transitional adaptations of a homogeneous vesicle pool to an ever-changing pattern of electrical activity.

Introduction

In the present study we searched for a unifying molecular mechanism by which neuromuscular transmission adapts dynamically to the ongoing pattern of electrical activity. Four aspects of transmission were analyzed in detail. i. Spontaneous release at rest (Fatt and Katz, 1952); ii. Calcium-dependent evoked release on an impulse (Katz and Miledi, 1979), iii. Facilitation, namely a non-linear increase of release upon rapid subsequent stimulation (Feng, 1940; Eccles, 1941; Liley and North, 1953; del Castillo and Katz, 1954b; Katz and Miledi, 1968), and iv.

Depression, namely a reduction of release on stimulation at extended intervals under high release probability (Eccles, Katz, Kuffler, 1941; Lundberg and Quilish, 1953; Del Castillo and Katz, 1954b; Betz, 1970).

Understanding release requires a collective analysis of the events regulating vesicle fusion. An essential study by del Castillo and Katz (1956) showed that release may occur from any region of presynaptic terminals. It is also well-accepted that vesicle fusion requires a mature also called “primed” molecular assembly established by each vesicle with the plasma membrane (for review see Sudhof, 2013). Maturation of the fusion complex follows a stereotyped sequence of molecular transitions that can be resumed as follows: i. Docking (*D*) is the early tethering of vesicles with the plasma membrane upon establishment of boundaries between vesicle, membrane and soluble proteins; ii. Pre-priming (*pP*) occurs upon stabilization of the molecular complex; iii. Priming (*P*) occurs when vesicles become competent for fusion. Fusion (*F*) is evoked by calcium activation of the primed complex, mediated by the vesicle protein synaptotagmin. After fusion, vesicles are recycled to a new docked state ($F \rightarrow D$) (del Castillo and Katz, 1956; Heuser and Reese, 1973; Betz and Angleson, 1998; Dulubova et al., 2005; Andrews-Zwilling et al., 2006; Kittel et al., 2006; Sudhof, 2013; Weimer et al., 2006; Imig et al., 2014; Gan and Watanabe, 2018). That only a small (~3%) fraction of the vesicle pool fuses on an impulse (Fatt and Katz, 1952, Katz and Miledi, 1979) has suggested that most vesicles rest in immature docked or pre-primed states.

Based on the stereotyped molecular transitions that render a primed vesicle and on the calcium-dependence of some such transitions (Neher and Sakaba, 2008; Burgoyne, 2007; Craxton, 2010; Corbalan-Garcia and Gómez-Fernández, 2014; Burgoyne et al., 2019), we put forward the hypothesis schematized in Figure 1, according to which the dynamic adaptations in the number of vesicles that fuse upon variations in nerve activity express a calcium-dependent, collective and reversible maturation of the fusion complex.

Our hypothesis considers that the $D \rightleftharpoons P \rightleftharpoons P$ transitions are bidirectional, with characteristic forward (α) and backward (β) rate constants. The α values are similar for all transitions; both β values are also similar but different from α . Reversibility is supported from the continuous docking and undocking of vesicles in ribbon synapses (Zenisek et al. 2000) and from experiments and modeling of pre-primed to primed transitions in crayfish neuromuscular junction (Pan and Zucker, 2009). On an action potential, calcium evokes fusion and promotes further maturation of fusion complexes. Rapid arrival of a subsequent impulse evokes facilitation. Backward transitions gradually reduce facilitation and return vesicles to resting levels. After intensive release, slow vesicle recycling new docked state plus the reversible transitions produce depression.

The experimental exploration of our hypothesis exceeds the current technical possibilities. However, mathematical modeling provides a solid alternative (Varela et al. 1997; Dittman et al. 2000; Shahrezaei, et al., 2006; Pan and Zucker, 2009; Dittrich et al., 2013; Herman and Rosemund, 2015; Neher, 2015). Here we used a master equation based on the Gillespie (1976) stochastic algorithm to simulate the sequence of maturation transitions shown in Figure 1. Each vesicle with its fusion complex is a unit of a large homogeneous pool that responds collectively to each presynaptic impulse. The model was fed with experimental data from the literature. Undetermined parameters were fitted for convincing reproduction of well-known experiments of neuromuscular transmission in frog or cat. The code used for the simulations in this study is freely available in: <https://github.com/alexini-mv/kinetic-neurotransmission>

Results

Spontaneous and evoked presynaptic vesicle fusion were accurately reproduced by a theoretical sequence of four maturation kinetic states of the vesicle fusion complex, provided that forward transitions had the same α value and were calcium-

dependent, while the backward transitions had a β value 50-100 times larger than α . A three-state model failed to reproduce transmission. By contrast, five or six sequential kinetic steps reproduced every form of release provided a proportional increase in α and reduction in β . The parameters that reproduced cat and frog neuromuscular transmission are resumed in Table 1.

Preparation	Kinetic transitions	α (s ⁻¹)	β (s ⁻¹)	λ (β/α)	ρ (s ⁻¹)
Cat	4	0.62*	62	100	1.0
Frog	4	0.3	15.0	50	1.0
Frog	5	0.62	13.0	21	1.0
Frog	6	1.43	9.5	13	1.0

Table 1. Kinetic parameters that reproduce neuromuscular transmission in frog and cat. * from Boyd and Martin, 1956a.

Spontaneous quantal release

The spontaneous quantal release in cat presynaptic neuromuscular junction reported by Boyd and Martin (1956a), was fairly reproduced by our model fed with an $\alpha = 0.62$ s⁻¹ value, obtained as the inverse of the experimental 1.61 s time constant (τ) of the time interval distribution of miniature end plate potentials (mepp_s). An unexpectedly large $\beta = 100\alpha$ ($\lambda = \beta/\alpha = 100$ coefficient) and a $\rho = 1.0$ s⁻¹ recycling rate constant produced 148 ± 2 mepp_s at 1.40 ± 0.10 s⁻¹ frequency (n=250 simulations), quite similar to the 143 mepp_s recorded at a 1.43 ± 0.88 s⁻¹ in the original paper (Figure 2A). The experimental distribution of the intervals between mepp_s was fitted by the function $n = n_T (\Delta t / \langle t \rangle) e^{-t/\langle t \rangle}$ (Fatt and Katz, 1952), where n_T is the number of quanta released and $\Delta t = 0.5$ s is the bin size.

The mepp_s frequency (Figure 2B) was proportional to α and inversely proportional to β . Our best explanation to this result is that the large β value reduces the pool of primed vesicles, therefore, the probability of spontaneous fusion. Figure 2C

compares simulations of cat and frog spontaneous release. The $\beta = 62 \text{ s}^{-1}$ ($\lambda=100$) that reproduced the 1.43 s^{-1} meep frequency in cat recordings at 37°C (Boyd and Martin 1956a) quadruples the $\beta = 15 \text{ s}^{-1}$ ($\lambda= 50$) coefficient that reproduced the 2.5 s^{-1} meep frequency commonly recorded from frog synapses at 20°C (see Fatt and Katz 1952). The larger rate constant values in mammalian neuromuscular synapses may reflect their characteristic higher physiological temperature.

The previous results may be explained in the following way. First, spontaneous release reflects the spontaneous $P \rightarrow F$ transitions; second, the small probability of spontaneous release depends on the large λ coefficient, which maintains a small pool of primed vesicles. Since a majority of experimental evidence used here proceeds from experiments in frog, the simulations that follow used the $\alpha=0.3$ and $\beta=50$ values, unless otherwise indicated.

Kinetic steps contributing to spontaneous release

Figure 2D and E shows that a three-step version of the model failed to reproduce spontaneous release. In addition, data in Figure 2D predicts that each kinetic step reduces the α -dependence of spontaneous release by two logarithmic units. By contrast, a five-step version of the model reproduced spontaneous release provided an increase of α (Figure 2D) and a reduction of β (Figure 2E). This means that a four-step $D \rightleftharpoons pP \rightleftharpoons P \rightarrow F \rightarrow D$ transition cycle is necessary and sufficient to explain spontaneous release.

Number of necessary kinetic steps for spontaneous release

Figure 2D and E shows that a three-step version of the model failed to reproduce spontaneous release. In addition, data in Figure 2D predicts that each kinetic step reduces the α -dependence of spontaneous release by two logarithmic units. By contrast, a five-step version of the model reproduced spontaneous release provided an increase of α (Figure 2D) and a reduction of β (Figure 2E). This means that a four-step $D \rightleftharpoons pP \rightleftharpoons P \rightarrow F \rightarrow D$ transition cycle is necessary and sufficient to explain spontaneous release.

187

188 ***Calcium and evoked release***

189 A useful experimental strategy to study statistical fluctuations of quantal release
190 consists of reducing the extracellular calcium concentration and adding
191 extracellular magnesium (Del Castillo and Katz, 1954a; Boyd and Martin, 1955).
192 Under such conditions, the number of quanta released by a presynaptic impulse is
193 drastically reduced and can be predicted precisely by the Poisson distribution (Del
194 Castillo and Katz, 1954a; Boyd and Martin, 1955). The theory states that the probability
195 “ p ” of releasing “ x ” number of quanta ($x=0, 1, 2, 3, \dots, n$) in a series of trials is low,
196 while the number “ n ” of vesicles in the pool remains large. Even when p and n are
197 experimentally elusive, the product $m=pn$, which is the average number of quanta
198 released per impulse is measurable from the recordings and provides a direct
199 means for the calculations.

200 To reproduce such experimental observations, impulses were coupled to a “calcium
201 elevation” whose amplitude and duration was adjusted to evoke release of small numbers
202 of quanta (see methods). The hypothesis that nerve impulses induce forward transitions in
203 each maturation was tested by coupling the calcium transient to every α rate constant.
204 Based the observation by Katz and Miledi (1968 and 1979) that the amount of release
205 increases with the duration of depolarization, i.e., with the duration and amount of calcium
206 entry, we adjusted the decay time (τ_e) of the artificial calcium signal as a way to control the
207 amount of release. With such approximation the m value increased in proportion to τ_e . The
208 simulations in Figure 3A reproduce the experimental calcium-dependence according to the
209 equation by Dodge and Rahamimoff (1966; see also Smith et al., 1985; Augustine and
210 Charlton, 1986) expressing third ($R^2=0.999$) or fourth order ($R^2=0.998$) cooperativities.

211 Another series of trials simulated calcium-dependent release under high release
212 probability by using either, a long τ_e value or a large calcium transient amplitude.
213 The normalized number of quanta as a function of the normalized time (t/τ_e) in
214 Figure 3B displays a similar trend for different τ_e and amplitude values. The
215 combined effects of the amplitude and duration of the calcium transient in Figure
216 3B can be represented empirically by a sum of two exponential decays based on
217 the hypothesis that kinetic transitions are represented by exponential equations in

the form $m/m_{max} = (1 + A)e^{-t/\tau_e} - Ae^{-t/x\tau_e}$. The detachment from a single exponential distribution originates from the second exponential component of the Equation, in which x expresses the combined contribution of α (Figure 3C) and the number of kinetic steps in the model (Figure 3D). Figure 3D shows that each step in the kinetic chain delays release presumably due to recruitment of vesicle fusion from every kinetic state. The lack of effect of β and ρ is attributed to the recovery of the vesicle pool between subsequent stimulation pulses.

Evoked quantal release under low probability.

Our model reproduced quantal release under low release probability in frog neuromuscular junction (Del Castillo and Katz, 1954a). Brief 0.05 to 0.15 ms τ_e values produced mepp_s amplitude distributions with two ($\tau_e = 0.05$ ms) to five ($\tau_e = 0.15$ ms) amplitude classes including failures (Figure 4A). The Poisson equation reproduced such distributions when $\tau_e \leq 0.5$ (Pearson $\chi^2 > 0.05$ coefficients). Larger τ_e values produced a reduction in the number of failures and an increase in the number of classes in the distribution. τ_e values longer than 0.25 produced amplitude distributions with marked deviations from the Poisson predictions (Figure 4B and C), as in experimental observations made under higher release probability (Boyd and Martin, 1956b).

Role of the backward rate constant on the release probability

Simulations of frog experiments made under low probability conditions (Del Castillo and Katz, 1954a; Katz and Miledi, 1968) allowed a further analysis on the contribution of β to quantal release. The λ coefficients of the $D \rightleftharpoons Pp$ (λ_1) and $Pp \rightleftharpoons P$ (λ_2) transitions were varied independently, while the $\alpha=0.3$ s⁻¹, $\rho = 1.0$ and $\tau_e = 0.15$ ms remained fixed. The $\lambda_1=\lambda_2=50$ values reproduced transmission, as seen in the central chart of Figure 5.

The λ_1 value markedly influenced the number of quanta discharged per impulse. A small $\lambda_1=5$ ($\beta = 5\alpha$; top panels in Figure 5) that decelerates the $D \leftarrow Pp$ transition extended the range of classes in the distribution, which deviated from the predictions of the Poisson equation ($p \leq 0.05$). Even the largest $\lambda_2=500$ tested failed to compensate for the affection caused a reduced λ_1 . By contrast, a large $\lambda_1=500$ value constrained the amplitude meep distribution to a small-class range that was predicted by the Poisson distribution regardless of the λ_2 value (bottom plots in Figure 5). As will be seen below, this result only applies to release under single impulses, for the large $\lambda_1=500$ values failed to reproduce short-term plasticity. In spite of that, these results underscore the essential contribution of the backward $D \leftarrow Pp$ transition to maintain a small resting pool of primed vesicles.

Facilitation and depression.

Stimulation under experimental high release probability, for example by blocking acetylcholine receptors with curare (del Castillo and Katz, 1956; Betz, 1972), produces facilitation to turn into depression after a while, presumably because of depletion of the releasable vesicle pool (Otsuka et al., 1962; Mallart and Martin, 1968; Betz, 1970). In this section we reproduced the experimental transition from facilitation to depression in frog neuromuscular junction upon a conditioning train of three impulses followed by a test pulse applied 250 ms later, as in Mallart and Martin (1968). A $\tau_e=1.3$ ms reproduced the high release probability. The number of quanta, which is hard to estimate from experimental records, was predicted here by the model. An alternative approximation can be obtained from the relationship between the number of quanta and the τ_e value in Figure 3.

Relevant to our study is that the same parameters that reproduce spontaneous and evoked release also reproduced the facilitation-depression balance. In addition, our simulations reproduced the asynchronous release that follows the bulk of evoked release on electric impulses in frog (Figure 6; Miledi, 1966) and other peripheral

and central synapses (Zengel et al., 1980; Goda and Stevens, 1994; Atluri and Regehr, 1998; Hui et al., 2005; Best and Regehr, 2009).

The balance from facilitation to depression

How the sequence of transitions affects the balance from facilitation and depression in frog was analyzed with an alternative protocol used by Betz (1970). In this case, experiments were made with high extracellular calcium concentration to enhance depression and curare was added to block acetylcholine receptors and produce subthreshold transmission. Such conditions were reproduced here by $\tau_e = 1.5$ ms (Figure 7). The rest were our conventional parameters for frog neuromuscular transmission. A briefer $\tau_e = 0.5$ ms increased facilitation and abolished depression while a longer $\tau_e = 2.0$ ms eliminated facilitation while depression remained.

Effects of λ on short-term plasticity.

Contrary to the dominant effect of λ_1 on low probability release, the λ_2 coefficient dominated facilitation (Figure 7B). A small $\lambda_2 = 5$, which decelerates vesicle return to previous states elongated facilitation by 450%, from 90 to 500 ms, without affecting its peak amplitude. However, large $\lambda_1 = \lambda_2 = 500$ values reduced and shortened facilitation (arrowheads in Figure 7B and C). Increasing or decreasing any λ coefficient reduced depression without affecting its time course (Figure 7B and C).

Effect of vesicle recycling on short-term plasticity

It has long been hypothesized that depression occurs when the releasable vesicle pool is reduced by large release and slow recycling dynamics (Elmqvist and Quastel, 1965; Kusano and Landau, 1975). The mild effects of λ on depression in our simulations support such hypothesis. Figure 8 shows that a ten-fold acceleration of the mean recycling time ($\rho = 10$ s⁻¹) while keeping $\lambda_1 = \lambda_2 = 50$, increased the amplitude and duration of facilitation while depression was

eliminated. Facilitation decayed biexponentially with a rapid $\tau_1=30.19 \pm 2.56$ ms followed by a slower $\tau_2=169.55 \pm 23.1$ ms ($R^2=0.997$). Conversely, a ten-fold reduction of ρ to slow down vesicle recycling had no effect on facilitation but increased depression from the $N_{\text{test}}/N_{\text{Icond}}=0.6$ in the experimental data to a 0.25 sustained value by 450 ms.

Effect of the number of kinetic steps on short-term plasticity.

The three-step model fed with the regular parameters of frog experiments failed to reproduce facilitation but maintained depression levels similar to those already described (Figure 9A). By contrast, a five-step kinetic model by addition of a D state reproduced short-term plasticity provided a larger $\alpha = 0.62 \text{ s}^{-1}$ (as in mammalian neuromuscular junction), and a reduced $\lambda = 21$, accounting for a $\beta = 13 \text{ s}^{-1}$ value. Depression was less susceptible to the λ variations. A six-step model also reproduced the experimental data provided an even larger $\alpha = 1.43 \text{ s}^{-1}$ and smaller $\lambda = 13$, reflecting $\beta = 9.5 \text{ s}^{-1}$. These results support that the four-state kinetic sequence fed with one common set of parameters is necessary and sufficient to reproduce the dynamics of release from spontaneous to short-term plasticity.

Activity-dependent dynamics of the vesicle pool

The plastic dynamics of transmission in response to a conditioning train followed by a test pulse were graphically plotted in Figure 10 (Figure 5; Mallart and Martin 1968). The fraction of vesicles in each state is normalized to $N_0=10,000$. At rest, ~98% vesicles are docked, the remaining being decreasingly distributed in pre-primed and primed states. About 300 vesicles (3%) fuse in response to the first impulse, as estimated by Katz and Miledi (1979) at 6°C. About 66% of the vesicles that fuse were primed, while the other 34% fuse upon rapid series transitions from immature states. The remaining vesicles become accommodated reversibly in immature states. Sudden arrival of a second impulse encounters an increased population of pre-primed and primed vesicles, thus evoking facilitation plus further forward transitions. After the third conditioning pulse, about 25% of the

vesicle pool has fused. Such large release along with the slow recycling (F/N_0 panel in Figure 10) reduce the vesicle pool and produce a depressed response to the test impulse (Figure 10).

Discussion

Spontaneous release, evoked release and short-term plasticity, were reproduced here by a mathematical model of vesicles bound to a dynamic molecular fusion complex with four kinetic states. Our model provides a unifying mechanistic interpretation to the adjustable activity-dependent forms of release in a homogeneous vesicle pool. The backward rate constant and the much smaller forward rate constant values concentrate a vast majority of vesicles in docked state. Spontaneous and asynchronous fusion reflect spontaneous $P \rightarrow F$ occurrence in a small pool of primed vesicles. The dynamics of evoked release are influenced by electrical activity, which determines the momentary proportion of vesicles in the different maturation states. We also predict that the duration of facilitation depends largely on the backward transitions, with a contribution of the recycling time constant, whereas the duration of depression depends on the slow vesicle recycling.

Functional advantage of the similar rate constant values

The accurate dynamics of transmission described here rely on collective transitions that result from the similar rate constants in each direction. Such similarity has been shown for the forward rate constants (Li et al., 2007; Chapman, 2008; Sudhof and Rothman, 2009). The synchronous collective transitions in either direction prevent the large accumulations of pre-primed and primed vesicles for long periods that occur when $\lambda_1 \neq \lambda_2$. Such observation implies that the pP state is an essential buffer for adaptation of the vesicle pool of primed vesicles to the dynamics of motoneuron electrical activity. It was also shown that evoked release occurs from vesicles that were primed at the time of the impulse arrival with addition of vesicles reacting from immature states.

368

369 **Timing of facilitation and depression**

370 The balance between the forward and backward transitions explains the timing of
 371 the non-linear fluctuations in the quantal output during facilitation and depression.
 372 The sequential transitions of the fusion complex produces that the pool of primed
 373 vesicles increases largely after the bulk of synchronous exocytosis. Therefore
 374 here, facilitation is due to the calcium-dependence delayed increase of pre-primed
 375 and primed vesicles on an impulse. Such delayed recruitment of previously
 376 immature vesicles is confirmed by the increased latency for fusion upon addition of
 377 a docked state to the model. A corollary to this observation is that the amount of
 378 facilitation relies on the balance between the duration of the calcium signal and the
 379 velocity of the forward transitions. Faster forward transitions would increase
 380 synchronous release and prevent facilitation. Slower forward transitions would
 381 produce small amounts of evoked release and rapid uncoupling of motoneuron and
 382 muscle responses.

383

384 **Relationship between facilitation and asynchronous release**

385 Previous observations have suggested that facilitation and asynchronous
 386 neuromuscular release rely on the exact same mechanism (Rahamimoff and Yaari,
 387 1973; Zucker, 1996). Our data suggest that such mechanism is the spontaneous
 388 fusion of primed vesicles. However, the dynamics of asynchronous release reflect
 389 the reversible activity-dependent maturation of vesicles from previous states.

390 **Recycling and depression**

391 Our results confirm the essential role of vesicle recycling on depression and predict
 392 that backward transitions contribute to the amplitude of depression. Two or more
 393 recycling modes in the neuromuscular junction (Rizzoli and Betz, 2005) and central
 394 synapses (Wu and Borst, 1999; Sakaba and Neher, 2001) suggest equal numbers
 395 of recycling vesicle pools (for review see Alabi and Tsien 2012). However, with a

single recycling rate constant, our model reproduced convincingly the balance between facilitation and depression as studied by Betz (1970). However, we cannot exclude that the slow time constant of recycling in our model is masking faster events including some displaying a calcium-dependence (Sakaba and Neher, 2001).

A single type of calcium sensor for fusion

Our data are consistent with one type of calcium sensor producing all forms of release, in contrast to the proposed contribution of different forms of synaptotagmin controlling release in central synapses (Volynski and Krishnakumar, 2018; Sudhof, 2013; Keser and Regher, 2014; Kavali, 2015). Central synaptic vesicles seem to carry different types of synaptotagmin (Jahn and Sudhoff, 1994; Takamori et al., 2006), and fast synchronous release is produced by synaptotagmin 1, 2 or 9 (For review see Kaeser and Regehr, 2014) asynchronous release depends on the high calcium affinity synaptotagmin 7 (Bacaj et al., 2013, 2015; Turecek and Regehr, 2018). Accordingly, theoretical models of transmission considering two or three calcium sensors reproduce the electrophysiological data (Dutta Roy et al., 2014; Goda and Stevens, 1998). Although neuromuscular terminals of the genetically-accessible *Drosophila*, zebrafish and mice express synaptotagmins 1 and 2 (Pang et al., 2006), careful experiments in *Drosophila* neuromuscular junction have shown that synaptotagmin 1 mutants cannot evoke fast release (Shields et al., 2020), supporting that synaptotagmin 2 is functionally enough for fast fusion, in consistency with our results.

Calcium-sensors for early transitions.

In addition to the catalyzing role of calcium on vesicle fusion, we predict that calcium also promotes the $D \rightarrow pP \rightarrow P$ transitions. A natural question emerging from such hypothesis is if the same calcium sensor producing fusion may also

produce the previous transitions. The residual calcium hypothesis for paired pulse facilitation by Katz and Miledi (1968) and the third or fourth order calcium-dependence of release (Dodge and Rahamimoff, 1966; Smith et al., 1985; Augustine and Charlton, 1986) predict that the low residual calcium levels activate high-affinity calcium sensors to produce the supralinear vesicle fusion in facilitation (Zucker and Lara Estrella, 1983; Yamada and Zucker, 1992; Van der Kloot and Molgó, 1993; Vyshedskiy and Lin, 1997; Regehr and Zucker, 2002; Ma et al., 2014). Such high affinity sensor must be other than the low affinity synaptotagmins 1, 2 or 9 (For review see Kaeser and Regehr, 2014). Synaptotagmin 7 has emerged again as a candidate in central synapses (Sugita et al., 2002; Pan and Zucker, 2009; Wen et al., 2010; Bacaj et al., 2013, 2015; Jackman and Regehr, 2014; Li et al., 2017; Turecek and Regehr, 2018). However, the possibility of a single sensor producing all serial transitions from pre-priming to fusion is feasible in neuromuscular synapses by considering that electron tomography experiments have shown that from the moment of docking, the fusion complex forms intimate boundaries with calcium channels (Harlow et al., 2001; Nagwaney et al., 2009; Szule et al., 2012). Such configuration may permit a single calcium sensor to catalyze every transition of the chain, as opposed to central synapses in which calcium channels may be separated from the fusion complex at early maturation stages (Neher, 2015).

Methods

Design of the mathematical model

The four-state kinetic model with six kinetic transitions shown in Figure 1 set the bases to analyze the collective behavior of a pool of 10,000 identical vesicles (Rizzoli and Betz, 2005). The six transitions R_j , correspond to those in Figure 1, with j being a stochastic discrete variable that takes the values $j=1, 2, \dots, 6$, that correspond to each transition. Each transition occurs with an equal probability $a_j(x)$. The term $a_j(x)dt$ is the probability that an R_j transition will occur in an

infinitesimal time interval $t+dt$, when the system is in a state $X(t)=(D(t), pP(t), P(t), F(t))=x$. Each R_j transition is characterized by two quantities: One is the system state $x = D(t), pP(t), P(t), F(t)$, which reflects the number of vesicles at each kinetic state; the second quantity is the vector $V_j(v_{D_j}, v_{pP_j}, v_{P_j}, v_{F_j})$, which represents the change in the total number of vesicles over time at each state. At rest, a vast majority of vesicles lay in the D state. The effect of larger numbers of molecular states on transmission was analyzed by adding states with their corresponding bidirectional rate constants, between the D and pP states. For a three-state possibility the pP state was eliminated from the model.

The stochastic kinetic model considers that fusion requires vesicles to arrive at the P state. Since the classical kinetic differential equations do not describe correctly the collective kinetics of a small number of vesicles ($\sim 10,000$ as compared to Avogadro's number), we used instead the master Equation (1) for the probability distribution $P(x, t; x_0, t_0)$ (Gillespie, 1976), whose solution describes the temporal evolution of the six transitions between kinetic states. The rate constants are conventional probabilities per time unit (Gillespie, 1992):

$$\frac{\partial P(x, t; x_0, t_0)}{\partial t} = \sum_{j=1}^6 \left(a_j(x - v_j) P(x - v_j, t | x_0, t_0) - a_j(x) P(x, t | x_0, t_0) \right) \quad (1)$$

Because calculating an analytical solution of Equation 1 is extremely difficult, the solution of Equation 1 was obtained using the Gillespie algorithm (Gillespie, 1976), which emulates random transitions connecting different $X(t)$ states. The fundamental equation of the Gillespie algorithm for the time evolution of the system is:

$$p(j, \tau) d\tau = a_j(x) \exp \left[- \sum_{j=1}^6 a_j(x) \tau \right] d\tau \quad (2)$$

Equation 2 predicts the probability that at a state $X(t) = x$, the next kinetic transition R_j , will occur at the next infinitesimal time $[t + \tau, t + \tau + d\tau]$. The random

continuous variable τ advances the time in the simulations by the amount:

$$\tau = - \left[\frac{1}{\sum_{j=1}^6 a_j(x)} \right] \ln(r1) \quad (3)$$

with $r1$ being a random number distributed uniformly in the interval (0, 1).

The probability distribution $p(j, \tau)d\tau$ mimics the solution of the stochastic kinetic Equation 1 and plays a key role in the implementation of the stochastic algorithm. Thus, the random trajectories that connect different kinetic states, $X(t) = x$, describe the kinetic evolution of the vesicle pool.

The algorithm for the kinetic sequence can now be summarized as follows: (i) The simulation begins by setting the initial state of the system X_o at time t_o . (ii) The propensity functions $a_j(x)$ and their sum $a_o(x) = \sum a_j(x)$ are calculated for each different time t . (iii) The values of the discrete random variables j is chosen as the smallest integer that satisfies, $\sum_{k=1}^j a_k(x) > r_2 a_o$ with r_2 a random number distributed uniformly in the interval [0,1], and the continuous random variable τ is generated by applying equation (3). (iv) The transition to the next kinetic state $x \rightarrow x + v_j$ and the time shifts to $t \rightarrow t + \tau$ are calculated. (v) A new state (x, t) is obtained, and the procedure returns to step (i).

The simulation starts with $N_o = 10,000$ vesicles accumulated in the D state. In such conditions $X(t = 0) = X_o = (D(t = 0) = N_o)$, and $pP(t = 0) = 0$, $P(t = 0) = 0$, $F(t = 0) = 0$. As the simulation progresses, the vesicles distribution among the different states becomes stationary in about 5 min. This is the time in which our measurements in the simulations are made.

Estimates of kinetic values

The activation energies involved in the molecular transitions from docking to exocytosis lay in the same order of magnitude (Li et al., 2007; Sudhof and Rothman, 2009). Therefore, we initially considered that $\alpha_1 = \alpha_2 = \alpha_3 = \alpha$, and $\beta_1 = \beta_2 = \beta$.

Such strategy proved successful for reproducing every release mode. The α value used in cat simulations was estimated from the frequency distribution of spontaneous miniature potentials (Boyd and Martin, 1956a, b). The β and ρ values were fitted independently. The model was simplified by using the coefficient $\lambda=\beta/\alpha$, which permitted to evaluate the kinetic behavior in terms of the combination of ρ and the relative magnitudes of α and β . The code used in this study is available in the following repository: <https://github.com/alexini-mv/kinetic-neurotransmission>

Modeling the calcium-dependence

Presynaptic calcium elevations upon brief depolarizations were modeled by adding a function $f(t)$ to the forward rate constants, which acquired the form $\alpha_s = \alpha + f(t)$. The kinetics of the calcium current decay in squid giant synapse experiments (Llinas et al., 1981a and b) served as baseline. The onset of the calcium transient was considered as instantaneous for the calcium channels in presynaptic neuromuscular terminals are tightly bound to the fusion complex (Harlow et al., 2001; Nagwaney et al, 2009). Adjustments in the amplitude (in arbitrary units) and decay time (ms) of the artificial calcium elevation rendered successful results.

For our simulations it was more convenient to express the decay time τ_e of the calcium elevation instead of the decay time of the current, since according to the residual calcium hypothesis (Katz and Miledi, 1968; Kamiya and Zucker, 1994; Matveev et al., 2006), it is the remnant free intracellular calcium after the impulse what promotes facilitation. The decay time of the calcium elevation was defined as:

$$f(t) = \begin{cases} 0 & \text{if } t < t_s \\ I_e \exp\left(-\frac{t-t_s}{\tau_e}\right) & \text{if } t \geq t_s \end{cases} \quad (4)$$

where t_s is the stimulation time. The τ_e value was adjusted for each experimental protocol in a range of 0.05 - 1 ms. Once adjusted, the parameters of the calcium signal remained the same for each experiment. Calcium currents in certain central

synapses may facilitate or depress upon subsequent stimulation (Borst and Sakmann, 1998; Forsythe et al., 1998; Cuttle et al., 1998; Inchauspe et al., 2004; Ishikawa et al., 2005; Xu and Wu, 2005; Mochida et al., 2008). However, our model rendered accurate results without any such modulation.

Acknowledgements

We wish to express our gratitude to Mr. Bruno Mendez and to Mrs. Sara Flores González for their excellent laboratory assistance. Our research was funded by a DGAPA-UNAM grant IN200914 and a CONACYT grant 130031 to FFDM and by a DAGAPA-UNAM grants IN118410 and IN108916 to GRS. AMV acknowledges support from CONACYT as a master's degree fellowship at Posgrado en Ciencias Físicas at UNAM.

References

- Alabi AA & Tsien RW (2012). Synaptic vesicle pools and dynamics. Cold Spring Harb Perspect Biol 4, a013680.
- Atluri PP & Regehr WG (1998). Delayed release of neurotransmitter from cerebellar granule cells. J Neurosci 18, 8214–8227.
- Andrews-Zwilling, Y.S., Kawabe, H., Reim, K., Varoqueaux, F., and Brose, N. (2006). Binding to Rab3A-interacting molecule RIM regulates the presynaptic recruitment of Munc13-1 and ubMunc13-2. J. Biol. Chem. 281, 19720–19731.
- Augustine, G. J., & Charlton, M. P. (1986). Calcium dependence of presynaptic calcium current and post-synaptic response at the squid giant synapse. The Journal of physiology, 381, 619–640.
- Bacaj, T., Wu, D., Yang, X., Morishita, W., Zhou, P., Xu, W., Malenka, R. C., & Südhof, T. C. (2013). Synaptotagmin-1 and synaptotagmin-7 trigger synchronous and asynchronous phases of neurotransmitter release. Neuron, 80(4), 947–959.
- Bacaj, T., Wu, D., Burré, J., Malenka, R. C., Liu, X., & Südhof, T. C. (2015). Synaptotagmin-1 and -7 Are Redundantly Essential for Maintaining the Capacity of the Readily-Releasable Pool of Synaptic Vesicles. PLoS biology, 13(10), e1002267.
- Becherer U & Rettig J (2006). Vesicle pools, docking, priming, and release. Cell and Tissue Research 326, 393–407.

Best, A. R., & Regehr, W. G. (2009). Inhibitory regulation of electrically coupled neurons in the inferior olive is mediated by asynchronous release of GABA. *Neuron*, 62(4), 555–565.

Betz WJ (1970). Depression of transmitter release at the neuromuscular junction of the frog. *The Journal of Physiology* 206, 629–644.

Betz, W. J., & Angleson, J. K. (1998). The synaptic vesicle cycle. *Annual review of physiology*, 60, 347–363. <https://doi.org/10.1146/annurev.physiol.60.1.347>

Borst, J. G., & Sakmann, B. (1998). Facilitation of presynaptic calcium currents in the rat brainstem. *The Journal of physiology*, 513 (Pt 1)(Pt 1), 149–155.

Boyd IA & Martin AR (1955). Miniature endplate potentials in isolated mammalian muscle. *J Physiol* 28, 30-1P

Boyd IA & Martin AR (1956a). Spontaneous sub threshold activity at mammalian neuromuscular junctions. *The Journal of Physiology* 132, 61–73.

Boyd IA & Martin AR (1956b). The end-plate potential in mammalian muscle. *The Journal of Physiology* 132, 74–91.

Burgoyne RD. (2007). Neuronal calcium sensor proteins: Generating diversity in neuronal Ca²⁺ signalling. *Nat Rev Neurosci* 8: 182–193.

Burgoyne, RD, Helassa, N., McCue, H. V., & Haynes, L. P. (2019). Calcium Sensors in Neuronal Function and Dysfunction. *Cold Spring Harbor perspectives in biology*, 11(5), a035154.

Catterall WA, Leal K & Nanou E (2013). Calcium channels and short-term synaptic plasticity. *J Biol Chem* 288,10742-10749.

Chapman ER (2002). Synaptotagmin: “A Ca²⁺-sensor that triggers exocytosis?”, *Nature Reviews. Mol Cell Biol* 3, 1–11.

Chapman ER (2008). How Does Synaptotagmin Trigger Neurotransmitter Release? *Annual Review of Biochemistry* 77,615-641.

Corbalán-García, S., & Gómez-Fernández, J. C. (2014). Classical protein kinases C are regulated by concerted interaction with lipids: the importance of phosphatidylinositol-4,5-bisphosphate. *Biophysical reviews*, 6(1), 3–14.

Craxton M. (2010). A manual collection of Syt, Esyt, Rph3a, Rph3al, Doc2, and Dblc2 genes from 46 metazoan genomes--an open access resource for neuroscience and evolutionary biology. *BMC genomics*, 11, 37.

Cuttle, M.F., Tsujimoto, T., Forsythe, I.D., and Takahashi, T. (1998). Facilitation of the presynaptic calcium current at an auditory synapse in rat brainstem. *J. Physiol.* 512, 723–729.

del Castillo J, Katz B (1954a). Quantal components of the end-plate potential. *The Journal of Physiology* 124, 560–573.

del Castillo J, Katz B (1954b). Statistical factors involved in neuromuscular facilitation and depression. *The Journal of Physiology* 124, 574–585.

del Castillo J, Katz B (1956). Localization of active spots within the neuromuscular junction of the frog. *The Journal of physiology*, 132(3), 630–649.
<https://doi.org/10.1113/jphysiol.1956.sp005554>

Delgado R, Maureira C, Oliva C, Kidokoro Y & Labarca P (2000). Size of vesicle pools, rates of mobilization, and recycling at neuromuscular synapses of a *Drosophila* mutant, *shibire*. *Neuron* 28,941–953.

Dittman, J. S., Kreitzer, A. C., & Regehr, W. G. (2000). Interplay between facilitation, depression, and residual calcium at three presynaptic terminals. *Journal of neuroscience* 20(4), 1374–1385.

Dittrich, M., Pattillo, J. M., King, J. D., Cho, S., Stiles, J. R., & Meriney, S. D. (2013). An excess-calcium-binding-site model predicts neurotransmitter release at the neuromuscular junction. *Biophysical journal*, 104(12), 2751–2763.
<https://doi.org/10.1016/j.bpj.2013.05.023>

Dodge FA Jr & Rahamimoff R (1967). Co-operative action of calcium ions in transmitter release at the neuromuscular junction. *J Physiol* 193,419-432.

Dulubova, I., Lou, X.L., Lu, J., Huryeva, I., Alam, A., Schneggenburger, R., Sudhof, T.C., and Rizo, J. (2005). A Munc13/RIM/Rab3 tripartite complex: from priming to plasticity? *EMBO J.* 24, 2839–2850.

Dutta Roy, R., Stefan, M. I., & Rosenmund, C. (2014). Biophysical properties of presynaptic short-term plasticity in hippocampal neurons: insights from electrophysiology, imaging and mechanistic models. *Frontiers in cellular neuroscience*, 8, 141.

Eccles JC, Katz B & Kuffler SW (1941). Nature of the “endplate potential” in curarized muscle. *J Neurophysiol* 4, 362–387.

Elmqvist, D., & Quastel, D. M. (1965). A quantitative study of end-plate potentials in isolated human muscle. *The Journal of physiology*, 178(3), 505–529.

Fatt P & Katz B (1952). Spontaneous subthreshold activity at motor nerve endings. *The Journal of Physiology* 117,109–128.

Feng TP (1940). Studies on the neuromuscular junction. XVIII. The local potentials around n-m junctions induced by single and multiple volleys. *Chin J Physiol* 15, 367–404.

Forsythe, I.D., Tsujimoto, T., Barnes-Davies, M., Cuttle, M.F., and Takahashi, T. (1998). Inactivation of presynaptic calcium current contributes to synaptic depression at a fast central synapse. *Neuron* 20, 797–807.

648 Gan, Q., & Watanabe, S. (2018). Synaptic Vesicle Endocytosis in Different Model
649 Systems. *Frontiers in cellular neuroscience*, 12, 171.
650 <https://doi.org/10.3389/fncel.2018.00171>

651 Gillespie DT (1976). A general method for numerically simulating the stochastic
652 time evolution of coupled chemical reactions. *J Comp Phys* 22, 403 – 434.

653 Gillespie DT (1992). A rigorous derivation of the chemical master equation.
654 *Physica A: Statistical Mechanics and its Applications* 188, 404 – 425.

655 Goda Y & Stevens CF (1994). Two components of transmitter release at a central
656 synapse. *Proc Natl Acad Sci USA* 91, 12942–12946.

657 Harlow, M. L., Ress, D., Stoschek, A., Marshall, R. M., & McMahan, U. J. (2001).
658 The architecture of active zone material at the frog's neuromuscular junction.
659 *Nature*, 409(6819), 479–484. <https://doi.org/10.1038/35054000>
660

661 Hessler, N.A., Shirke, A.M., and Malinow, R. (1993). The probability of transmitter
662 release at a mammalian central synapse. *Nature* 366, 569–572.
663

664 Herman, M. A., & Rosenmund, C. (2015). On the brink: a new synaptic vesicle
665 release model at the calyx of held. *Neuron*, 85(1), 6–8.
666 <https://doi.org/10.1016/j.neuron.2014.12.038>
667

668 Heuser JE & Reese TS (1973). Evidence for recycling of synaptic vesicle
669 membrane during transmitter release at the frog neuromuscular junction. *J Cell Biol*
670 57, 315–344.

671 Hui, E., Bai, J., Wang, P., Sugimori, M., Llinas, R. R., & Chapman, E. R. (2005).
672 Three distinct kinetic groupings of the synaptotagmin family: candidate sensors for
673 rapid and delayed exocytosis. *Proceedings of the National Academy of Sciences of*
674 *the United States of America*, 102(14), 5210–5214.

675 Imig, C., Min, S. W., Krinner, S., Arancillo, M., Rosenmund, C., Südhof, T. C.,
676 Rhee, J., Brose, N., & Cooper, B. H. (2014). The morphological and molecular
677 nature of synaptic vesicle priming at presynaptic active zones. *Neuron*, 84(2), 416–
678 431.

679 Inchauspe, C.G., Martini, F.J., Forsythe, I.D., and Uchitel, O.D. (2004). Functional
680 compensation of P/Q by N-type channels blocks short-term plasticity at the calyx of
681 held presynaptic terminal. *J. Neurosci.* 24, 10379–10383.

682 Ishikawa, T., Kaneko, M., Shin, H.S., and Takahashi, T. (2005). Presynaptic N-type
683 and P/Q-type Ca²⁺ channels mediating synaptic transmission at the calyx of held
684 of mice. *J. Physiol.* 568, 199–209.

685 Jahn, R., & Südhof, T. C. (1994). Synaptic vesicles and exocytosis. *Annual review*
686 *of neuroscience*, 17, 219–246.

687 Jackman, S. L., & Regehr, W. G. (2017). The Mechanisms and Functions of
688 Synaptic Facilitation. *Neuron*, 94(3), 447–464.
689 <https://doi.org/10.1016/j.neuron.2017.02.047>

690 Kaeser PS & Regehr WG (2014). Molecular mechanisms for synchronous,
691 asynchronous, and spontaneous neurotransmitter release. *Annu Rev Physiol* 76,
692 333-363.

693 Kamiya, H., & Zucker, R. S. (1994). Residual Ca²⁺ and short-term synaptic
694 plasticity. *Nature*, 371(6498), 603–606. <https://doi.org/10.1038/371603a0>

695 Katz B & Miledi R (1968). The role of calcium in neuromuscular facilitation". *The*
696 *Journal of Physiology* 195, 481–492.

697 Katz, B., & Miledi, R. (1979). Estimates of Quantal Content during "Chemical
698 Potentiation" of Transmitter Release. *Proceedings of the Royal Society B:*
699 *Biological Sciences*, 205(1160), 369–378.

700 Kavalali ET (2015). The mechanisms and functions of spontaneous
701 neurotransmitter release. *Nat Rev Neurosci* 16, 5-16.

702 Kaeser, P. S., & Regehr, W. G. (2014). Molecular mechanisms for synchronous,
703 asynchronous, and spontaneous neurotransmitter release. *Annual review of*
704 *physiology*, 76, 333–363.

705 Kittel, R. J., Wichmann, C., Rasse, T. M., Fouquet, W., Schmidt, M., Schmid, A.,
706 Wagh, D. A., Pawlu, C., Kellner, R. R., Willig, K. I., Hell, S. W., Buchner, E.,
707 Heckmann, M., & Sigrist, S. J. (2006). Bruchpilot promotes active zone assembly,
708 Ca²⁺ channel clustering, and vesicle release. *Science (New York, N.Y.)*,
709 312(5776), 1051–1054. <https://doi.org/10.1126/science.1126308>

710 Kusano, K., & Landau, E. M. (1975). Depression and recovery of transmission at
711 the squid giant synapse. *The Journal of physiology*, 245(1), 13–32.

712 Li F, Pincet F, Perez E, Eng WS, Melia TJ, Rothman JE & Tareste D (2007).
713 Energetics and dynamics of snarepin folding across lipid bilayers. *Nat Struct Mol*
714 *Biol* 14, 890–896.

715 Liley, AW and North, KAK (1953). An electrical investigation of the effects of
716 repetitive stimulation on mammalian neuromuscular junction. *J Neurophysiol.* 16,
717 509-527.

718 Llinas R, Steinberg IZ & Walton K (1981a). Presynaptic calcium currents in squid
719 giant synapse. *Biophysical Journal* 33, 289–321.

720
721 Llinás R, Steinberg IZ, Walton K. (1981b) Relationship between presynaptic
722 calcium current and postsynaptic potential in squid giant synapse. *Biophys J.*
723 1981;33(3):323-351.
724

725 Lundberg, A., Quilisch, H. (1953). On the effect of calcium on presynaptic
726 potentiation and depression at the neuro-muscular junction. *Acta physiologica*
727 *Scandinavica*. S 111, 121–129.
728

729 Ma, J., Kelly, L., Ingram, J., Price, T. J., Meriney, S. D., & Dittrich, M. (2015). New
730 insights into short-term synaptic facilitation at the frog neuromuscular junction.
731 *Journal of neurophysiology*, 113(1), 71–87. <https://doi.org/10.1152/jn.00198.2014>
732

733 Mallart A & Martin AR (1968). The relation between quantum content and
734 facilitation at the neuromuscular junction of the frog. *The Journal of Physiology*
735 196,593–604.

736 Matveev, V., Bertram, R., & Sherman, A. (2006). Residual bound Ca^{2+} can
737 account for the effects of Ca^{2+} buffers on synaptic facilitation. *Journal of*
738 *neurophysiology*, 96(6), 3389–3397. <https://doi.org/10.1152/jn.00101.2006>

739 Miledi, R. (1966) Strontium as a Substitute for Calcium in the Process of
740 Transmitter Release at the Neuromuscular Junction. *Nature* 212, 1233–1234.

741 Mochida, S., Few, A.P., Scheuer, T., and Catterall, W.A. (2008). Regulation of
742 presynaptic $\text{CaV}2.1$ channels by Ca^{2+} sensor proteins mediates short-term
743 synaptic plasticity. *Neuron* 57, 210–216.

744 Nagwaney S, Harlow ML, Jung JH, Szule JA, Ress D, Xu J, Marshall RM &
745 McMahan UJ (2009). Macromolecular connections of active zone material to
746 docked synaptic vesicles and presynaptic membrane at neuromuscular junctions of
747 mouse. *J Comp Neurol* 513, 457–468.

748 Neher E. (2015) Merits and Limitations of Vesicle Pool Models in View of
749 Heterogeneous Populations of Synaptic Vesicles. *Neuron*. 23;87(6):1131-1142.
750

751 Neher, E., & Sakaba, T. (2008). Multiple roles of calcium ions in the regulation of
752 neurotransmitter release. *Neuron*, 59(6), 861–872.
753

754 Otsuka AM. Endo, HI, Nomura Y. (1962). Presynaptic nature of neuromuscular
755 depression. *Jap J Physiol* 12,573-584.

756 Pan B & Zucker RS (2009). A general model of synaptic transmission and short-
757 term plasticity. *Neuron* 62,539-554.

758 Pang ZP, Melicoff E, Padgett D, Liu Y, Teich AF, Dickey BF, Lin W, Adachi R,
759 Südhof TC. (2006). Synaptotagmin-2 is essential for survival and contributes to
760 Ca^{2+} triggering of neurotransmitter release in central and neuromuscular
761 synapses. *J Neurosci*. 27;26(52):13493-504.

762 Rahamimoff, R., & Yaari, Y. (1973). Delayed release of transmitter at the frog
763 neuromuscular junction. *The Journal of physiology*, 228(1), 241–257.

764 Rizzoli SO & Betz WJ (2005). Synaptic vesicle pools. *Nat Rev Neurosci* 6, 57–69.

765 Rosenmund, C., Clements, J.D., and Westbrook, G.L. (1993). Nonuniform
766 probability of release at a hippocampal synapse. *Science* 262, 754–757.

767 Sakaba T & Neher E (2001). Calmodulin mediates rapid recruitment of fast
768 releasing synaptic vesicles at a calyx-type synapse. *Neuron* 32, 1119-1131.

769 Shahrezaei, V., Cao, A., & Delaney, K. R. (2006). Ca²⁺ from one or two channels
770 controls fusion of a single vesicle at the frog neuromuscular junction. *The Journal*
771 *of neuroscience* 26(51), 13240–13249.

772
773 Schneggenburger R, Meyer AC & Neher E (1999). Released fraction and total size
774 of a pool of immediately available transmitter quanta at a calyx synapse. *Neuron*
775 23,399-409.

776
777 Schneggenburger R, Sakaba T, Neher E. (2002) Vesicle pools and short-
778 term synaptic depression: lessons from a large synapse. *Trends*
779 *Neurosci.* 25(4):206-12.

780
781 Shields, M. C., Bowers, M. R., Kramer, H. L., Fulcer, M. M., Perinet, L. C., Metz, M.
782 J., & Reist, N. E. (2020). The role of the C2A domain of synaptotagmin 1 in
783 asynchronous neurotransmitter release. *PloS one*, 15(5), e0232991.

784
785 Shahrezaei, V., Cao, A., & Delaney, K. R. (2006). Ca²⁺ from One or Two Channels
786 Controls Fusion of a Single V esicle at the Frog Neuromuscular Junction. *Journal*
787 *of Neuroscience*, 26(51), 13240–13249.

788
789 Smith SJ, Augustine GJ, Charlton MP. (1985) Transmission at voltage-clamped
790 giant synapse of the squid: evidence for cooperativity of presynaptic calcium
791 action. *Proc Natl Acad Sci U S A.*;82(2):622-625.

792
793 Sudhof TC (2013). Neurotransmitter release: the last millisecond in the life of a
794 synaptic vesicle. *Neuron* 80, 675- 690.

795
796 Sudhof TC & Rothman JE (2009). Membrane fusion: Grappling with SNARE and
797 SM proteins. *Science* 323, 474–477.

798
799 Sugita, S., Han, W., Butz, S., Liu, X., Fernández-Chacón, R., Lao, Y., & Südhof, T.
800 C. (2001). Synaptotagmin VII as a plasma membrane Ca(2+) sensor in exocytosis.
801 *Neuron*, 30(2), 459–473.

802 Szule JA, Harlow ML, Jung JH, De-Miguel FF, Marshall RM & McMahan UJ
803 (2012). Regulation of synaptic vesicle docking by different classes of
804 macromolecules in active zone material. *PLoS ONE* 7, e33333.

805 Takamori, S., Holt, M., Stenius, K., Lemke, E. A., Grønborg, M., Riedel, D., Urlaub,
806 H., Schenck, S., Brügger, B., Ringler, P., Müller, S. A., Rammner, B., Gräter, F.,
807 Hub, J. S., De Groot, B. L., Mieskes, G., Moriyama, Y., Klingauf, J., Grubmüller, H.,

808 Heuser, J., ... Jahn, R. (2006). Molecular anatomy of a trafficking organelle. *Cell*,
809 127(4), 831–846. <https://doi.org/10.1016/j.cell.2006.10.030>

810 Takeuchi A (1958). The long-lasting depression in neuromuscular transmission of
811 frog. *Jap J Physiol.* 8, 102-113.

812 Turecek J & Regehr WG (2018). Synaptotagmin 7 Mediates Both Facilitation and
813 Asynchronous Release at Granule Cell Synapses. *J Neurosci* 38,3240-3251.

814 Van der Kloot, W., & Molgó, J. (1993). Facilitation and delayed release at about 0
815 degree C at the frog neuromuscular junction: effects of calcium chelators, calcium
816 transport inhibitors, and okadaic acid. *Journal of neurophysiology*, 69(3), 717–729.
817 <https://doi.org/10.1152/jn.1993.69.3.717>

818 Varela, J. A., Sen, K., Gibson, J., Fost, J., Abbott, L. F., & Nelson, S. B. (1997). A
819 quantitative description of short-term plasticity at excitatory synapses in layer 2/3 of
820 rat primary visual cortex. *J Neuroscience* 17(20), 7926–7940.

821 Volynski KE & Krishnakumar SS (2018). Synergistic control of neurotransmitter
822 release by different members of the synaptotagmin family. *Curr Opin Neurobiol*
823 51,154-162.

824 Vyshedskiy, A., & Lin, J. W. (1997). Change of transmitter release kinetics during
825 facilitation revealed by prolonged test pulses at the inhibitor of the crayfish opener
826 muscle. *Journal of neurophysiology*, 78(4), 1791–1799.
827 <https://doi.org/10.1152/jn.1997.78.4.1791>

828 Walmsley, B., Edwards, F.R., and Tracey, D.J. (1988). Nonuniform release
829 probabilities underlie quantal synaptic transmission at a mammalian excitatory
830 central synapse. *J. Neurophysiol.* 60, 889–908.

831 Weimer, R.M., Gracheva, E.O., Meyrignac, O., Miller, K.G., Richmond, J.E.,
832 and Bessereau, J.L. (2006). UNC-13 and UNC-10/Rim localize synaptic vesicles to
833 specific membrane domains. *J. Neurosci.* 31, 8040–8047.
834

835 Wen H, Linhoff MW, McGinley MJ, Li GL, Corson GM, Mandel G, Brehm P. (2010)
836 Distinct roles for two synaptotagmin isoforms in synchronous and asynchronous
837 transmitter release at zebrafish neuromuscular junction. *Proc Natl Acad Sci U S A.*
838 3;107(31):13906-11.

839 Xu, J.H., and Wu, L.G. (2005). The decrease in the presynaptic calcium current is
840 a major cause of short-term depression at a calyx-type synapse. *Neuron* 46, 633–
841 645.

842 Wu, L. G., & Borst, J. G. (1999). The reduced release probability of releasable
843 vesicles during recovery from short-term synaptic depression. *Neuron*, 23(4), 821–
844 832.

845 Yamada, W. M., & Zucker, R. S. (1992). Time course of transmitter release
846 calculated from simulations of a calcium diffusion model. *Biophysical journal*, 61(3),
847 671–682.

848 Zengel JE, Magleby KL, Horn JP, McAfee DA, Yarowsky PJ. (1980) Facilitation,
849 augmentation, and potentiation of synaptic transmission at the superior cervical
850 ganglion of the rabbit. *J Gen Physiol*. 76(2):213-231.

851 Zenisek, D., Steyer, J. A., & Almers, W. (2000). Transport, capture and exocytosis
852 of single synaptic vesicles at active zones. *Nature*, 406(6798), 849–854.

853 Zucker R. S. (1996). Exocytosis: a molecular and physiological perspective.
854 *Neuron*, 17(6), 1049–1055.

855 Zucker RS & Lara-Estrella LO (1983). Posttetanic decay of evoked and
856 spontaneous transmitter release and a residual-calcium model of synaptic
857 facilitation at crayfish neuromuscular junctions. *J Gen Physiol* 81,355–372.

858 Zucker RS & Regehr WG (2002). Short-term synaptic plasticity. *Annu Rev Physiol*
859 64, 355-405.

860

Figure Legends

Figure 1. Kinetic model of molecular transitions of the fusion complex in individual vesicles. *D*, docked; *pP*, pre-primed; *P*, primed; *F*, fusion; α , forward rate constant; $f(t)$, calcium time dependence of the forward transition; β , backward rate constant; ρ , recycling rate constant. $D \rightleftharpoons pP \rightleftharpoons P$ are bidirectional; $P \rightarrow F \rightarrow D$ are unidirectional; Spontaneous transitions occur following the corresponding rate constant; On electrical activity, the calcium-dependence accelerates the $Dp \rightarrow P \rightarrow P \rightarrow F$ transitions.

Figure 2. Spontaneous quantal release. A. Experimental (black) and simulated (red) time distributions of spontaneous mepp_s from 5-min recording intervals. The experimental distribution of 143 mepp_s was obtained with license from Boyd and Martin (1956b); the simulation contains 148 mepp_s. The 1.54 s decay half time of the experimental probability rendered the $\alpha=0.62 \text{ s}^{-1}$ value used in simulations of cat neuromuscular transmission along the paper. B) Predicted contributions of the α and λ values on the meep frequency. Arrowheads point to values that gave the best fittings in simulations of frog and cat transmission. C. Predicted mepp_s frequency as a function of the λ coefficient in frog (vermillion) and cat (black) synapses. The $\rho = 1.0$ value was equally successful in all simulations. Arrowheads point to experimental meep frequencies at the indicated temperatures, from Boyd and Martin (1956a). D. Effect of α on release with different number of kinetic steps. E. Effect of λ on release with different number of kinetic steps. The grey lines in D and E are the α and λ values that reproduce all forms of release in frog neuromuscular junction. Arrowheads indicate the α and λ values that reproduce release with 5 kinetic steps. Three kinetic steps failed to reproduce spontaneous release regardless on the α and λ values.

Figure 3. “Calcium-dependence” of quantal release. A. The mean number of quanta (*m*) depends on the mean decay time (τ_e) of the intracellular calcium increase. The dots are model predictions; the lines were obtained with the equation by Dodge and Rahamimoff (1966) with third and fourth order cooperativities. B. The normalized number of quanta ($m/m_{\max}$) depends on the normalized t/τ_e duration of the calcium signal. The traces are superimpositions of curves obtained using two different amplitudes (I_e , arbitrary units) of calcium signal. The semilogarithmic chart in the inset shows the dispersion from a single exponential behavior below $t/\tau_e=1$. C. Increasing the α value accelerated release. D. Adding kinetic steps to the model increased the latency of release.

Figure 4. Evoked quantal release at low probability experimental conditions. A. Amplitude distributions of quantal release in frog neuromuscular junction. Counts are the number of quanta; the black lines link the discrete Poisson classes. The τ_e values are above in each plot. B.- Amplitude distributions at increasing probabilities by use of larger τ_e values. The discrepancies between the simulations and the Poisson predictions are clear with τ_e values above 0.25 ms. Each plot contains data from 250 stimuli mediated by a 5 s recovery interval. C. Pearson’s

significance (p) dependence on the τ_e value. The horizontal line indicates the 0.05 significance.

Figure 5. Contribution of the backward rate constant to quantal release. Data are presented in terms of the $\lambda=\beta/\alpha$ coefficients. λ_1 corresponds to $D \rightleftharpoons pP$; λ_2 corresponds to $pP \rightleftharpoons P$. The plots are as in Figure 4. The Pearson's significance (p) appears in each chart. The central chart was obtained with $\lambda_1=\lambda_2=50$, which fitted every form of release in frog synapses. Other parameters were $\alpha = 0.3 \text{ s}^{-1}$, $\rho=1.0 \text{ s}^{-1}$ and $\tau_e = 0.15 \text{ ms}$.

Figure 6. Sequence of facilitation and depression in frog neuromuscular junction. A) Number of quanta released in response to a train of three conditioning pulses 30 ms apart, followed by a test pulse 250 ms later (Mallart and Martin, 1968). Facilitation on the second and third impulses was followed by depression on the test pulse. B) Asynchronous release followed the conditioning impulses (square in A). The simulation parameters were $\alpha = 0.3 \text{ s}^{-1}$; $\lambda = 50$; $\rho = 1.0 \text{ s}^{-1}$, and $\tau_e = 1.3 \text{ ms}$.

Figure 7. Calcium and rate constants influence short-term plasticity. A. The duration of the calcium signal (τ_e) determines the balance between facilitation and depression. B. The λ_2 coefficient determines the duration of facilitation. C. The λ_1 coefficient reduces facilitation and depression. $N_{\text{test}}/N_{1\text{cond}}$ is the ratio between the amplitude of the response to the test pulse (N_{test}) and the conditioned pulse ($N_{1\text{cond}}$). Values above 1.0 indicate facilitation; below 1.0 indicate depression. Experimental data obtained with license from Betz (1972).

Figure 8. Vesicle recycling affects depression. The arrowhead denotes increased facilitation at high ρ value. Note the similar time course of the facilitation-depression sequence upon extreme ρ values. Conventional α and λ values for frog synapse were used.

Figure 9. Short-term plasticity with different numbers of kinetic steps. A. Three kinetic steps produced depression without facilitation. B. Five kinetic steps reproduced short-term plasticity by using a larger $\alpha = 0.62 \text{ s}^{-1}$ and a smaller $\lambda = 21$.

Figure 10. Vesicle dynamics in frog neuromuscular junction. The stimulation protocol is shown above (Mallart and Martin, 1968). The proportion of vesicles in each state is normalized to a pool of 10,000 (N_0) vesicles. Calcium produces rapid $D \rightarrow pP \rightarrow P \rightarrow F$ transitions. Reversibility contributes to rapid recovery to resting state. Recycling contributes to slow recovery and depression.

$$D \xrightleftharpoons[\beta]{\alpha+f(t)} pP \xrightleftharpoons[\beta]{\alpha+f(t)} P \xrightarrow{\alpha+f(t)} F \xrightarrow{\rho} D$$

bioRxiv preprint doi: <https://doi.org/10.1101/2021.09.15.460525>; this version posted September 17, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Calcium-dependence

λ_2 -dependence λ_1 -dependence