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A Comparison between Direct and Indirect Measurements of Neurotransmitter Vesicle Release Dynamics: A computational Study

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Abstract

Presynaptic vesicular release of neurotransmitters is a stochastic process involving complex mechanisms triggered by an elevation of calcium concentration. The mechanisms behind neurotransmitters release play a critical role in synaptic function and plasticity. Understanding its properties, both in term of its dynamics and its underlying mechanisms, may therefore help further our understanding of synaptic plasticity. However, measuring vesicle release dynamics is experimentally challenging. One experimental protocol used to determine the dynamic properties of vesicle release is to measure postsynaptic current. However, this method inherently not only captures properties of the release itself, but also the contributions from the postsynaptic receptors. Here we propose to use a synapse simulation platform known as EONS/RHENOMS to capture the functional properties of vesicle release, separate from the dynamics known to be associated with postsynaptic receptors, and compare the results with those determined experimentally. We find that despite attempts to reduce interference of postsynaptic dynamics, the receptor channel properties, particularly desensitization, may influence the overall measured results significantly. Re-estimating release rate by taking into account the contributions of postsynaptic receptors may give further insight into release dynamics and further our overall understanding on synaptic plasticity.

1. Introduction

Presynaptic vesicular release of neurotransmitters is a stochastic process that strongly depends on the dynamics of calcium concentration levels in the presynaptic terminal. Changes in voltage due to an action potential can cause voltage-gated calcium Channels

(VDCC) to open, leading to extracellular calcium flowing into the presynaptic terminal[1]. Calcium then binds to synaptic proteins which fuse the synaptic vesicles to the membrane, triggering the release of neurotransmitter. Importantly, the release process is highly modulated not only by calcium concentration increases due to the current action potential, but also by activity that preceded the present action potential, (residual calcium hypothesis). Residual calcium is believed to accumulate with successive events and ultimately affects release probability [2]. This process can induce an increase of the probability of release, or a decrease due to vesicle depletion, mechanisms known as release facilitation or depression. These complex dynamics strongly modulate the rate of vesicle release changes over time. However, experimental measurement of these release dynamics are not straight-forward. A few methods allow for indirect study of neurotransmitter release dynamics. Calcium, as earlier stated, directly relates to the rate of vesicle release, so the measurement of calcium through fluorescence imaging is a valid technique used to understand vesicle release dynamics [3][4]. Alternatively measure of postsynaptic current via patch-clamp methods is used to determine release events [5]. Unfortunately, both methods have their drawbacks. In calcium fluorescence imaging, one can quantify the calcium levels within presynaptic terminals but calcium indicators can act as buffers, thereby altering intracellular calcium dynamics[3], and, used independently, this method does not allow for determination of vesicles release events. Patch clamp measurements provide a means of determining the occurrence and amplitude of postsynaptic events triggered by release events, thereby allowing indirect determination of modifications in release probability without influencing presynaptic intracellular calcium concentration. The drawback of this method is that the dynamics observed in postsynaptic currents are not solely due to release events at the presynaptic terminal, but inherently combine postsynaptic dynamics. In experimental protocols NMDA and GABA receptors are therefore typically blocked to minimize interference. With NMDA and GABA receptors blocked, the major contribution of the postsynaptic current then comes from quick acting AMPA receptors. However, AMPA receptors themselves are not simply on or off switches when glutamate is bound. AMPA receptors also undergo non-linear processes such as saturation and desensitization, which can reduce the amplitude of postsynaptic responses in subsequent events [6]. To account for desensitization, experimental protocols may include application of desensitization-blocking drugs such as CTX [5]. In parallel, there have also been attempts at quantifying the desensitization process using direct measurements on individual AMPA receptors [6].

Multilevel modeling of the nervous system from biomolecular to higher levels of complexity provides a means to quantify and analyze the impacts of individual components at the molecular scale, such as receptor and/or channel kinetics, on higher levels of complexity with physiological results on the cellular, multicellular, or tissue level. Through multilevel simulations it is possible to validate and/or correct for experimental protocols ranging from the molecular level (i.e. individual receptor dynamic analyses from [6]) up to the cellular level such as the patch clamp experiments as done in [5]. The mechanistic synaptic modeling platform we developed provides for a number of readouts and allows for direct measurement of presynaptic release rate instead of attempting its estimation through other means. Additionally, our platform allows for direct estimation of the non-linearities of other factors, for example, how postsynaptic current is affected not only by release rate but also by

more complex receptor dynamics, e.g. saturation, desensitization. Based on the advantages of the simulation platform outlined above, we pursued the development of functional models, which can efficiently replicate the nonlinear dynamics of the mechanistic receptor models while decreasing the underlying computational complexity. These functional models can then be used in models encompassing higher level of complexity such as network models of interconnected neurons and provide non-trivial information on the non-linear temporal dynamics of the system modeled. Such analysis would be infeasible in a standard experimental setup. As previously mentioned, vesicle release cannot be easily measured. In the present simulation protocol we use the facilitation/diffusion vesicle release model based on the vesicle release dynamics analysis conducted by Song et al [5] and implement functional receptor models (specifically, AMPA receptor). We then compare simulated postsynaptic currents to experimental one, describe and discuss on the differences observed.

II. Methods

In our simulations we used the EONS (Elementary Objects of the Nervous System)/ RHENOMS (Rhenovia Modeling and Simulation) platform as a detailed glutamatergic synapse model and the CA1 pyramidal cell NEURON model described by Jarsky et al. [7] simulated within the NEURON simulation environment[8]. The EONS synaptic platform comprises both the pre- and postsynaptic sides of the synapse with models such as voltage dependent calcium channels, neurotransmitter release and diffusion, and postsynaptic glutamate receptors (ionotropic such as AMPA/NMDA as well as metabotropic receptors). The kinetic models in the EONS platform used for training the functional model include neurotransmitter diffusion [9], the 16 state AMPA receptor model developed by Robert and Howe [10] and the 8 state NMDA receptor model by [11]. For more details on the EONS/ RHENOMS synaptic platform please see [12] and [13].

The EONS functional model (fEONS) is a representation of the nonlinear dynamics predicted by the EONS/RHENOMS synaptic platform and/or its subcomponents, including the subdivision of pre- and post-synaptic components. To investigate postsynaptic dynamics of EONS/RHENOMS, we used the platform to measure the simulated AMPAr response and NMDAr response to a given release event. (Note that the NMDA model was trained but was not used for this simulation.) For AMPAr, the receptor conductance derived from the EONS platform was used to train a functional model using the Volterra models and Laguerre basis functions [5]. In the case of NMDAr, the Magnesium block affects channel conductance depending on voltage. For this reason, the open state probability of NMDA receptor kinetics is the output of the functional model rather than overall conductance. The conductance was then calculated using the equations described in [14] based on open state probability, as implemented in the original platform. In order to fully capture nonlinearities up to the third order, a 2 Hz Poisson random interval train of length 500 seconds (1000 events) was used as input to the simulation. Responses derived from the functional model were used to analyze the nonlinear properties of the AMPA receptor conductance and NMDA receptor open state kinetics. For validation, another set of inputs (2 Hz, Poisson random interval train) was used to determine the accuracy of the functional model compared to the original synaptic platform. The comparisons were done in the NEURON simulation environment, where 16 EONS/fEONS synapses were randomly placed on the Jarsky CA1 pyramidal neuron model.

Non-linear presynaptic release was modeled using a modified version of the facilitation/depression (FD) model developed by Dittman et al.[4] as described in [5]. The FD model was calibrated to previous experimental results from [5] with the assumption that their results represented the actual vesicle release rate. The vesicle release model served as the presynaptic component to the functional synapse model, as shown in Figure 2a, labeled "FD". Presynaptic release parameters were calibrated according to experimental results, as shown in Figure 1. To analyze the nonlinear effects on synapses due to release, multiple synapses were simulated (numerical count of 1000) due to the probabilistic nature of release events. The AMPA conductance values from all simulations were then averaged and deconvolved, leaving only the averaged postsynaptic amplitudes for each input event. The deconvolved amplitudes were used to estimate a discrete-time Poisson-Volterra model. The responses of the resulting PV model were compared to the probability of release derived directly from the Dittmann FD model (Figure 2b).

Finally, to test whether desensitization has an effect on overall postsynaptic response, desensitization was blocked in the AMPA kinetic scheme by adjusting the model so that the receptors would never enter the desensitized states. The functional AMPA model was then re-estimated with new coefficients and replaced the previous functional AMPA model coefficients in the functional EONS synapse. AMPA conductance results were then re-analyzed in the same manner as described earlier.

III. Results

In the paper by Song et al. 2009 [5], the vesicle release rate was approximated by measuring AMPA-mediated postsynaptic current (NMDA blocked) from clusters of synapses. Figures 1 and 2 show the comparisons between various representations of vesicle release from simulation results. Figure 1c shows relative change in the vesicle release probability for a given event, derived from Dittman's Facilitation-Depression model (solid), manually calibrated to approximate the response of the Volterra functional model based on Song's experimental results (dashed). In figure 2b, the relative change in probabilistic release rate in the FD model (solid) is compared with relative amplitude change derived from measured AMPAr conductance from the functional EONS model (dotted). The conductance is the result of averaged vesicle release in a group of synapses using the FD model combined with the nonlinear dynamics from AMPA receptors and undergoes the same analysis used to approximate release rate in [5]. If vesicle release rate directly correlates to the postsynaptic current as established in [5], then the release rate and the AMPAr conductance should have similar relative amplitude assuming AMPAr is the only contributor to postsynaptic current, with NMDA receptors blocked. However, predicted release rate measured from AMPA conductance in our model is shown to have a much lower relative change in amplitude than the predicted release rate measured directly from our FD model.

In [5] it was established that blocking desensitization did not significantly change the profile of the vesicle release curve based on measured postsynaptic current. Similarly, we blocked AMPA desensitization in the simulated mechanistic model. Relative release rate variations derived from AMPA conductance with desensitization blocked in the simulated model is shown in figure 2c (dash-dot), along with probabilistic release rate from the FD model for

comparison (solid). Blocking desensitization increased the effects of facilitation (and to a more moderate extent decreased depression) almost similar to the levels seen from the probabilistic release rate from the FD model. From this result it is presumed that AMPA receptor desensitization plays a significant role in postsynaptic conductance and can influence interpretations of vesicle release through measuring postsynaptic current.

IV. Conclusion

Experimental measurement of vesicle release is challenging due to the short duration of the release event along with the narrowness of the synaptic cleft. Measurement of the postsynaptic signal provides a means to derive information on vesicle release, but other mechanisms may obscure the true interpretation of vesicle release rate. Song et al. 2009 [5] attempt to reduce these effects as much as possible through the use of drugs, both to block NMDA receptors as well as AMPA desensitization.

Our results obtained using a detailed mechanistic model of AMPA receptor in the EONS/RHENOMS integrated synaptic modeling platform indicate that AMPA kinetics influence the overall interpretation of vesicle release dynamics based off of postsynaptic conductance. When AMPA is desensitized in the simulation, the measured change in release probability is much less than its actual value. This suggests that AMPA receptor kinetics should be accounted for and properly adjusted when taking measurements based on postsynaptic release. Interestingly, experimental results in [5] showed that CTX, an AMPA desensitization blocker, did not have a significant effect on measured postsynaptic activity, while our simulations indicate that blocking desensitization allows the measured postsynaptic conductance to return to the initial release dynamics derived from the Dittman model. Further investigation will be needed to determine if there may be other mechanisms involved during experimentation that would influence the postsynaptic signal, or the methods used for blocking AMPA desensitization (ie, CTX application) did result in complete blockade of desensitization. Also, results obtained experimentally may be corrected for through the analysis of such simulations by considering that the postsynaptic signal is composed of both vesicle release dynamics and receptor kinetics, and by the same respect, use simulations to derive the corrected vesicle release rate by filtering the receptor dynamics from the postsynaptic signal. Future work will attempt to derive vesicle release rate by optimizing the FD model to ultimately reproduce the postsynaptic response as seen in the experimental protocols. Such a model may help give us additional insight into vesicle release dynamics.

One additional aspect to note is the replication of the experimental protocol in a simulated environment. For the patch-clamp setup, the dynamics of vesicle release were not measured in individual synapses, but instead measured from the dendrite branches most likely containing multiple spines. Also, because vesicle release is inherently probabilistic, measuring individual synapse responses cannot fully capture the change in probabilistic release rate; instead, many synapses must be measured concurrently and averaged out to get a reasonable approximation. One of the aspects of the functional EONS model is the capability of running many different instances numbering in the thousands, parallel to each other – a feature that would be infeasible with a detailed mechanistic model such as EONS,

due to the amount of computational power it requires. This inherently constitutes one of the advantages of functional models, as it allows for the elaboration of more complex simulation protocols potentially spanning multiple hierarchical and temporal scales. Future applications of the EONS functional model will therefore include incorporation into large scale models while preserving the complex nonlinear dynamics of synapses, leading to the implementation of more complete map of the brain's mechanistic properties.

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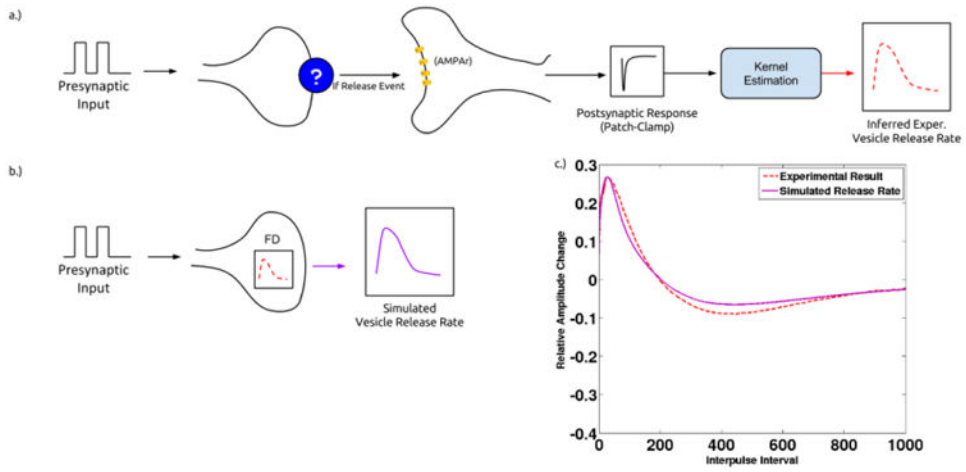

Figure 1.

Calibration of the Presynaptic Release Model based on Experimental Data. (a) the vesicle release rate was inferred from experimental data by analyzing kernel responses from patch clamp experiments, according to [1]. Release events are captured through postsynaptic responses, which are then analyzed through Volterra kernel estimation techniques. (b) The Facilitation/Depression (FD) model from [2] was calibrated with the inferred probabilistic release rate derived from the experimental data. (c) a comparison between the experimentally derived release rate (dashed) and the (calibrated) simulated release rate (solid). The graph shows the change in amplitude of a 2^{nd} event relative to the 1^{st} event response based on the time interval elapsed between the two events.

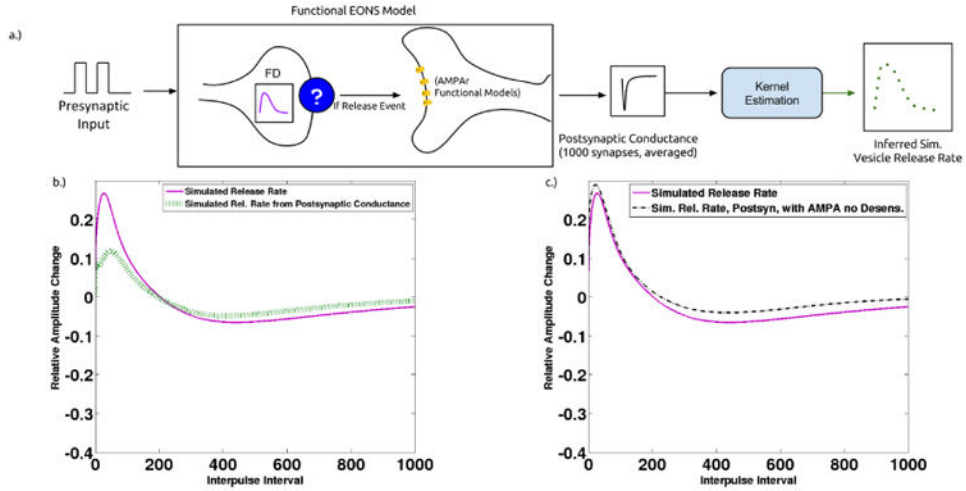

Figure 2.

Simulations using the EONS functional model. (a) Representation of the EONs functional model and simulation techniques for estimating vesicle release rate. Postsynaptic responses were analyzed similar to the experimental setup as explained in Fig 1. The Functional EONS model utilizes the FD model calibrated from Fig. 1 and AMPA receptor models based on AMPA receptor kinetics. (b) comparison between the simulated release rate from the FD model (solid) and the inferred release rate from the kernel estimation of the postsynaptic response (dotted). (c) uses the same setup as (a) except that the kinetics of the AMPA receptors were modified such that AMPA receptor desensitization was inhibited (results shown in the dash-dotted line).