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Presynaptic fluctuations and release-independent depression

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Abstract

Although vesicle depletion contributes to short-term depression, several studies have reported that the release probability can be transiently depressed even if an action potential fails to evoke release. Here we argue that stochastic fluctuation in the release machinery can give rise to apparent release-independent depression as a result of sampling bias. The relationship between this apparent depression and the interstimulus interval provides a window on the kinetics of state transitions of the release apparatus.

Synapses almost universally show a dependence of release probability on the recent history of activity: they facilitate or depress, and a number of mechanisms contribute to this phenomenon, most prominent of which are postspike elevations of the calcium concentration in the presynaptic terminal and the depletion of readily releasable vesicles¹. However, an intriguing phenomenon has been revealed by several studies that looked at individual synapses with very short interstimulus intervals (<10–15 ms): the probability of observing a quantal release event seems to be profoundly decreased following the second stimulus, not only when the first stimulus resulted in a release event, but also sometimes when it resulted in a failure of release (Fig. 1 and refs. 2,3).

'Release-independent depression' is typically demonstrated by repeatedly probing the synapse with paired stimuli and sorting the results into several outcomes. Let us denote the probability of observing two failures as $P(0,0)$, a failure followed by a success as $P(0,1)$, a success followed by a failure as $P(1,0)$, and two successes as $P(1,1)$. The reported result is that the success rate for the second response, if the first stimulus resulted in a failure, is lower than the overall success rate for the first stimulus: that is, $P(0,1)/(P(0,0) + P(0,1)) < P(1,0) + P(1,1)$. In other words, $P[R_2|F_1]$, the conditional probability of observing release for the second stimulus when there was a failure at the first stimulus, is smaller than $P[R_1]$, the overall average probability of release for the first stimulus (Fig. 1b).

Several mechanisms have been proposed to underlie release-independent depression, including failure of action potential invasion⁴ and Ca^{2+} channel inactivation^{2,3}, although none of these has been conclusively demonstrated. We argue here that release-independent depression may actually be illusory, resulting from the assumption that the initial state of the synapse is constant across all trials.

To illustrate this idea, let us first consider the following analogy. Let us assume that you like to fish, but because you are also a busy scientist, you set yourself a rule that each time you go fishing you will only cast the line twice before packing up to go back to the lab. You may

soon notice that if you catch a fish the first time you cast, the chance of catching one the second time is slightly reduced. You infer, quite reasonably, that this is simply because you have just removed one of the available fish, and the river is temporarily depleted of stock. However, you may also notice another pattern. On those days when you fail to catch a fish on the first attempt, you are also less likely to do so on the second attempt. Do you conclude that casting the line the first time (but not catching anything) somehow frightens off the fish? Perhaps, but an alternative explanation is that from one day to the next, the number of fish willing to bite the bait fluctuates, because of the weather, food abundance and so on. The initial state of the river on those days when you failed to catch anything the first time you cast the line is biased away from the average day toward a lower abundance of hungry, inquisitive or foolhardy fish.

What is the presynaptic analog of a fluctuation in the abundance of such fish? It is commonly accepted that at a single synapse, the probability of vesicular exocytosis in response to an action potential is determined by the number of release-ready vesicles (RRVs), n , and by the average probability of release for a single RRV, P_{ves} . Application of a binomial model allows us to calculate the probability that at least one vesicle will be released as $P = 1 - (1 - P_{\text{ves}})^n$. Accumulating data suggest that the probability of release at a given synapse can fluctuate from trial to trial due to fluctuations in both n and P_{ves} . For example, at CA3-CA1 glutamatergic synapses in the neonatal rat, the number of RRVs immediately available for release has been estimated to fluctuate between 0 and 4 on a timescale of < 100 ms (refs. 5,6). Considering that the average vesicle release probability in this preparation has been estimated as $P_{\text{ves}} \sim 0.43 \pm 0.28$ (ref. 5), such fluctuations in the number of RRVs are expected to lead to trial-to-trial variability in P across the entire possible range (that is, 0 to 1). Among likely mechanisms underlying fluctuations in n or P_{ves} is nonstationarity in the distance between Ca^{2+} channels and the vesicular Ca^{2+} sensors that trigger fusion⁷ and in the states of the proteins that make up the release machinery^{8–10}. The recent demonstration that somatic membrane potential propagates passively to presynaptic varicosities provides a further mechanism for rapid fluctuations in presynaptic release probability^{11,12}.

Thus it is reasonable to consider nerve terminals, in common with other organelles, as highly dynamic systems undergoing stochastic state fluctuations, resulting in rapid fluctuations in the probability of neurotransmitter release even in response to a fixed action potential-evoked Ca^{2+} influx. If so, by analogy with the fishing experience alluded to above, the probabilities of neurotransmitter release at the first and second stimuli are unlikely to be independent. Selecting trials with failures following the first stimulus will result in a bias toward those trials where the immediate probability of release was lower than average. The degree of this bias will depend on the distribution of $P[R_1]$ (a formal derivation is given in Supplementary Data online).

To explore quantitatively how stochastic fluctuations in the instantaneous probability of release can contribute to apparent release-independent depression, we performed Monte Carlo simulations of vesicle exocytosis in response to pairs of action potentials. We assumed that, at any given moment, the release site can exist in a state characterized by an instantaneous action potential-dependent probability of release, P_{inst} . We allowed P_{inst} to be a random variable uniformly distributed between 0 and 1, which either remained constant or adopted a new value drawn from the same interval if the release site underwent a spontaneous state transition. This state transition was governed by a Poisson process characterized by a time constant τ (arbitrarily set to 20 ms in Fig. 2). (This nonstationarity in P_{inst} could, for instance, represent a stochastic change in the number of primed RRVs or in the phosphorylation of a key component of the release apparatus.) We then sampled the system twice in rapid succession (equivalent to paired-pulse stimulation in the absence of

facilitation or depression) for 10,000 trials, and processed the outcome as follows. First we calculated the average probability of release by dividing the number of successes for the first action potential by the total number of trials. Then we calculated the average probability of release for the second action potential in the same way, but specifically focusing only on those trials where we observed a failure at the first action potential. We calculated the ratio of the second probability to the first (using the notation above—that is, $P[R_2|F_1] / P[R_1]$) to ask whether apparent release-independent facilitation or depression occurred. (Strictly speaking, this should be called failure-dependent facilitation or depression.) Finally, we repeated this entire simulation for each of a range of interspike intervals (0–100 ms).

At first sight one might expect the above ratio to be equal to 1, because no genuine facilitation or depression was allowed. In fact, for short interstimulus intervals ($<\tau$), the ratio was consistently less than 1. This is because restricting attention to those trials resulting in a failure on the first action potential biased the sample toward times when P_{inst} was lower than average, and there was only a small chance that a state transition occurred by the time of the second action potential (details in Supplementary Data). In other words, $P[R_2|F_1] < P[R_1]$, which is characteristic of the phenomenon reported as release-independent paired-pulse depression. Notably, this result was obtained in the absence of any ‘memory’ effect of presynaptic stimulation and simply resulted from the selection bias inherent in the sampling method. As the interval between the stimuli was increased, the apparent release-independent depression attenuated with a monoexponential time constant equal to τ (Fig. 2a).

Experimental data suggest that at short interstimulus intervals (<10 – 15 ms), release-independent depression can be detected even at some synapses that normally facilitate at longer intervals (20–200 ms; refs. 2,3). As a result, the ratio $P[R_2|F_1] / P[R_1]$, has a characteristic bell-shaped dependence on time, with depression at short intervals, followed by facilitation and then relaxation to 1 (ref. 2). We asked whether this time course could be reproduced in Monte Carlo simulations incorporating a simple action potential-dependent facilitation phenomenon (consistent with the residual Ca^{2+} model of facilitation). We repeated the simulations, but instead of letting P_{inst} vary over the same range for both first and second spikes (0–1, average 0.5), we rescaled the range for the second stimulus. The upper bound of this range remained 1, but the lower bound was described by an arbitrarily chosen alpha function ($0.025te^{-50t}$), peaking at 0.46 (corresponding to an average P_{inst} of 0.73) 50 ms after the first stimulus (inset in Fig. 2b). For any given interspike interval, if the terminal had undergone a spontaneous transition (again modeled by a Poisson process with $\tau = 20$ ms), the new P_{inst} for the second stimulus was sampled randomly from the narrower range. If, on the other hand, no transition had taken place, P_{inst} for the second stimulus was obtained by linearly mapping P_{inst} for the first stimulus to the narrower range corresponding to the interstimulus interval. The results of these simulations (Fig. 2b) again show apparent release-independent depression at short times, followed by an overshoot and relaxation reproducing the bell-shaped form of the paired-pulse ratio curve at facilitating synapses².

Finally, we examined the behavior of a population of five independent synapses innervating the postsynaptic neuron (Fig. 2c). For simplicity we assumed that each synapse (equivalent to that described in Fig. 2a) contributes to the postsynaptic amplitude in a binary manner: 1 if there was a release and 0 if there was a failure. (This situation also describes multivesicular release at a single synapse in the special case where there is no interaction among release sites.) We sampled the system twice in rapid succession (interstimulus interval 8 ms), without allowing any use-dependent facilitation or depression, and repeated this 10,000 times. As expected, the response to the first stimulus fluctuated between different numbers of release events, corresponding to a binomial distribution with a mean of 2.5 releases (Fig. 2c, top). Restricting the analysis to those trials where there was a failure at all five synapses for the first stimulus, we found that the response to the second stimulus was

biased to a smaller average size: 1.93 releases (Fig. 2c, bottom). This simulation thus again reveals apparent release-independent depression.

The argument outlined here does not rule out the possibility that genuine release-independent depression occurs. However, it removes the necessity to invoke such a phenomenon to explain the available experimental data. Notably, the shape of the relationship between the paired-pulse ratio and interstimulus interval is sensitive to the kinetics of the stochastic state transitions governing the release machinery (assumed, for convenience, to be a simple Poisson phenomenon here, although likely to be more complex in reality). Briefly, the slower the stochastic state transitions, the longer the interval over which apparent release-independent depression is expected to occur. In the simulation where neither facilitation nor depression occurred (Fig. 2a), the time constant describing the shape of the apparent release-independent depression was simply the Poisson. Thus, the time course of the relationship between the apparent release-independent depression and interstimulus interval potentially provides a new window on the kinetics of state transitions of the release apparatus.

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Figure 1.

Apparent release-independent depression observed in CA1 pyramidal neurons, in response to minimal stimulation of Schaffer collaterals (interstimulus interval, 8 ms; details in ref. 13 and Supplementary Data). (a) Sample traces of two successes (1,1), success and failure (1,0), and failure and success (0,1). (b) Histogram showing the average success probability for the first of two stimuli (that is, $P[R_1] = P(1,1) + P(1,0)$), and the conditional success probability for the second stimulus, given a failure in response to the first (that is, $P[R_2|F_1] = P(0,1) / \{P(0,1) + P(0,0)\}$). In 6 of 7 experiments where the overall success probability for the second response was lower than the first, we observed apparent release-independent depression (open circles; $P < 0.006$, $n = 7$; paired t -test).

Figure 2.

Monte Carlo simulations yielding apparent release-independent short-term synaptic depression (details in text). **(a)** Dependency of apparent release-independent facilitation or depression on interspike interval. No synaptic plasticity was incorporated into the model. The facilitation or depression ratio was estimated from the success rate for the second action potential, restricting the analysis to those trials where the first action potential failed to evoke release, divided by the overall success rate for the first action potential: that is, $P[R_2|F_1] / P[R_1]$. The apparent release-independent depression at short intervals results from sampling bias toward times when the release probability was lower than average. **(b)** Simulation results obtained when paired-pulse facilitation was incorporated, modeled by increasing the lower bound for instantaneous probability of release using the alpha function $0.025te^{-50t}$ (inset). **(c)** Apparent release-independent depression observed for multiple synapses. Top, binomial distribution of postsynaptic responses to the first stimulus, obtained for five synapses as in **a**. Bottom, distribution of responses to the second stimulus, delivered after 8 ms, restricting the analysis to failures in response to the first stimulus (gray bar in top histogram). The distribution of responses is shifted to the left, implying release-independent depression ($P[R_2|F_1] / P[R_1] = 0.77$).