Supporting Information

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SI Text

Derivation of the Equation Describing EPSC Rundown After Baf. The rundown of the autaptic response after Baf treatment reflects the loss of vesicular glutamate content. In principle, glutamate can be lost by stimulus-dependent and stimulus-independent mechanisms. Stimulus-dependent loss of glutamate results from action potential-triggered exocytosis of vesicles, whereas stimulus-independent loss may occur in Baf-treated vesicles through a passive leakage mechanism. The aim was to model the rundown of the autaptic response incorporating expressions for both contributions to glutamate loss and to extract a rate constant for passive leakage by fitting this model to the experimental data.

First, we determined an expression for just the stimulus-dependent rundown of the autaptic response after Baf treatment. It is assumed that vesicles are identical and noninteracting. Immediately after Baf application, when all vesicles are full, each vesicle has a probability (p) of being released. (p is sometimes called p_{ves}; e.g., ref. 1). Assuming there are N vesicles, the total number of vesicles released is given by Np. The EPSC amplitude (A) is proportional to the product of Np and the vesicular glutamate content. For simplicity, in this model the initial vesicular glutamate content is assumed to be 1. After the first stimulus, $A_1 = Np$. Because Baf-treated vesicles recycle normally, N will remain constant from stimulus to stimulus, even though some of these vesicles will now be empty (as released vesicles cannot be refilled).

In Baf, p should also remain constant. Thus, the probability of a vesicle being released on the second stimulus is still p. However, after Baf treatment, only full vesicles will contribute to the postsynaptic response. Therefore, when determining the EPSC amplitude on the second stimulus, we must determine the probability that a vesicle was not released on the first stimulus but is released on the second (so it is still full on the second stimulus). The probability that this vesicle was not released on the first stimulus is given by $(1 - p)$. Thus, the total probability that a vesicle which had not been released on the first stimulus is released on the second is given by $p(1 - p)$. Again, because recycling occurs normally and N remains constant, the amplitude of the EPSC for the second stimulus is given by $A_2 = Np(1 - p)$. A general expression for the amplitude on the jth stimulus is given by $A_j = Np(1 - p)^{j-1}$.

The stimulus-dependent amplitude rundown described above assumes that all vesicles are full of glutamate before exocytosis. What happens if glutamate leaks out of the Baf-treated vesicles over time? We assume that a stimulus-independent loss of glutamate through passive leakage will lead to an exponential loss of glutamate: e^{-kt}, where k is the rate constant for glutamate leakage. At $t = 0$ (immediately after Baf application) all vesicles are full. The first stimulus is delivered at $t = 1/f$ and the transmitter content in the vesicles will have decayed by $e^{-k/f}$. The EPSC amplitude incorporating this time-dependent decay, in addition to the stimulus-dependent component (described above), for the first stimulus is then $A_1 = Npe^{-k/f}$. When the second stimulus is delivered, the glutamate content in the vesicles will have decayed by $e^{-2k/f}$, and the amplitude is given by $A_2 = Np(1 - p)e^{-2k/f}$. When the jth stimulus is delivered, $e^{-jk/f}$ glutamate will have leaked out. Generalizing both stimulus-dependent and stimulus-independent components of the autaptic response after Baf application we obtain:

$$A_j = Np(1 - p)^{j-1}e^{\frac{-kj}{f}} \quad [1]$$

We now wish to derive a difference equation describing the amplitude rundown per stimulus. The amplitude of the response to the $(j - 1)$th stimulus is

$$A_{j-1} = Np(1 - p)^{j-2}e^{\frac{-k(j-1)}{f}}$$

Forming the difference, we get

$$A_j - A_{j-1} = Np(1 - p)^{j-2}e^{\frac{-k(j-1)}{f}}((1 - p)e^{\frac{-k}{f}} - 1)$$

after factoring out $Np(1 - p)^{j-2}e^{-k(j-1)/f}$ on the right.

Recognizing that $Np(1 - p)^{j-2}e^{-k(j-1)/f} = A_{j-1}$ we then have

$$A_j - A_{j-1} = -(1 - (1 - p)e^{\frac{-k}{f}})A_{j-1}$$

or

$$A_j - A_{j-1} = -p_{effective}A_{j-1} \quad [2]$$

where

$$p_{effective} = 1 - (1 - p)e^{\frac{-k}{f}} \quad [3]$$

The difference Eq. **2** is a discrete version of the equation that describes an exponential decay

$$\frac{dA}{dj} = -p_{effective}A$$

which has the solution

$$A_j = A_{j0}\, e^{-p_{effective}\, j}$$

where A_{j0} is a constant. This solution is more convenient if we write it in terms of time (t) rather than stimulus number (j). Using

$$t = \frac{j}{f} \therefore j = ft$$

the solution now becomes

$$A(t) = A_0 e^{-p_{effective}\, ft} \quad [4]$$

Hence, the rundown of the EPSC amplitude versus time for each stimulus frequency f is described by the exponential in Eq. **4**, and the reciprocal of the fitted decay time constant (τ) for this rundown is given by

$$\frac{1}{\tau} = p_{effective}\, f \quad [5]$$

Therefore, a plot of $1/\tau$ versus f will be described by Eq. **5**, where $p_{effective}$ is given by Eq. **3**.

The fit of Eq. **5** to the observed data allows us to estimate p and k (Fig. 3*B*). The estimated p is nearly identical to that found by Schikorski and Stevens (2) by using a completely different method ($\approx$0.03), whereas the estimated k is close to zero. The expected effect of leakage is apparent in Eq. **3**. At higher frequencies, $e^{-k/f}$ approaches 1 and so $1/\tau \rightarrow [1 - (1 - p)]f = pf$ which is independent of leakage. This is expected, because leakage is a minor factor as the stimulus frequency becomes large.

1. Garcia-Perez E, Wesseling JF (2008) Augmentation controls the fast rebound from depression at excitatory hippocampal synapses. *J Neurophysiol* 99:1770–1786.

2. Schikorski T, Stevens CF (1997) Quantitative ultrastructural analysis of hippocampal excitatory synapses. *J Neurosci* 17:5858–5867.