Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5359–5362, July 1990
Neurobiology

Origin of variability in quantal size in cultured hippocampal neurons and hippocampal slices

(miniature synaptic currents/patch clamp/brain slice)

J. M. Bekkers*, G. B. Richerson†, and C. F. Stevens*

*The Salk Institute, Howard Hughes Medical Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037; and †Yale University School of Medicine, Department of Neurology, 333 Cedar Street, New Haven, CT 06510

Contributed by C. F. Stevens, May 1, 1990

ABSTRACT **The size of synaptic quanta has been found to display considerable variation in cultured hippocampal neurons, but the source of this variability was previously unknown. We have now compared the properties of locally evoked miniature excitatory postsynaptic currents in cultured hippocampal neurons and in thin hippocampal slices using whole-cell patch-clamp recordings. The variability in miniature excitatory postsynaptic current size was similar in both preparations and occurred in cultured neurons when only one or a few synaptic boutons were stimulated. Thus, the variability in miniature excitatory postsynaptic current amplitude is not an artifact of cultured neurons and arises predominantly from variability within a single bouton. Possible origins of this variability are discussed.**

The elementary synaptic event, the miniature excitatory postsynaptic current (mepsc), recorded in cultured hippocampal neurons has been reported (1) to vary significantly from one occurrence to the next, rather than having a fixed size. Consequently, random variations in the mepsc amplitude must contribute to variability in the size of evoked macroscopic synaptic currents. As a result of this variability in quantal size, distinct peaks in the histogram of evoked macroscopic synaptic current amplitudes are absent (1), and an accurate quantal analysis is difficult without specific information about the distribution of the sizes for individual mepscs.

The previous report on fluctuations in mepsc size left two important questions unanswered. (*i*) How much of the mepsc size variability arises from fluctuations at a single bouton, and how much is due to variations in the quantal size from one bouton to the next? (*ii*) Is the large variability in quantal size an artifact of the culture system or is it also typical of neuronal circuits *in vivo*? Here we report that most of the variability in quantal size occurs at a single bouton rather than reflecting the distribution of sizes characteristic of a population of boutons. Further, the characteristics of mepscs observed in culture are similar to those recorded in hippocampal slices.

MATERIALS AND METHODS

Cell Culture. Pyramidal cells from region CA1 of the hippocampi of 1- to 2-day-old Long–Evans rat pups were dissociated and maintained in cell culture as described (1). Whole-cell patch recordings were made from the soma under voltage clamp with an Axopatch 1A amplifier at a holding potential of −60 mV or −70 mV. The bath solution was 137 mM NaCl/5 mM KCl/3 mM $CaCl_2$/10 mM glucose/5 mM Hepes, pH 7.4/1 μM tetrodotoxin (to block Na action potentials)/100 μM picrotoxin (to block γ-aminobutyric acid type A receptors), adjusted to 310 mOsm with sorbitol. The patch electrodes contained 150 mM potassium methylsulfate/5 mM KCl/5 mM K_4BAPTA [bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid]/10 Hepes, pH 7.2, 300 mOsm. Sometimes Cs replaced the K. Experiments were done at room temperature (22–25°C). Immunohistochemistry was done as described (1).

Slices. Slice experiments were performed by using a modification of the technique of whole-cell patch clamping in thin brain slices (2). Hippocampal slices (100 μm) were prepared from 14- to 21-day-old Sprague–Dawley rats using a vibratome. The slices were maintained at 22–25°C in a solution containing 120 mM NaCl/5 mM KCl/2.5 mM $CaCl_2$/1.2 mM $MgCl_2$/23 mM $NaHCO_3$/1.2 mM NaH_2PO_4/11 mM dextrose/0.5 μM tetrodotoxin/100 μM picrotoxin, pH 7.4. The slices were continuously superfused with oxygenated solution at a rate of 1–2 ml/min. Recordings were made from the soma of CA1 pyramidal cells with 2- to 3-MΩ fire-polished patch pipettes under direct observation with Nomarski optics on an inverted microscope. Electrodes were filled with a solution containing 140 mM KF/10 mM KCl/10 mM Hepes/11 mM EGTA, pH 7.25, 280 mOsm. Most recordings were made from neurons near the surface of the slice after cleaning the cell membrane with a jet of solution from the same electrode used for recording. Evoked mepscs were recorded under voltage-clamp conditions using an Axopatch 1A amplifier; holding potential was −60 mV or −70 mV.

Local Stimulation of mepscs. The main difficulty in studying mepscs in central neurons is that spontaneous mepscs can occur anywhere in the dendritic tree of the neuron, so that the observed size and time course depend jointly on the form of the mepsc at its origin and on the cable filtering that occurs in dendrites (3). What appear to be size fluctuations may just be mepscs differentially attenuated by the dendritic cable. The earlier study (1) circumvented this difficulty by evoking local mepscs through the application of hypertonic solution to a limited region of dendrite.

In culture, the hypertonic solution (bath Ringer's solution to which 0.5 M sucrose had been added) was applied by puffer pipette and removed by a nearby suction pipette, yielding a visible gray stream 15–20 μm wide. As shown (1), this elicited mepscs only when boutons were contained within the plume of solution. This fact was confirmed in the present experiments for one cell in which two clusters of boutons were separated by 30 μm. Application of the hypertonic solution at either of these clusters elicited mepscs, whereas solution applied halfway in-between yielded no response. This result indicates that boutons >15 μm from the point of solution application are not stimulated.

In slices, the same technique was used to apply hypertonic solution to the surface of the slice, except a suction pipette was not used; instead, the continuous bath perfusion was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: mepsc, miniature excitatory postsynaptic current; CV, coefficient of variation, or SD/mean.

relied upon to remove the hypertonic solution. The effect of this solution was limited to a local region because moving the puffer pipette by only 20–30 μm was always sufficient to abolish the mepsc response.

RESULTS

Basic Properties of mepscs in Slices and Culture. Preliminary experiments were aimed at confirming that mepscs are mediated by excitatory amino acid receptors (see also ref. 1). Hypertonic solution-evoked mepscs in both slices and culture were collected while voltage clamping the soma at a range of potentials (−100 to −20 mV for slices, −80 to 80 mV for culture). In each case, a plot of average mepsc amplitude at each potential versus holding potential gave a straight line with a reversal potential near 0 mV, as expected for near-symmetric monovalent cation concentrations. The mepscs in both slice and culture were reversibly blocked by bath addition of 1 mM kynurenic acid, a glutamate-receptor antagonist (4).

mepsc Variability in Culture. Our strategy for estimating the relative contributions of within-bouton and between-bouton variability to the overall mepsc size fluctuation was to evoke quantal release locally in young (7–14 days postplating), sparsely populated (≈ 5 neurons per mm^2) cultures that have relatively small numbers of synapses. After eliciting mepscs from specific locations, we used anti-synapsin I immunohistochemistry to visualize the boutons we had studied electrophysiologically. With appropriate cultures we could find locations at which only a few boutons were present within the 15- to 20-μm length of dendrite subjected to the hypertonic solutions. If the variation in mepsc size at a single bouton is small, then we should, in general, see a discrete peak in the amplitude histogram for each bouton present. On the other hand, if most of the variability in quantal size originates from a single bouton, then stimulation of just one bouton should produce the typical variability seen for a population of boutons.

We have correlated electrophysiological and immunohistochemical data at nine locations in six neurons. Examples of individual mepscs elicited from a circumscribed region on one cell are shown in Fig. 1*A* (top three traces) together with the mean of 21 such currents (bottom trace). The rate of spontaneously occurring mepscs in this cell, as in all cells studied here, was very low (<0.2 sec^{-1}) compared with the rate of evoked mepscs (>5 sec^{-1}). Note the large variability in amplitude of individual mepscs, which is further illustrated

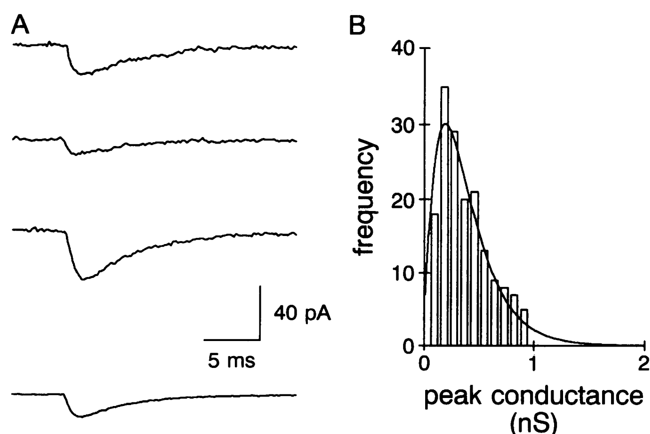

FIG. 1. Variability of mepscs evoked in cultured hippocampal neurons. (*A*) Three individual mepscs (top three traces) and the average of 21 such mepscs (bottom trace). (*B*) Frequency histogram of peak conductances of mepscs, including those in *A*, evoked from a single location in one neuron. The theoretical curve is superimposed on the histogram (see text).

in the histogram of conductances in Fig. 1*B*. The mean mepsc conductance for all cells was 1.0 nS, the mean decay time constant was 5.1 msec, and the mean of the coefficients of variation (CV = SD/mean) of the amplitudes of all cells was 0.55. (These figures include a single exceptional cell in which the mepscs were ≈ 3 times larger than normal; see below.) Note that hypertonic solution was applied to dendrites in more than half the cases, so that the mean mepsc conductance and decay time constant given here partly result from cable filtering. The average number of boutons at each site was 3.0 (range, one to seven), with five locations each containing three boutons. In no case were peaks observed in the mepsc conductance histograms that coincided with the number of boutons present. For example, the data in Fig. 1 were obtained from two boutons. If the CV for mepscs arising at a single bouton were <≈0.3, two distinct peaks should be apparent in the conductance histogram in Fig. 1*B*. This absence of peaks is consistent with the notion that much of the mepsc variability arises at individual boutons.

In most of the above-mentioned instances, boutons were located on distal dendrites, resulting in cable filtering of the mepscs as recorded at the soma (2, 5). However, in three instances application of hypertonic solution to the soma evoked quantal release, permitting us to measure the properties of mepscs in the absence of cable distortion. In each of these three cases immunohistochemistry revealed the presence of three somatic boutons. The average of the individual mean mepsc conductances for these three cells was 0.95 nS (SD of the individual means equals 0.02 nS), the mean decay time constant was 3.7 msec (SD = 0.95 msec), and the average of the amplitude CVs for the three cells was 0.57.

Fig. 2 shows the distribution of mepsc conductances and a photomicrograph of the stimulation site in the only experiment for which a single bouton was within the region of hypertonicity. The center of the region of hypertonicity is 15 μm from the closest bouton on the soma, and boutons are also seen at a dendritic site 23 μm distal to the site of hypertonic-solution application. These other boutons would not have been stimulated by the hypertonic solution due to its localized effect (see *Materials and Methods*). In this case, the mean mepsc conductance was 0.82 nS, the decay time constant was 6.5 msec, and the amplitude CV was 0.65. Note that there is as much variability in the mepsc size in this case (Fig. 2*B*) as when more than one bouton was stimulated. This fact confirms the conclusion that the majority of the mepsc-size fluctuations arises at individual boutons.

The variation of mepsc size from one bouton to the next is difficult to calculate from our data; we can only conclude that the contribution to the overall variability seems small for a particular cell. For the three cases of stimulation on the soma, where amplitude should be unaffected by cable attenuation, the SD of the individual means was 0.02 nS. Because three boutons were present at each stimulation site, the SD of the mepsc distribution for a population of single boutons is estimated by using a standard result from mathematical statistics (6) to be $\sqrt{3}$ = (1.7) times this value. This estimate seems implausibly small to us and is probably the chance result of a small sample.

Some boutons were clearly functionally larger than others. In a single case where one large elongated or two large and nearly fused boutons were present, we observed a mean mepsc conductance of 2.7 nS, a value outside the usual range. In this case the decay time constant was 7.2 msec (the synapse was on a dendrite). The amplitude CV was 0.50, a usual value.

In another series of experiments (without histochemical localization of boutons) in which hypertonic solution was applied to the somas of 19 cells, so that cable attenuation of mepsc amplitudes should have been minimal, the overall mean conductance was 0.76 nS with the mean of the indi-

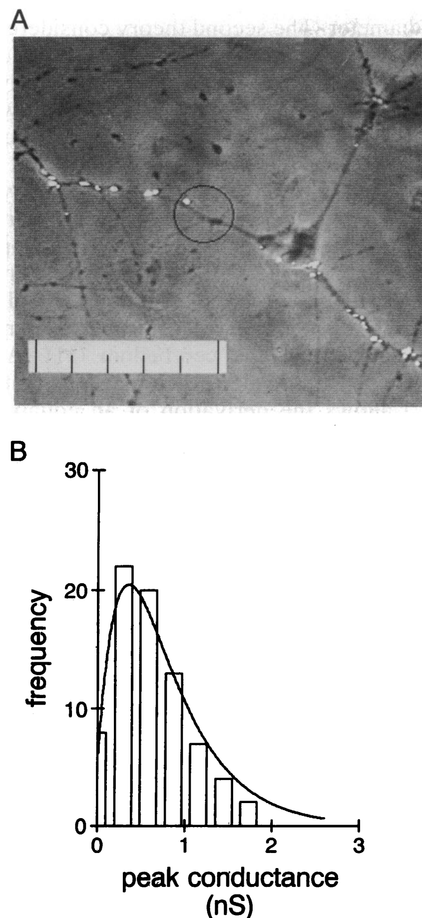

FIG. 2. Variability in mepsc amplitude in a case in which a single bouton was stimulated by application of hypertonic solution. (*A*) Photomicrograph of synapsin I immunohistochemical staining of a neuron previously used to record evoked mepscs. The recordings were made from the cell soma. Hypertonic solution was applied on the dendrite only within the circled region. The synapsin I-stained synaptic boutons are displayed as white dots; only one bouton is within the region of hypertonic solution. (10 μm per minor line division.) (*B*) Frequency histogram of peak conductances of mepscs evoked from the location shown in *A*. The theoretical curve is superimposed on the histogram (see text).

vidual coefficients of variation equal to 0.37. The age of these cells in culture ranged from 7 to 19 days, and the mepsc size was negatively correlated with culture age (r = −0.45); a regression line relating age in days to size in nS had a slope of −0.035 nS per day and an intercept 1.276 nS, so that a 7-day-old cell would have a mean mepsc size of 1 nS, and a 3-week-old cell would have a size of 0.54 nS. Clearly, then, boutons do not have all mepscs of the same size, but the exact contribution to synaptic current variability remains uncertain.

The mean mepsc conductances ranged from 0.49 to 2.7 nS. Amplitude of mepsc was not correlated with amplitude CV (r = −0.1), so that the CV can be taken, over the range of mepsc sizes studied here, as constant. This means that the variance of the mepsc-amplitude distribution grows approximately like the mean value squared, an observation that places some limits on acceptable models for the source of mepsc variability.

mepsc Variability in Slices. Due to the small size of mepscs, they have been difficult to resolve in the past by using standard intracellular microelectrode recordings from brain slices, where the background noise is relatively large (7, 8). The technique of whole-cell patch clamping in thin slices permits recordings to be made with lower access resistance and much

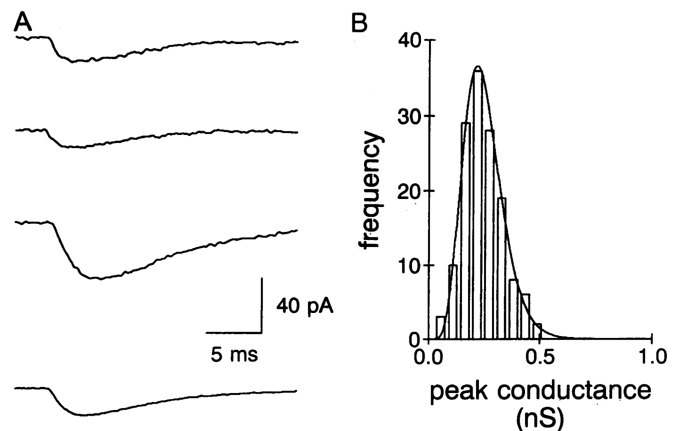

FIG. 3. Variability of mepscs evoked in neurons in the hippocampal slice. (*A*) Three individual mepscs (top three traces) and the average of 12 such mepscs (bottom trace). (*B*) Frequency histogram of peak conductance of multiple mepscs, including those in *A*, evoked from a single location in one neuron. The theoretical curve is superimposed on the histogram (see text).

higher shunt resistance (2, 9). As a result, even small mepscs can be easily resolved from the background noise, allowing more quantitative analysis of their size distribution in the same manner as was done in cultured neurons.

By carefully searching through the dendritic field of a neuron maintained under voltage-clamp conditions, specific locations were found at which application of hypertonic solution produced mepscs. These locations are presumably where synaptic contacts are made. Spontaneously occurring mepscs were rare in the CA1 region of hippocampal slices, so they caused little contamination of mepscs evoked by the hypertonic solution. Examples of evoked mepscs and the histogram of mepsc conductances are shown in Fig. 3. The mepscs varied considerably in amplitude (Fig. 3*B*) with a distribution similar to that seen in cultured neurons (Fig. 1*B*). The mean conductance of mepscs evoked at 27 locations in 10 neurons was 0.21 nS, and the mean time constant for the declining phase was 7.3 msec. The average CV for the distribution of amplitudes was 0.42. The largest mean mepsc observed in the slice was 0.32 nS with a mean decay time constant of 3.2 msec (the shortest value found in the slice). Hypertonic solution applied to the soma did not elicit mepscs. This is compatible with there being no excitatory synapses on the soma. Thus, all mepscs originated in dendrites, usually >100 μm from the cell body.

Table 1 presents a comparison between the mepsc characteristics in the slice and in culture. Note that some of the figures for culture exclude an unusual cell, mentioned above, in which the mean mepsc size was outside the usual range.

Table 1. Comparison of some mean properties of mepscs in hippocampal neurons in slices and culture

	Culture (n = 9)*	Slice (n = 27)
Mean conductance, nS	0.81 ± 0.17†	0.21 ± 0.12
Mean charge/holding potential, fC/mV	4.9 ± 1.8†	1.54 ± 0.51
Mean decay time constant, msec	5.1 ± 1.9	7.3 ± 2.0
Coefficient of variation of conductance	0.55 ± 0.13	0.42 ± 0.16

Values are given as mean (± SD).

*n = 8 for mean conductance and mean charge/holding potential values for culture.

†Values exclude an exceptional case in which the mean conductance was 2.7 nS and mean charge/holding potential was 23.6 fC/mV.

The table includes means of the charges carried by mepscs (normalized to the holding potential), which is a quantity less affected by cable attenuation (5). Although the CV of conductance is about the same for slice and culture, the mean conductance and the mean ratio of charge to holding potential in the slice were both one-third to one-fourth as large as those in culture. This result suggests that the average amount of cable attenuation was not greatly different in the two preparations. However, the decay time constant for mepscs in the slice was ≈ 1.4 times longer than in culture, so average cable filtering may have been slightly greater in the former, consistent with the fact that all the slice mepscs were elicited on distal dendrites.

DISCUSSION

Our main conclusions are that mepsc sizes vary considerably, even at single boutons, and that this variability is not an artifact of culture but occurs to a similar degree in slices. These conclusions depend on our having accurately correlated the immunohistochemical location of boutons with the physiology and on having stimulated primarily local occurrence of mepscs in slices.

Three arguments support the conclusion that mepscs were, indeed, of local origin in the slice. (*i*) We found that the pipette used for applying hypertonic solutions could be moved by only 20 to 30 μm and leave the regions where quantal release was evoked. (*ii*) The coefficients of variation of mepsc sizes in slices approximately matched those in culture, and that this could occur by two different mechanisms seems unlikely. (*iii*) We have shown previously for cultured neurons that mepsc amplitude and decay time constant change systematically with the distance between the mepsc origin and the site of recording, owing to cable attenuation, provided the distance is large enough (typically >50 μm) for cable effects to be significant (5). If release occurs at widely separated sites, then the amplitude of the mepsc should be negatively correlated with the decay time constant. In our slice data, however, no such correlation was observed, a finding that supports the conclusion that mepscs originated within, at most, a 50-μm length of dendrite (assuming similar cable properties in slices and culture).

Previous authors (7, 8) have noted the occasional appearance of miniature epsps in hippocampal neurons using intracellular electrodes. These miniature epsps were reported to have an amplitude distribution that was positively skewed but with its peak buried in baseline noise (8). However, those authors had no way of determining how much of this skew was from cable attenuation.

Our primary goal in the studies reported here has not been to determine the mechanism of the mepsc variability but, rather, to discover whether or not the variability occurs *in vivo* and whether it arises at single boutons or requires a population of boutons. Nevertheless, we have made some preliminary observations about the mechanism in order to obtain an equation, needed for other investigations, that describes the mepsc size distribution. We have considered two theories and have settled on the simpler one as a description, without necessarily accepting its physical plausibility.

Because the major part of the mepsc size variability occurs at single boutons, we have considered only mechanisms that vary the quantity of neurotransmitter released for each mepsc. The first possible mechanism considered was variation in the quantity of neurotransmitter contained in each vesicle. If vesicle diameters vary and if the molecular mechanisms for loading transmitter into vesicles operated until a fixed final concentration is achieved, then the quantity of transmitter released would vary in proportion to the cube of the vesicle diameter. The second theory considered was that vesicle-release sites are not independent, so that cooperative releases of multiple vesicles (each containing nearly the same quantity of transmitter) occurs in a probabilistic way. Other mechanisms are possible—such as variability in quantity of neurotransmitter loaded into each vesicle, but we have focused on these two because they are potentially testable.

We have measured vesicle diameters in electron micrographs of terminals from our cultures with appropriate corrections for sampling artifacts (10). Diameters were found to be normally distributed with a mean of 34 nm and SD of 3.8 nm. This result is comparable with the mean of 35 nm and SD of 4.5 nm reported for the cerebellum (11). A standard theorem on the probability density for a function of a random variable (12) allows the derivation of an equation for the probability $P(x)$ that a mepsc will have amplitude x, on the assumption of a linear dose–response curve over the range of transmitter quantities encountered:

$$P(x) \propto \exp\left[\frac{-(x^{1/3} - m)^2}{2\sigma^2}\right] \cdot x^{-2/3}, \qquad [1]$$

where m and σ^2 are the mean and variance, respectively, of $a^{1/3}$ where the a values are the observed mepsc amplitudes. This equation is fitted to the conductance histograms in Figs. 1*B*, 2*B*, and 3*B* and provides an adequate description of the data.

On occasion our mepsc conductance histograms seemed to have discrete peaks; however, the number of peaks was always greater than the number of boutons subjected to the hypertonic solution. We have not been able to determine whether these peaks are real or merely statistical artifacts. If peaks are actually present, this presence would suggest that mepsc-size variation could come from the cooperative and concerted fusion of various numbers of vesicles.

Because mepscs amplitudes at even a single bouton vary considerably and because this same degree of variability occurs in slices as well as in cultured neurons, attempts to interpret evoked macroscopic excitatory postsynaptic current variability, as for quantal analysis, should take account of this effect.

This work was supported by the Howard Hughes Medical Institute (C.F.S.) and Grant NS12961 from the National Institutes of Health to C.F.S.

1. Bekkers, J. M. & Stevens, C. F. (1989) *Nature (London)* **341,** 230–233.
2. Edwards, F. A., Konnerth, A., Sakmann, B. & Takahashi, T. (1989) *Pflugers Arch.* **414,** 600–612.
3. Rall, W. (1977) in *Handbook of Physiology*, eds. Brookhart, J. M. & Mountcastle, V. B. (American Physiological Society, Bethesda, MD), pp. 39–97.
4. Perkins, M. N. & Stone, T. W. (1982) *Brain Res.* **274,** 184–187.
5. Bekkers, J. M. & Stevens, C. F. (1990) in *Understanding the Brain Through the Hippocampus: The Hippocampal Region as a Model for Studying Brain Structure and Function*, eds. Storm-Mathisen, J., Zimmer, J. & Ottersen, O. P. (Elsevier, Holland), in press.
6. Hoel, P. G. (1954) *Introduction to Mathematical Statistics* (Wiley, New York), pp. 101–103.
7. Yamamoto, C. (1982) *Exp. Brain Res.* **46,** 170–176.
8. Brown, T. H., Wong, R. K. S. & Prince, D. A. (1979) *Brain Res.* **174,** 194–199.
9. Sah, P., Hestrin, S. & Nicoll, R. A. (1989) *Science* **246,** 815–818.
10. Froesch, D. (1972) *J. Microsc.* **98,** 85–89.
11. Palay, S. L. & Chan-Palay, V. (1974) *Cerebellar Cortex: Cytology and Organization* (Springer, New York), p. 96.
12. Papoulis, A. (1965) *Probability, Random Variables, and Stochastic Processes* (McGraw-Hill, New York), p. 118.