Spontaneous quantal currents in a central neuron match predictions from binomial analysis of evoked responses

(quantal responses/miniature currents/Cl⁻ channels/synaptic noise)

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ABSTRACT Inhibitory postsynaptic currents occurring spontaneously in the teleost Mauthner cell were analyzed with the single-electrode voltage-clamp technique. They were collected during depolarizing steps and were outward-going; this procedure allowed them to be isolated from possible excitatory currents flowing in the opposite direction. Their amplitude histograms were found to exhibit regularly spaced multiple peaks, each of which had a Gaussian distribution of the same width. These compound inhibitory postsynaptic currents represent responses evoked by background firing of presynaptic neurons, and when tetrodotoxin was applied topically, only the first peak in the frequency histogram, which can be attributed to single exocytotic events, remained. The mean conductance of this quantal unit equaled 46.0 nS, which corresponds to the opening of 1000–2000 Cl⁻ channels activated by glycine— the transmitter at these synapses. Its waveform and those of the larger units were essentially the same. Furthermore, in each set of data provided by a given Mauthner cell, the size of a quantum was quite constant, with its variance and those of further peaks being equivalent to that of background noise. These properties, which characterize the quantal events on the basis of spontaneous synaptic activity, were strikingly similar to those of the basic units derived by the simple binomial analysis of unitary postsynaptic potentials, thus validating the use of this statistical model to quantify the quantal nature of release at central connections. The quite straightforward method used here to extract single miniature currents from complex signals should be applicable to the other systems of the central nervous system, where the pertinence of this probabilistic model of release has yet to be demonstrated.

The notion of quantal transmitter release (1), as demonstrated at the neuromuscular junction, relied upon the linkage of two findings. One is that evoked responses are composed of unitary elements of equal amplitudes. The second, which was as crucial, is that these basic units correspond to the so-called spontaneous miniature potentials or quanta. In the central nervous system, however, it has not yet been possible to establish the same correlation. Small spontaneous potentials, presumed to be miniatures, have been detected in a number of systems, including spinal neurons (2–4) and hippocampal cells (5). But their definite identification as quanta has remained debatable despite early attempts with motoneurons (6). In that case, fluctuation analysis was applied to spontaneous rather than evoked postsynaptic potentials; furthermore, the Poisson model used appeared later to be inadequate (7).

In fact, quantal release models can be applied satisfactorily to the central nervous system, as shown, for instance, with inhibitory synapses on the teleost Mauthner cell (M-cell). In this system, binomial predictions adequately fit the distribution of fluctuating unitary potentials evoked by a single presynaptic cell; furthermore, the relevance of this statistical model was shown by the finding that the binomial term n, which defines the "total number of units available" (8), has a physical counterpart. That is, n equals the number of active zones established by one presynaptic neuron on its target (9–11). On the basis of this equivalence and of conductance measurements, several conclusions could be drawn concerning g, the quantum extracted from the binomial statistics. Specifically, its amplitude, which averaged 1.2% of the full-sized inhibitory postsynaptic potential (IPSP) produced by stimulating the M-cell's recurrent collateral network, or 0.6% of the collateral conductance (12), would be due to the opening of 1000–2000 glycine-activated Cl⁻ channels (10). It remained to be demonstrated that miniatures due to spontaneous release share the above-defined properties of the mathematically defined quantum and, therefore, constitute the basic unit of evoked responses. On the basis of voltage-clamp measurements of synaptic noise, we report here that such is the case.

MATERIALS AND METHODS

Intracellular recordings with KOAc-filled microelectrodes (4 M, 2–5 MΩ resistance) were obtained from the M-cell soma of goldfish (Carassius auratus) anesthetized with MS-222 and immobilized with d-tubocurarine. This neuron is subjected to a powerful chemically mediated Cl⁻-dependent inhibition brought about by activation of at least two groups of interneurons (13), including (i) cells belonging to the crossed vestibulo-vestibular pathway, which innervates both M-cells; and (ii) cells in the strictly ipsilateral recurrent collateral network. The latter circuit was activated by antidromic stimulation of the Mauthner axon (M-axon) in order to obtain the maximum-sized collateral response as a reference for data analysis (12). Single-electrode voltage-clamp techniques (Axoclamp, Axon Instruments, Burlingame, CA; chopping rate, 15–25 kHz) allowed measurements of synaptic currents during 0.5- to 2.5-sec depolarizing steps of 25–80 mV, which equaled the driving force because the Cl⁻ equilibrium potential is close to the resting level. A major advantage of this procedure is that the inhibitory postsynaptic currents (IPSCs) are thus outward and can be distinguished from spontaneous excitatory ones, which would be in the opposite direction. In most experiments, tetrodotoxin (TTX; 35 µg/ml) was applied topically (after control data were collected) with the expectation that it would reduce or abolish presynaptic impulses, thereby isolating synaptic noise due to single exocytotic events (6, 14).

Abbreviations: IPSC, inhibitory postsynaptic current; TTX, tetrodotoxin; M-cell, Mauthner cell; M-axon, Mauthner axon; IPSP, inhibitory postsynaptic potential.

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Continuous current records were stored on tape and subsequently fed to a computer (DEC 11/23 with INDEC data acquisition system) for digitalization at a sampling interval of 25 μsec and measurements. Successive segments of data occurring 200 msec or more after the onset of the depolarization were viewed to select all presumptive IPSCs, which were recognized as positive-going current transients above a threshold amplitude. A running average, 0.5 msec in length, provided the reference level for detecting such transients, and the threshold amplitude was taken as twice the standard deviation ($\sigma_N$) of the background noise. The latter parameter was determined by measuring the mean and variance of continuous (quiet) sections of the current that were at least 5 msec in duration and did not appear to contain any clear spontaneous synaptic events (i.e., remained relatively flat). In previous current and voltage-clamp experiments, the time courses of the unitary responses evoked by stimulation of identified interneurons were asymmetrical, with the average time-to-80%-peak amplitude and decay-time constant being about 0.5 and 6.7 msec, respectively (12, 15–17). Therefore, the criteria for accepting a transient as an IPSC were that it had a peak time in the range of 0.4–1.0 msec and lasted at least another 1 to 2 msec. (With this technique, it is possible, however, that some small-amplitude high-frequency noise signals that occurred just prior to actual IPSCs were mistaken in the analysis.) The peak amplitudes of the IPSCs and $\sigma_N$ were expressed both in terms of nanoamperes and as fractions of the full-sized collateral IPSC (Fig. 1A).

As expected, outward-going IPSCs could readily be distinguished from the inward excitatory currents, with the latter being, in addition, shorter lasting (Fig. 1B), but assessment of their amplitude, which was always made automatically, could require corrections due to baseline variations. Two different situations were encountered: (i) isolated events arising from a relatively flat baseline could be directly measured from onset to peak (e.g., see Fig. 1B), and (ii) in periods of intense synaptic noise, these were closely spaced responses with one riding near the top of the other or during its decay phase. Because the tail of the preceding IPSC was then curtailed, a correction procedure was designed that was based on the fact that the unitary synaptic current decays exponentially with a rather-fixed time constant (14, 17). This method is illustrated in Fig. 1 C and D. A series of clear-cut and isolated responses was first averaged, and the mean decay time was determined by a best-fit (least squares) method (Fig. 1C). Extrapolation of the decay of any leading response could then be obtained on the basis of an exponential function, the maximum of which corresponded to the peak of the IPSC ($A_0$ at $t = 0$), while the time constant $\tau$ equalled that of the averaged response, according to the relation $A(t) = A_0e^{-t/\tau}$. In cases of several overlapping IPSCs, the reference or “zero” points for their respective summits ($A_0$s) for calculations of their extrapolated decay phases was the same—i.e., that of the first response. When the onset of

![Fig. 1. Evoked and spontaneous inhibitory currents recorded from the M-cell soma. Single-electrode voltage-clamp records were obtained with the indicated chopping rate and during depolarizing steps ($\Delta V$) of 70 mV. (A) Full collateral response produced by antidromic activation of the M-axon. (B) Discrimination of spontaneous excitatory (filled circles) and inhibitory (arrow) synaptic currents, which are in opposite directions during the depolarizing command. Vertical dashed lines designate the onset and peak of the IPSC. (C and D) Illustration of the method used to determine the size of IPSCs occurring on the falling phase of preceding ones. (C) Determination of the mean decay time of IPSCs. Average of 26 isolated responses fit with a single exponential having the indicated time constant $\tau$. (D) Measurement of two IPSC amplitudes from their peaks to the projected tails of previous events, extrapolated from an exponential function of the same $\tau$ as in C. The pairs of vertical lines indicate onsets and peaks of the measured responses; note the translation of the fitted curve for the first superimposed IPSC. (E) Sample trace, showing the amplitude range and frequency of ongoing synaptic activity. Individual components, presumed to represent spontaneous IPSCs on the basis of their time to peak, are signaled by arrows. The accompanying values are the amplitude of each peak, expressed in nA, as measured by the computer. The baselines of responses measured to projected tails are indicated. Crossed arrows indicate possible responses that were not included in the measurements either because of baseline uncertainties or decay phases that were too rapid, although events of this nature were not systematically rejected. Note the extreme variability of the responses and the low standard deviation ($\sigma_N$) of background noise. In this and the following figures, inhibitory currents are outward because the Cl\(^-\) driving force is positive and effectively equal to $\Delta V$. All four figures are from the same experiment.}
the second response was displaced from the extrapolated function because of noise, the projected baseline was translated to intersect the beginning of that response. This point was taken automatically as the minimum preceding the peak of the second IPSC and, in most cases, coincided with the rapid upswing within the 0.4- to 1.0-msec time-window defined above. This assignment was monitored continuously to guarantee that the onset would not be incorrectly identified because of preceding noise, as could have been the case for the first labeled response of Fig. 1D. In such instances, the beginning of the IPSC was reset manually to correspond with the inflection point on the rising phase.

RESULTS

The results reported here are illustrated by Figs. 1–4, which are from the same experiment. The value of the collateral IPSC in this case was 200 nA during the 70-mV depolarizing steps (Fig. 1A). Some isolated IPSCs arising from a stable baseline were present throughout the recordings, and selected spontaneous events with amplitudes ranging from 1.94 to 18.25 nA (n = 26, m = 9.76, SD = 3.75, or, stated otherwise, m = 4.88% of the collateral IPSC) were averaged. The average decay time constant, \( \tau \), was 3.2 msec (SD = 0.32), with extreme values for individual traces of 2.8 and 3.5 msec. The exponential curve determined by such a time constant gave good fits whenever required for precisely projecting the tails of spontaneous responses (Fig. 1D). In confirmation we checked the constancy of this decay-time constant during the experiment: it remained constant for IPSCs collected during eight depolarizing steps and pooled into two groups corresponding to the first 320–960 msec (n = 425) and 1600–2240 msec (n = 277) after the onset of the depolarizations.

The synaptic noise, which could be intense (Fig. 1E), was composed of a succession of numerous events whose peaks nevertheless could be distinguished and measured. They presumably were brought about by presynaptic firing, the variability of which can be inferred from successive traces obtained in the control before TTX application. It should be noted that the larger responses could be identified with assurance, whereas some smaller currents, which may have been IPSCs, had to be neglected whenever their waveforms were questionable. The record in Fig. 1E also indicates that the synaptic noise was dominated by inhibitory responses because inward-going excitatory postsynaptic currents occurred infrequently.

The frequency histogram of current amplitudes for responses measured during eight steps before and after the collateral IPSC is illustrated in Fig. 2A. At least six, and possibly eight, equally spaced peaks stand out. The interval between them is about 2 nA, and their distributions overlap, making it difficult to precisely assess their limits. However, even without further manipulations, it was already apparent that the variance about each peak was essentially the same; indeed, Fig. 2B confirms that their distribution was Gaussian with equivalent standard deviations. The relative constancy of the variance suggests that this parameter mainly reflects fluctuations in background recording noise and not variability intrinsic to the quantal events themselves (see also the Discussion). This overall distribution strongly implies that the larger responses are integral multiples of a basic unit, which had an amplitude corresponding to that of the first peak, but had to be isolated for better definition.

Superfusion with TTX provided a tool for such an isolation, since it rapidly eliminated all the large responses (Fig. 3). The effect of the drug on impulse activity was confirmed by the complete block both of antidromic invasion of the M-cell's initial segment and axon hillock and of the recurrent collateral IPSP (Fig. 3A) within a few minutes. At that time, the first peak of the histogram was unaffected, and there remained but a few IPSCs of the second class (Fig. 3B), which

![Fig. 2.](image)

**FIG. 2.** Evidence that the amplitudes of spontaneous IPSCs are grouped by increments of constant size. (A) Frequency histogram of 1635 events, the magnitudes of which are expressed in terms of percentage of the full collateral IPSC (abscissa). The currents were recorded during the control prior to TTX application (\( V = 70 \) mV). Eight clear peaks, regularly spaced at intervals of about 1%, can be distinguished. Note that essentially no responses were measured in the first 0.5%, a gap that equals half of the quantum size and is twice the standard deviation of the background noise. (B) Gaussian fit of the components of the histogram; their amplitudes were determined by the relative number of events, which were 33.3, 22.4, 16.5, 12.6, 7.0, 3.8, 2.6, and 1.8% of the total amount, respectively. The separation between the peaks of the theoretical curve equals the mean amplitude of the first peak, as determined after TTX application (see text). \( x^2 = 155.73, P > 0.55 \) (degrees of freedom = 159). For each, \( \sigma = 0.24\% \) of the collateral IPSC, or 0.48 nA, which is close to the standard deviation of the recorded noise.

![Fig. 3.](image)

**FIG. 3.** Unimodal distribution of synaptic currents in conditions designed to abolish presynaptic spike activity. (A) Superimposed traces obtained from the M-cell before and after TTX administration, which completely suppressed the antidromic action potential and the subsequent IPSP evoked by the spinal stimulation, except for the early axon spike. (B) Frequency histogram of 735 IPSCs recorded under the same conditions as for Fig. 2 but shortly after topical application of TTX. There remains only one peak, the domain of which lies within the two arrows. Note its correspondence with the first peak of Fig. 2. A few larger events were also detected, but only at the beginning of the recording session (see the text). (C) Computer average of the 671 smaller IPSCs, whose amplitudes fell within the limits defined by the arrows in B. The standard deviation of this class was close to that of the background noise, indicating a rather invariant quantal size. As shown by the exponential fit of the mean IPSC falling phase (\( \tau = 3.2 \) msec), the decay time of the responses remained constant throughout the experiment.
totally disappeared shortly. That is, the latter represented 16% of the first 100 counts, averaged 4% of the next 500 counts, and then vanished, leaving the distribution of the first peak unambiguous. Averaging all of these basic units indicated a peak with the expected waveform (Fig. 3C) equal to 2.08 ± 0.42 nA (SD), which was 1.02 ± 0.21% of the full collateral IPSC recorded in the control. Note that this peak is well above the limitations posed by the measurement technique, as its amplitude is almost 3 times greater than the detection threshold (2σN = 0.74 nA). Once the TTX effect was complete, the range of the remaining spontaneous responses was taken as indicative of the extreme values of the distribution of a single quantal event, and the control data of Fig. 2 was subdivided according to these boundaries, with the averages and standard deviations being, in nA, 2.10 ± 0.43 (number of events, n = 545), 3.99 ± 0.52 (n = 367), 6.05 ± 0.48 (n = 270), 8.01 ± 0.48 (n = 206), 10.07 ± 0.46 (n = 115), 12.0 ± 0.48 (n = 62), 14.10 ± 0.48 (n = 42), and 16.04 ± 0.45 (n = 28) for the first eight successive peaks. There is a strict correspondence between these means and integer multiples of the first component in the distribution. Thus, each increment is the same and equally spaced in amplitude to the basic unit.

As expected, the shape of composite responses did not significantly differ in appearance from that of the individual unit isolated by exposure to TTX (Fig. 4). This observation was already apparent in sample traces exhibiting currents produced most likely by single, double, and triple quanta (Fig. 4A) and is confirmed by the superimposed averages of the presumed single and double IPSCs obtained after TTX administration and of triplets recorded before (Fig. 4B). Their times to peak—0.63, 0.83, and 0.86 msec, respectively—were also comparable.

Data from the six other experiments were consistent with those of the illustrated one, in that each exhibited equally spaced multiple peaks, with the smallest one, isolated by using TTX, having properties matching our previous binomial predictions. Pooled (n = 7), the mean quantal amplitude was 0.59 ± 0.2% ±SD) of the collateral IPSC, and its conductance averaged 46.0 ± 16.5 nS, which is within 30% of the derived binomial estimate of 35 nS.

**DISCUSSION**

An important outcome of this study is that high-frequency controlled synaptic events in a polyinnervated cell can be resolved into individual quantized components. This advance presumably is in part due to advantages offered by the voltage-clamp approach, which allows for a clear differentiation of excitatory and inhibitory responses during depolarization and still provides a favorable signal-to-noise ratio. Thus, it can be anticipated that the herein described method could be successfully applied to other central neurons for the purpose of quantal analysis, at least in conditions where the spontaneous activity is dominated by a homogeneous population of active contacts that are electrotonically close to each other. If, however, such junctions are widely distributed over the neuronal surface or diverse classes of inputs with different physiological properties (transmitter–receptor kinetics) all contribute significantly to the background noise, quantal amplitudes would be expected to vary, and the resultant amplitude histograms might not exhibit such distinct peaks.

On the other hand, this approach to the analysis of spontaneous PSCs might provide an independent means for testing the intriguing hypothesis that, while transmission at contacts between cat Ia fibers and motoneurons may be quantal (18), in contrast to earlier claims (7, 19, 20), all quantal amplitudes are the same at the soma regardless of the dendritic locus of the active synapses.

One possible source of uncertainty merits consideration: were there smaller synaptic currents buried in noise and, thus, undetected? The finding of a constant increment between peaks equal to the amplitude of the first one makes this alternative unlikely. The effect of TTX also addresses this question. It cannot be concluded whether the remaining peak is due to truly spontaneous events or if some presynaptic electrogenic activity persisted, thus causing evoked release with a lowered probability (21). Regardless, the overall distribution within the first class remained constant, and smaller events were not unmasked by blocking the larger ones.

**Fig. 4.** Extraction of miniature IPSCs from the background activity. (A) Sample recordings of IPSCs (arrows) obtained in the presence of TTX, with their indicated amplitudes in nA. Three recordings show single events (Left), two contain doublets (Right Top and Middle), and one of which is followed by another single miniature (Right Top), and the remaining trace illustrates an exceptional triplet (Right Bottom). (B) Superimposed averages of IPSCs selected by their amplitude classes. The two smallest ones are from recordings after TTX application, and the largest is from the third group of the control histogram, included for comparison. According to their indicated mean amplitudes and standard deviations, their sizes are consistent with the notion that they represent responses produced by one, two, and three quanta, respectively.
The decay time constant of miniature IPSCs in these experiments ranged from about 3 to 8 msec, which is comparable to that reported for evoked responses recorded at the resting potential (12), but it does not fit with the voltage-dependent increase in τ seen during brief (25–35 msec) depolarizations (17). The latter observation has been interpreted as suggesting that mean channel lifetime is prolonged by depolarizations of 20 mV or less. The mechanism underlying this apparent discrepancy is not clear, but it may be that (i) in the present study, the voltage dependency of the transmitter-receptor interactions was inactivated during the first 200 msec after the onset of the voltage steps, when spontaneous events were not measured; or (ii) τ is a bell-shape function of membrane potential, being maximal in the region of −50 to −60 mV and decreasing again with larger depolarizations of the magnitudes used in these experiments.

Measurements of the background noise, which can be treated as being Gaussian (see also ref. 10), indicated that its standard deviation (σn) ranged from 0.18 to 0.37 nA and was comparable to that of the first class, with the ratio of the two, σn/σε, averaging 1.15 ± 0.16 (n = 7). In other words, the size of a quantum is relatively invariant, with the distribution of values around a given mean being primarily due to the added contribution of noise, which provides the Gaussian appearance of each component of the amplitude histogram. In confirmation, we also found that the standard deviations (σε) of larger peaks in the controls were about the same as those of the first one. Such was the case for the illustrated example where, as mentioned above, σε equals 0.48 mA for four of the peaks and varied from 0.45 to 0.52 for the others. Yet, since the ratio σε/σn averaged slightly greater than 1.0, there may have been some secondary inherent fluctuations in quantum size, which could be calculated from the differences in the variances. The coefficient of variation derived from the relationship CV = (σ2/μ)1/2/mean (quantal size) ranged from 2.23% to 20%, with a mean of 11.35%. Assuming minimal error in σε estimation, this value is less than the 30% reported (22) for miniature end-plate potentials, although subunits of miniature end-plate potentials (23) and individual components of IA-evoked IPSPs (20) were reported to have a similar small variance. Whatever its cause, the uniformity of quantal size would have been surprising (given the number and distribution of inhibitory afferents) had it not been previously suggested by the adequacy of the binomial fits (10, 24).

The measured quantal currents can be converted to their underlying conductances because, as indicated, the driving force equals the magnitude of the depolarizing voltage step in these experiments. This conductance varied from 27 to 69 nS, with a mean of 46 ± 16 nS (n = 7), which is not statistically different from the estimates (35 ± 14 nS) based upon the binomial analysis of fluctuating IPSPs (10). The >2.5-fold variability in calculated quantal size from one M-cell to the next, coupled with the relative invariance of this parameter in individual experiments, may reflect a global regulation of the neuron’s responsiveness to glycine (25). Finally, assuming that the conductance of a single glycine-activated Cl− channel is 25 pS (26), these values indicate that, on the average, 1840 ± 610 channels are opened at the peak of a miniature IPSC, which is not inconsistent with the hypothesis discussed elsewhere (10, 11) that when a presynaptic action potential causes exocytosis at a single active zone, only the contents of a single vesicle are released.

The values determined directly with voltage clamp for the size of a quantum, spontaneous or miniature, and its underlying conductance correspond to those derived with the indirect statistical analysis of unitary IPSP fluctuations, where the smallest events could not be visualized. Thus, these results give additional credence to the binomial model. The latter, in concert with the morphological data, led to the conclusion that all terminals are functional in the studied system (i.e., there are no “silent” synapses here), in contrast to what was postulated for la motoneuron synapses (18).

Since now two of the release parameters—namely, n and q—appear to be fixed, it follows that p, the average probability of release, is the only one that can be modified as a function of activity (24), at least on a short-term basis.

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