Central Synaptic Integration: Linear After All?

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Unitary synaptic currents in hippocampus show small variability. Experimental evidence suggests that the neuron is endowed with mechanisms to reduce location-dependent differences in amplitude and time course of synaptic events, contributing to small variability. These mechanisms may allow the neuron to count individual quanta and thereby linearize integration of unitary events.

About 50 years ago, Del Castillo and Katz (5) established the classical concept of quantal transmission at the frog neuromuscular junction (NMJ). The authors showed that transmission was quantized, that the size of the quantum corresponded to the spontaneous miniature end-plate potential, that the quantum had a coefficient of variation (variability normalized by the mean; CV) of ~0.2, and that, under their experimental conditions, the statistical nature of transmitter release could well be described by a Poisson-type process. Synaptic transmission at central synapses differs considerably from transmission at the NMJ, where muscle contraction is driven with a high safety factor. Transmission at central synapses is integrative: synaptic events produced over large dendritic trees are integrated over the total membrane area to eventually generate action potentials close to the soma. A key question is whether synaptic integration under these conditions is still linear or nonlinear.

One of the important features of the brain is that it is capable of either implicitly or explicitly performing complicated calculations. An example is the estimation of the trajectory of a football observed in air by a player and his immediate and skillful motor reaction in response to it. The implicit calculations performed require a repertoire of tools either at the cellular or at the network level to implement basic functions such as summation and subtraction. Although it is easy to implement additions and subtractions in neural terms by integrating synaptic charge over the whole of the membrane, a more complex calculation like multiplication requires nonlinearities, since a proportionally larger output per input current (scaling) is required to provide a multiplicative response. A similar line of thought holds true for divisions and correlations. Many investigators have described nonlinearities that could serve these computational requirements.

Theoretical predictions

Before going into details, the subsequent use of the term linear in conjunction with integration of unitary synaptic events needs a qualification. Linear not only expresses summation of currents relayed to the soma of different size but also summation of currents of uniform size. The subsequent text is also based on the concept that this uniform size is caused by a single vesicle from one release site (5).

If the neuron integrates synaptic input in a linear way, each synapse must have equal electrical access to the action potential encoding zone, which, in most cases, is located in the initial part of the axon (18). However, based on theoretical considerations, linear integration seems to be unlikely, because neurons receive thousands of synaptic inputs on elaborate dendritic trees. Based on purely passive dendritic trees, a prediction would be that synaptic potentials close to the soma are faster and larger than inputs generated at a remote dendritic location. This location-dependent attenuation is due to the dendritic cable structure, which filters voltage transients. Indeed, studies in the 1970s showed that, for small synaptic inputs, the dendritic tree behaves mostly passively (7). This means that the only parameters shaping the voltage transients are the leak and the surface area of the membrane. In recent years, however, an increasing amount of evidence accumulated showing that dendrites are not purely passive structures but are endowed with a variety of active conductances distributed along the dendrosomatic axis, among them conductances that have the potential to supply (inward currents) or remove charge (outward currents; for review, see Ref. 9).

To ensure an equal contribution to the generation of action potentials, the variability at a single synapse and between synapses must be small when observed at the soma. However, this is an unlikely scenario for the following reasons. Typically, the same presynaptic fiber establishes contacts on different points on the dendritic tree, potentially giving rise to spatial variability as the synapses are formed on locations with different electrotonic properties. Vesicles, the putative quantal resource, show significant variability in size, and therefore the postsynaptic responses should vary accordingly under conditions in which the postsynaptic receptors are not saturated. Indeed, recent studies show that, after a single stimulus, the occupancy of the postsynaptic receptors with transmitter is significantly less than one (19). In addition, the open probability of synaptic ligand-gated ion channels is less than one, indicating that they might not open, even though transmitter is bound to the receptor. This provides a source of variability in the peak amplitude and time course of the synaptic event, particularly when the number of channels is small. Serial reconstructions at the level of the electron microscope indicate that postsynaptic densities, within which the synaptic ion channels are located, are subject to large variability in size and shape, indicating a further source of variability. Considering all of these factors together, a significant amount of response variability for a vesicle of transmitter would be expected.

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Single-synapse recordings

The consequence of the factors outlined above is that a single quantum should be associated with large variability because, in mathematical terms, the variances of all potential sources add, if they are independent of each other. Indeed, at the beginning of the last decade, there was heated debate as to whether a single quantum showed large variability or not. The answer to this important point lies with the appropriate measuring technique and experimental design. In those days, the whole cell recording technique, which ensures low-noise recordings from cells due to the much smaller series resistance at the tip of the electrode compared with intracellular electrodes, in conjunction with good voltage-clamp amplifiers were introduced to measure unitary synaptic events with good resolution.

Ideally, estimating the variability associated with a single quantum relies on stimulating a single synapse. If any, only a few laboratories have achieved this result. The problem is that contacts between neurons are not made by a single synapse but with a set of a few synapses. In essence, even recordings of pairs of connected cells are not well suited, because in most cases three to eight release sites are found at which transmitter is released.

The issue of recording from a single synapse is confounded by the claim that extracellular stimulation could be capable of discerning single-synapse stimulation from multiple-synapse stimulation. This was particularly the case in experiments in hippocampus. The solution to this problem relies on key experiments in which paired recordings from connected CA3-CA1 pyramidal cells were achieved. With this approach, the presynaptic cell can be stimulated reliably with a short current pulse to generate an action potential, which then causes transmitter release from synapses, the effect of which can be measured in the postsynaptic cell. The average current amplitudes recorded under these conditions were on the order of a few picoamperes. However, extracellular single-synapse stimulations at the same synapse provided currents that were in the range of 20–40 pA, about an order of magnitude larger than the values obtained in paired recordings. Thus it is very unlikely that such recordings were from a single synapse. The conclusion is that recordings from single synapses are unlikely if based either on extracellular stimulation or even on paired recordings.

A compounding problem is encountered in the hippocampus, where it is very difficult to record from pairs of cells. Under such conditions, extracellular stimulation with currents, which produce unitary quantal responses, could hold the key to the answer regarding variability. The disadvantage with this approach is that, to be able to interpret the recorded data, a model is required that explains how the release from more than one synapse shapes the amplitudes observed. To evaluate whether the model is appropriate and capable of explaining the data, statistical techniques are required. We have developed such a statistical framework that allowed different models to be fitted to the data. A systematic evaluation of a hierarchy of models was used to identify the best-fitting model that could explain the data. The models were *1*) unconstrained in amplitude and location, *2*) unconstrained in amplitude with equal separation (quantal), *3*) the same as *2* but with the addition of quantal variance, *4*) equal separation and the amplitudes constrained to conform to a uniform binomial release process, and *5*) the same as *4* but with the release process governed by inhomogeneous probabilities (compound binomial). The competing alternative models relied on underlying densities, which reflected large quantal variance (gamma, Weibull, and cubic transform of a gaussian variable) and in essence provided densities with largely unimodal features. The scheme outlined above also allows the determination of whether there is significant variability. The inherent assumption in this hierarchical system of model comparison is that, if two models fitted the data equally well, the model with the smaller number of parameters is chosen (15).

Peaks and valleys: a tour to spot the quantum

Recordings from a few synapses provide fluctuating responses. If those fluctuations were indeed around multiple integers of the number of synapses involved, and if the signal-to-noise ratio were sufficiently large such that individual events could be distinguished, the densities of the amplitudes would normally show multiple peaks separated by valleys. In Fig. 1, a few distributions from CA1 pyramidal cells are shown (14, 16, 17). The quantum corresponds to the equidistant size between the peaks. If the quantum itself has trial-to-trial variability, the variances should add linearly from the first amplitude to the second and so forth, essentially starting to smear out the large-amplitude modes. In other words, variability has the effect of flattening the valleys between the peaks toward the larger amplitudes. If the variability is caused by location differences, the shape changes in the distributions are not easily anticipated. These rely on intricate interactions between the size at each location and the probability that a quantum was generated at each of those locations.

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Experimental evidence

While establishing the framework for model discrimination, objections to the quantal approach (4) were a stimulus to develop strict criteria for model evaluation and rejection. In fact, we systematically biased all statistical tests toward alternative explanations, which deviate considerably from the classical view. These served as the basis for the null hypothesis. Surprisingly enough, we were able to reject these hypotheses in more than two-thirds of the recordings done in hippocampus (14, 16, 17). A similar result was obtained in a comparable study, in which a slightly modified set of hypotheses was tested (10, 13).

In Fig. 1, a few densities of peak amplitudes are depicted, which were recorded from pyramidal cells in hippocampus

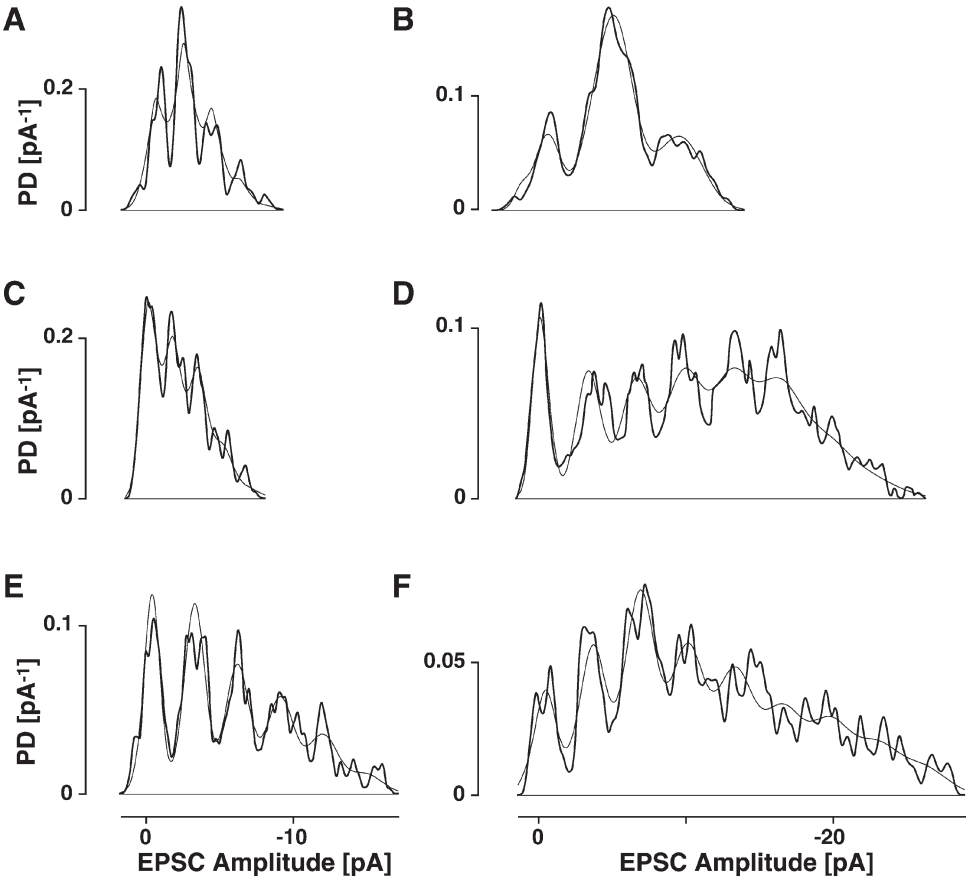

FIGURE 1. A set of 6 excitatory postsynaptic current (EPSC) amplitude distributions (thick line) with clear quantal separations and the best fit of the model to the data (thin line) in rat CA1 pyramidal cells. *A*: quantal separation is 1.9 ± 0.1 pA, with negligible quantal variability. *B*: quantal separation of 4.6 ± 0.2 pA and a variability normalized by the mean (CV) of 0.19 ± 0.03 are shown. *C* and *D*: recordings taken before (*C*) and after (*D*) the induction of LTP. Note the increase in quantal size from 2.7 ± 0.3 to 5.5 ± 0.5 pA. Toward the tail of the distribution in *D*, the amplitude modes start to become less clearly defined (CV 0.19 ± .12). *E* and *F*: same as in *C* and *D*; however, in this set of recordings, the EPSCs were recorded in the presence of 50 μM 2-amino-5-phosphonopentanoate to block *N*-methyl-D-aspartate currents. Note that there is no increase in quantal size (4.2 ± 0.1 vs. 4.4 ± 0.1 pA). In this case, the separation between successive peaks is retained from the control in *E* to the potentiated period in *F*. PD, probability density. Modified from Refs. 14, 16, and 17; reproduced with permission from The Physiological Society, London.

(14, 16, 17). Interestingly, the variability in the distributions is smaller than predicted by the arguments given earlier. The CV measured from such distributions was rarely >0.3; in fact, in most cases it was <0.2, a value close to that obtained for the NMJ. In Fig. 1*A*, there was no necessity for quantal variance. In fact, in many cases, the densities indicated slightly smaller variability than the fitted model suggested (17). In contrast, in Fig. 1*B* the modes did require variability; the CV was 0.19 ± 0.03. Some recordings, particularly those obtained after the induction of long-term potentiation (LTP, Fig. 1, *D* and *F*) (14, 16), a candidate mechanism for memory formation, showed a flattening of the valleys toward the larger amplitudes of the distribution, indicating that the variability associated with the quantum was slightly larger after the induction of LTP than before (Fig. 1, *C* and *E*). Since the expression of LTP is most likely associated with insertion of new ionotropic glutamate receptors of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) type into the postsynaptic membrane, the increase in variability might reflect some immaturity of the synapse. An alternative explanation could be that the occupancy of AMPA receptors by glutamate is smaller at new synapses where AMPA receptors have just been inserted. In fact, there was no systematic difference in quantal variability in our studies between the situations when quantal size increased after the induction of *N*-methyl-D-aspartate (NMDA)-dependent LTP (Fig. 1*D*; Ref. 16) and when the quantal size remained unchanged (Fig. 1*F*) in NMDA-independent LTP (14). These findings indicate that the scaling of the quantal amplitude is subject to precise regulation.

It is surprising how equally spaced the distributions in Fig. 1 are and how little variance is required, as indicated by the clear separation of the modes. This fact suggests that there must be mechanisms at work that compensate for dendritic location of the synaptic input.

A key question is how large the current generated at the synapse was compared with that recorded at the soma. To obtain insight into this question, we used a compartmental model of a CA1 pyramidal cell, with which we could estimate the conductance at the synapse once the electrotonic factors of the cells were determined (17). We calculated that the conductance change was ~1 nS at the synapse and that the number of AMPA channels involved in the generation of the quantum was ~100 channels, a value that was later confirmed by others (6, 12). At the same time, we estimated that <10% of the

current generated at the synapse was recorded at the soma, indicating that most of the current is lost along the dendritic surface. If this were indeed the case, synapses more remote on the dendritic tree would generate a smaller current at the soma than those more proximal. This would result in an increase in variability. However, as the distributions qualitatively suggest, this is not the case.

In fact, we found that if we sorted the excitatory postsynaptic currents (EPSC) into groups of the same amplitude, the rise times and half-widths of the currents were widely different. Remote synapses would have slower rise times and longer half-widths than those closer to the recording electrode. If we plotted the size of the quantum against the electrotonic location on the dendritic tree at which it was generated, we found no correlation. This can only be explained if the size of the quantum is scaled for electrotonic distance on the dendrite. This means that the size of the quantum increases as a function of distance from soma to synapse (17). This finding is illustrated in Fig. 2 and is in line with earlier reports, which showed a similar relationship in motoneurons of the cat spinal cord in vivo (7, 8).

Direct measurement of electrotonic scaling

The above-mentioned studies relied on the interpretation of recordings from the soma to estimate what was happening along the dendrite, closer to the synapse. The advent of the technique to record from soma and dendrites simultaneously allowed a direct investigation of the scaling of the quantum with dendritic distance. Since it is difficult to stimulate extracellular fibers with known synaptic locations on the dendrite, Magee and Cook (11) used spontaneous glutamatergic responses evoked using hyperosmolar solutions to assay the size of the synaptic event. The authors found that the size of the postsynaptic current increased from an average of ~7 pA near the soma to ~25 pA at a location 300 μm out on the dendrite. These distally evoked EPSCs had a faster decay time constant than proximal events, but when measured at the soma the time courses of the two EPSCs were not very different, indicating that not only the amplitude but also the time course of the EPSC were perfectly matched to counterbalance the effect of the dendritic filtering. This finding seems to be at odds with that depicted in Fig. 2*C*. The direct measurements of Magee and Cook were restricted to the apical dendrite and were confined to sampling from relatively small areas. It might be that different time courses, which we interpreted as electrotonic differences on the dendrite, actually reflect inputs from different dendrites. Thus this may indicate that time courses may not be matched perfectly under all conditions.

Potential mechanisms

The finding that the size of the quantum is scaled for electrotonic distance raises the question as to the mechanism(s) involved. There are a number of mechanisms by which location independence can be achieved. Some of these were very elegantly ruled out in the work by Magee and Cook. Among them are dendritic boosting by active conductances; an increase in input resistance in a tapering dendrite, which would result in an increase in the voltage transient caused by a synapse; a change in single channel conductance or kinetics of the AMPA receptors; and an increase in quantal content at a single synapse, suggesting that more than one vesicle per synapse might be released (19). The most likely candidate seems to be an increase in quantal size with distance from the soma. If the scaling of the quantal size were the mechanism, either an increase in the size of the postsynaptic densities or an increase in AMPA receptor density in those scaffolds would be observed morphologically. Unfortunately, to date, there are no systematic investigations into receptor density or the size and variability of excitatory (glutamatergic) postsynaptic receptor patches at different dendritic locations. However, the latter topic has been explored for inhibitory terminals. Triller et al. (20) found, using confocal microscopy with an antibody against glycine receptors, that, on the Mauthner cell in the spinal cord of the fish, the size of the postsynaptic density increased with dendritic distance from the soma. A similar result was found by Alvarez et al. (1) in cat spinal cord. Both

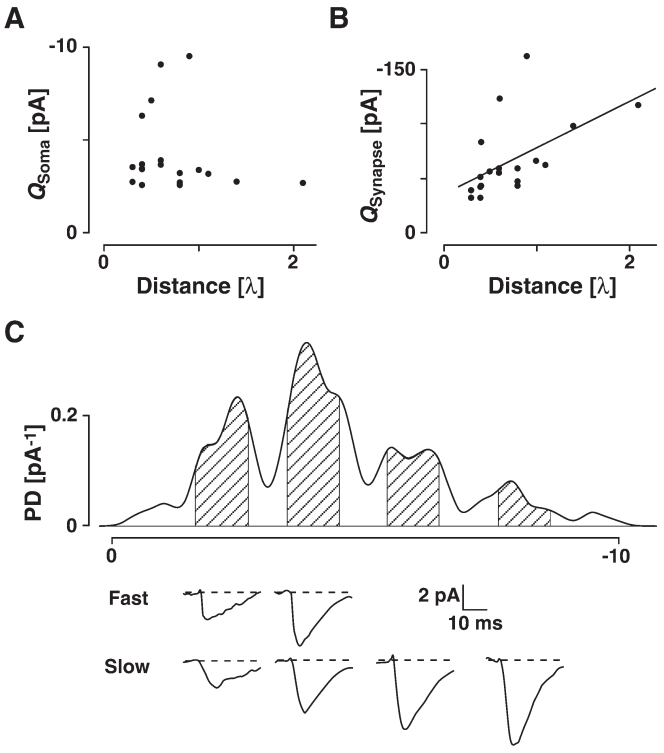

FIGURE 2. Quantal size scales for dendritic distance. *A*: relationship between quantal sizes measured at the soma vs. electrotonic distance (l). The lack of correlation indicates that the quantal current is scaled for electrotonic distance. *B*: this time, the current at the synapse is plotted against electrotonic distance. There is a significant correlation (straight line; $P = 0.02$), indicating that the current at the synapse is scaled for distance. *C*: different quantal amplitudes are separated into EPSCs of fast and slow time courses. The shaded areas indicate the boundaries, within which quantal amplitudes of sizes 1, 2, 3, and 4 were identified. Below are some individual filtered traces that fall within those boundaries. Note that fast amplitudes are only seen for small-sized EPSCs. If the kinetics of the EPSCs reflect electrotonic attenuation, this graph illustrates, in a different way, that the quantal size is independent of synapse location. Modified from Ref. 17; reproduced with permission from The Physiological Society, London.

studies strongly suggest that there are cellular mechanisms at work that determine the size of a postsynaptic density as a function of distance from the soma.

An interesting question that then arises is how the mechanism(s) for protein insertion into the dendritic membrane senses distance from the soma. A few potential candidates are at hand, which could serve to implement the rule observed. It is known from dual recordings of soma and dendrite that distal branches are slightly more depolarized than proximal regions of dendrites (2). This electrical gradient from dendrite to soma might serve to govern protein insertion. At the same time, the input resistance increases from soma to dendrite, implementing larger voltage changes per current injected at remote synapses. This could lead to differential activation of channels, through which a signaling molecule such as calcium could flow. An alternative view might be that trophic or metabolic factors in small dendrites are sufficiently different from those at the soma or express a gradient that could govern AMPA receptor insertion. The mechanism here is probably similar to the one that regulates the insertion of voltage-dependent channels. It is known, for example, that the density of the hyperpolarization-activated cation channel (I_h) is adjusted along a somatodendritic gradient, with the highest density found far out on the dendrite (2). Similar observations have also been made for other channels (for review, see Ref. 9).

The experiments in which LTP was induced (14, 16) also shed light on the time requirements and the precision with which the quantal size is scaled. After the induction of LTP, the increase in the quantal size is seen immediately after the induction paradigm, suggesting that the mechanism(s) operates on a second-to-minute time scale. Since in our recordings we started with small AMPA currents during the control period, AMPA receptors were most likely inserted as a consequence of induction of LTP. Therefore, newly inserted or already inserted but silent AMPA channels must immediately reach the same quantal size at newly inserted receptors as already-operating receptor patches, which produced a smaller quantal size during control than after the induction of LTP. This indicates that it is very likely that a common signal relays both the increase in and the insertion of AMPA channels into the membrane. Since no major increase in variability is seen after the induction of LTP, quantal variance remains small and, therefore, the mechanism works with surprising precision.

Since recent studies have shown that both AMPA and NMDA receptors are far from being saturated (19), at least one boundary condition arises. Either receptor saturation does not play a significant role (i.e., the variability is not as appreciable as predicted from fast-perfusion experiments) or there are compensatory mechanism(s) at work that set a ceiling to the quantal response, no matter how many receptors are involved or if they are saturated. This idea begs the question as to where quantization arises: from the presynaptic filling of vesicles, from the presynaptic emptying of the vesicle (kiss-and-run), from the size of the postsynaptic receptor patch, or from the postsynaptic response to transmitter (all-or-none). Even after 50 years of investigating synaptic transmission, this question is still largely unanswered.

Physiological implications

The finding that the quantum is associated with a small amount of variability because, among many potential factors, it is scaled for electrotonic distance suggests that the postsynaptic cell has the possibility of using a discrete system for transmission. It points toward a scenario in which the neuron uses intricate mechanisms to make synaptic transmission linear: an input close to the soma and one remote in the dendrite add up to a quantal value of 2. Above all, not only do the peak amplitudes add linearly, but the time courses do as well. This set of mechanisms is designed to linearize the postsynaptic response. It might be required to efficiently cancel imprecisions, which could arise during synapse formation.

It has to be stressed, however, that in the set of experiments reported here, small currents were evoked, which would have resulted in subthreshold activation of the neurons. It might well be that nonlinear interactions between synaptic inputs could arise if they were synchronous and sufficiently large to activate active dendritic conductances. Therefore, our experiments only covered a range within which the system performs almost linearly. To counter this argument, there are observations available that show that the interaction between large amplitudes is still close to linear and that, if some of the voltage-dependent conductances are blocked, significant deviations from linearity can be observed (3). These observations, together with the scaling of the quantal size for dendritic distance, suggest that, in fact, there are a number of nonlinear interactions employed to ensure an almost linear behavior of the neuron over a remarkably large range.

One functional consequence might be that more complex calculations like multiplications and divisions are not performed at the single-cell level, because these would require significant deviations from the linearity observed. This type of computation is most likely generated within small networks of neurons.

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