Doubts About Quantal Analysis

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TO THE EDITOR: In an impressive series of articles, Korn and co-workers (e.g., Faber and Korn 1988; Faber et al. 1992, 1995; Korn et al. 1981, 1982, 1987; reviews in Korn and Faber 1991, 2005) described electrophysiological recordings performed on goldfish Mauthner cells and adjacent inhibitory interneurons. In their founding article, published in the Journal of Neurophysiology, Korn and colleagues (1982) developed a formal analysis of the amplitude fluctuations of inhibitory postsynaptic potentials (IPSPs) produced by directly evoked presynaptic impulses. According to these analyses, the IPSPs follow a binomial distribution whose parameters can be determined unambiguously. In particular, Korn and co-workers claimed that the value of \( n \) obtained by selecting the binomial distribution that best fit the data was equal to the number of stained presynaptic boutons that had been counted anatomically (see Fig. 1, redrawn from Korn et al. 1982). They suggested that a single bouton can release only one quantum of transmitter per impulse; this idea has been influential but has remained controversial. The aim of this letter is to raise doubts about the validity of aspects of this seminal paper, rather than to review the subsequent literature on “one bouton one quantum,” which has accreted around it.

The very close agreement that Korn and co-workers obtained between the anatomically counted number of synapses and the \( n \) deduced from indirect analyses of electrophysiological recordings (see Fig. 1) is almost miraculous considering: 1) the difficulty of manually extracting precise slopes from zigzagging electrophysiological recordings that display fluctuations at two or three different scales (see Fig. 5 in Korn et al. 1982). Indeed, Korn and colleagues were ultimately led to develop automated procedures (Ankri et al. 1994; Korn et al. 1993) and 2) the lack of precision in the histograms that describe the slopes’ distributions. On average, one histogram represented data pooled from 160 events and such data were used to discriminate between, say, binomial parameters \( n = 17 \) and \( n = 18 \).

My main objection to the work of Korn et al. is derived from a close examination of their published theoretical curves. As I will show, several theoretical curves were simply not what Korn and co-workers claimed them to be. Other curves, given as unique solutions to an optimization algorithm on \( n \) and \( p \) were not unique because curves for other \( n \) and \( p \) values would have fit the data just as well. The inadequacy of the theoretical curves provided by the authors could not be perceived by the average reader because readers were not given the precise numerical values of the parameters needed to generate the curves. In the present work, an extensive exploration of the parameter space was performed, allowing me to evaluate the hidden parameters, and cast doubt on some of the authors’ claims.

METHODS

The experimental system

The electrophysiological recordings were performed by Korn et al. (1981, 1982) and Faber and Korn (1988) on Mauthner cells from goldfish and adjacent interneurons. The recordings pertained to the inhibitory postsynaptic potential (IPSP) in response to directly evoked presynaptic impulses.

Quantal analysis according to Korn et al. (1982)

Each electrophysiological recording provides an experimental IPSP amplitude. The distribution of the observed experimental values for a given cell is compared with either a Poisson distribution with the same mean or to various binomial distributions.

The binomial distributions used by the authors have four explicit parameters:
1) \( n \), the degree of the distribution
2) \( p \), the probability of synapse release
3) \( q \), the size of the quantum released by a synaptic vesicle, expressed in milli- or microvolts
4) \( \sigma \), a Gaussian spreading factor assumed to account for the fluctuations of the quanta

For a discrete binomial distribution with two parameters \( n \) and \( p \), and no spreading parameter \( \sigma \), the probabilities of having a number \( i \) of hits are given by the recurrence formulas:

\[
b(0, p) = (1-p)^n \quad (1)\\
b(i, p) = b(i-1, p)[(n+1-i)p/(1-p)] \\quad (2)
\]

In the notations of Korn et al. (1982), \( b(i, p) \) becomes \( \Phi_i(i) \).

For the continuous distribution with four parameters \( n \), \( p \), \( q \), and \( \sigma \), the probability density function is given by

\[
y(x) = \sum b(i, p) \exp\left(-\frac{(x-\text{iq})^2}{2\sigma^2}\right) \\quad (3)
\]

in which the sum (\( \Sigma \)) is computed from \( i = 0 \) to \( i = n \) and \( \delta \) has the value

\[
\delta = (2\pi)^{1/2}\sigma \\quad (4)
\]

Korn et al. 1982 introduce a further complexity in the models, by considering that as the number \( i \) of released quanta increases, the resulting IPSPs are initially proportional to \( i \), then follow a law of diminishing returns. Technically, they introduce a “quantal saturation” correction. When it is applied, the \( \text{iq} \) in \( \text{Eq. 3} \) is replaced by \( \text{iqE}/(\text{iqE} + E) \), where \( E \) is the driving potential (see \( \text{Eqs. 1}', 2', \text{and 3}' \), in Korn et al. 1982).

In the absence of a corrective factor, the distribution’s peaks must occur at the abscissa values 0, \( \text{q}, 2\text{q}, 3\text{q}, \ldots, n\text{q} \). Therefore the distance between the summits of consecutive peaks equals \( \text{q} \). In the presence of a corrective factor, the distances between consecutive summits must decrease as \( \text{iq} \) values increase. All peaks were perfectly spaced in the published theoretical curves, and well positioned with respect to the origin, implying that no corrective factor had been used in their generation. Actually, the authors do indicate rather late in their article that “Application of the correction factor for nonlinear summation of the quanta had little effects on the results except for 2 of the 26 neurons studied” (Korn et al. 1982) and none of the neurons for...
I investigated directly measured on the histograms, was used here to convert the and the theoretical curves can be expressed in events/mV. The bin width, heights of the bins of the histograms, these numbers are reliable. The of events used to generate the histograms. Judging from the relative follow may be checked directly by readers—although less precise—on the original figures. The authors always provided the total number which correction effects are documented (Table 4) belongs to the list of neurons for which histograms and theoretical curves are presented (Table 3).

Computer simulations

To reproduce the experimental histograms and generate the theoretical curves, an interactive computer graphics program was written in the C++ language with OpenGL graphic instructions and run on a Hewlett-Packard computer. This program will be sent on request. A simpler program, in C++ with a postscript output, is provided here as Supplementary material.\(^1\)

At any time, the curves and the histogram displayed on the screen could be saved in a postscript file. The diagrams shown here in Figs. 2 and 3 were thus generated on the fly. All four parameters of the binomial distributions could be incremented or decremented by pressing appropriate keys on the keyboard. The experimental data were obtained as follows: The original figures were enlarged using a photocopier machine and the histograms were measured under a magnifying glass with a finely divided ruler, then tabulated. Although photocopier can introduce some distortions, these will not affect the conclusions because what follows may be checked directly by readers—although less precisely—on the original figures. The authors always provided the total number of events used to generate the histograms. Judging from the relative heights of the bins of the histograms, these numbers are reliable. The graduations on the ordinate axes were made in events/millivolt (mV), instead of events/bin, which makes sense, because both the histograms and the theoretical curves can be expressed in events/mV. The bin width, directly measured on the histograms, was used here to convert the events/bin units into events/mV.

All theoretical curves were generated using the computer program. I investigated 1) whether the theoretical curve proposed by the authors could be simulated by a binomial distribution having the same \(n\) parameter and, if possible, \(p\) and \(q\) values compatible with those given by the authors. In other words, I tried to determine whether the published curves were what they were supposed to be; and 2) whether other binomial curves for different values of \(n\) could fit equally well the experimental histograms. In other words, I tried to determine the validity of the claim that quantal analysis could provide a unique estimate of \(n\).

Concerning \(\sigma\), Korn et al. (1982) mention that it is usually between 30 and 90 \(\mu\)V, and roughly equal to 10% of the mean amplitude of the evoked response, thus 10% of the \(npq\) product. Whatever the precise value of \(\sigma\), it has no incidence on the positions of the peaks: it affects only their widths and, indirectly, their heights (at constant surface of the histograms). Because the parameter \(\sigma\) was not generally provided by the authors, it had to be determined by simulation. In the graphical plots provided by the authors, the peaks of the binominal distribution are in general well separated (implying a relatively small \(\sigma\)). Because the interval between peaks must be equal to \(q\), this parameter is determined without ambiguity on the published theoretical curves. The parameter \(p\) has a strong influence on the relative heights of the successive peaks. So it affects the shape of the envelope’s distribution. Increasing \(p\) gives more weight to the rightmost peaks. Once \(q\) is fixed, \(\sigma\) controls the peak width. Thus having measured \(q\) on the figures, and allowing for only minor variations of \(q\), for each arbitrary value of \(n\) there is little latitude in the choice of \(p\) and \(\sigma\). The first parameter controls the relative heights of the successive peaks; the second parameter controls the resolution of the curve into distinct peaks.

RESULTS

Curve by curve analyses

There are two histograms in Korn et al. (1981); eight histograms in Korn et al. (1982); and three histograms in Faber and Korn (1988). They are labeled in abridged form according to the publication from which they are extracted (“Science” for Korn et al. (1981); “KTMF” for Korn et al. (1982) and “FK” for Faber and Korn (1988)) and according to the figure number and panel label in these publications. In each case, I indicate the number of events on which the histograms are based, as indicated by the authors, and the number of bins in the histograms. The two histograms of Fig. 15 in KTMF, in which the binominal curves use the anatomical \(n\), are left out of the present study.

SATISFACTORY CASES. Histograms in Science, Fig. 3A1 and KTMF, Fig. 7B (182 events, 19 bins).

They represent the same experiment. My simulations using a \(\sigma\) value (not provided by the authors) of 63 \(\mu\)V gives a visually best fitting curve with \(n = 6, p = 0.47, q \approx 305 \mu\)V accounts well for the published curve. In the legend to their Fig. 5, the authors indicate that \(\sigma = 61 \text{ or } 62 \mu\text{V}\). A very close fit to the published curve is also obtained with \(n = 5, p = 0.392, q = 262 \mu\text{V}\), and \(\sigma = 61 \mu\text{V}\).

CASES ALLOWING ALTERNATIVE BINOMIAL INTERPRETATIONS. Histogram in KTMF, Fig. 9D, bottom (133 events, 14 bins).

The binomal curve for \(n = 4, p = 0.46, q = 267 \mu\text{V}\) (rather than 280 \(\mu\text{V}\)), and \(\sigma = 60 \mu\text{V}\) accounts well for the published curve. In the legend to their Fig. 5, the authors indicate that \(\sigma = 61 \text{ or } 62 \mu\text{V}\). A very close fit to the published curve is also obtained with \(n = 5, p = 0.392, q = 262 \mu\text{V}\), and \(\sigma = 61 \mu\text{V}\).

HISTOGRAM IN KTMF, FIG. 11B (176 EVENTS, 20 BINS). The value of \(q\) derived from the interpeak distance is 147 \(\mu\text{V}\). It is significantly different from the \(q = 170 \mu\text{V}\) given by the authors. Furthermore, the highest peak has a summit at 750 \(\mu\text{V}\). With a 147-\(\mu\text{V}\) interpeak value for \(q\), we have \(5q = 735 \mu\text{V}\), whereas with \(q = 170 \mu\text{V}\), we would have \(5q = 850 \mu\text{V}\). The peak would thus be shifted by more than one bin with respect

\(^{1}\) The online version of this article contains supplemental data.
to its position in the published curve. I can reproduce satisfactorily the published curve with $n = 19$, $p = 0.281$ (instead of 0.260), $q = 148 \mu V$ instead of 170 \(\mu V\), and $\sigma$ (not provided by the authors) = 55 \(\mu V\). However, I also have a reasonably good simulation with $n = 16$, $p = 0.286$, $q = 177 \mu V$, and $\sigma = 70 \mu V$ [Fig. 2(a)]. The single-peak curve $n = 13$, $p = 0.409$, $q = 150.5 \mu V$, and $\sigma = 142 \mu V$ and the Poisson distribution $m = 5.5$, $q = 148 \mu V$, and $\sigma = 52 \mu V$ also give a reasonably good fit (figure not shown).

**HISTOGRAM IN KTMF FIG. 13C, BOTTOM (192 EVENTS, 14 BINS).** The parameters given by the authors for their theoretical binomial curve are $n = 11$, $p = 0.4$, and $q = 90 \mu V$. Taking $\sigma = 33 \mu V$, their curve is well simulated with $n = 11$, $p = 0.41$, and $q = 94.2 \mu V$. However, the histogram is just as well approached with the binomial curve $n = 13$, $p = 0.35$, and the same $q$ and $\sigma$ [Fig. 2(b)]. It is also well approximated with the single-peak distribution $n = 13$, $p = 0.402$, $q = 81.1 \mu V$, and $\sigma = 52 \mu V$ (figure not shown). In the legend to their Fig. 5, the authors indicate that $\sigma = 27.5$ to 31.5 \(\mu V\).

**HISTOGRAM IN FK FIG. 8B (350 EVENTS, 10 BINS).** The parameters given by the authors for their theoretical binomial curve are $n = 13$, $p = 0.55$, and $q = 260 \mu V$. Taking $\sigma$ (not provided by...
FIG. 3. Severe anomalies in quantal analyses. Two histograms [(a) and (b) represent the same data, taken from 2 different publications. Histogram in (a) was truncated in the original publication. It has been extended here to the right by similarity with the histogram in (b). From (a) to (b), the histogram was subjected by Korn and colleagues to linear transformations along both the x-axis and the y-axis. Yet the best-fitting binomial curve was presented with exactly the same parameters: $n = 11, p = 0.62$, and $q = 0.080 \text{ mV}$. (c) Continuous binomial curve for $n = 10, p = 0.547$ seems to match rather well the $n = 10$ binomial curve in KTMF, and the $n = 7$ dashed binomial curve also gives a very close fit. On the other hand, the dotted $n = 10, p = 0.51$ binomial curve is very close to that given in KTMF as Poisson distribution. Both the top curve and the bottom curve in KTMF are also very well approached for Poisson curves, for parameters given in the text. (d) Here, the value $n = 13$ proposed by Faber and Korn (1988) has special significance because their proposal that both $n$ and $p$ are unaffected by strychnine was the main conclusion of their article [compare with Fig. 2(c)]. However, their published curve is better simulated with the dashed $n = 12$ curve shown here.

HISTOGRAM IN FK FIG. 9B (152 EVENTS, 11 BINS). The parameters given by the authors for their theoretical binomial curve are $n = 17, p = 0.32$, and $q = 165 \text{ mV}$ (very close to the bin width). Taking $\sigma$ (not provided by the authors) $= 70 \text{ mV}$, their curve is well simulated with the same $n$ and $p$ values, and $q = 159 \text{ mV}$. However, the histogram is just as well approached with the binomial curve $n = 13, p = 0.372, q = 181 \text{ mV}$, and $\sigma = 76 \text{ mV}$ [Fig. 2(d)].

CASES RAISING SERIOUS PROBLEMS. Histogram in Science Fig. 1B5 (150 events, 10 bins). In this figure, the binomial theoretical curve has a main peak around 630 $\mu$V. With the value of $q$ given by the authors (80 $\mu$V), one expects to find a peak at 8q, thus 640 $\mu$V. However, the value of $q$ determined from the interpeak separation differs: it is close to 88 $\mu$V. With this value, the 7q peak should be at 616 $\mu$V. Refined fitting to the histogram to reproduce as closely as possible the theoretical
n = 11 curve presented by Korn et al. (1981) gives p = 0.62, as in their article, but q = 92 μV instead of 80 μV, and σ = 36 μV. On the other hand, if I stick to q = 80 μV, I can fit the data as well with n = 14, p = 0.556, and σ = 36 μV [Fig. 3(a)]. There is also a scaling problem on the y-axis. The indicated numbers of events/mV are too large by about 30% [compare with my Fig. 3(a)]. However, the scale cannot be changed arbitrarily because the surface of the histogram, using the “events/mV” scale on the y-axis, must give the total number of events.

HISTOGRAM IN KTMF, FIG. 13C, TOP (150 EVENTS, 13 BINS). It represents the same data as the previous histogram. However, the histogram has been contracted here horizontally by 10% and shifted to the right by 0.15 bin with respect to the previous one. The interpeak interval was also changed from 80 instead of 80 μV in the author’s first theoretical curve to 85 μV in the second one. The scaling of the y-axis was also altered, and it became consistent with the number of events. All these changes which affect the data were not justified by the authors. My simulation of the author’s theoretical curve gives n = 11 and p = 0.62, but q = 85 instead of 80 μV, and σ = 32 μV. (The smaller σ and q values here result from the contraction of the histogram.) The histogram is just as well approached with the single-peak curve n = 10, p = 0.685, q = 85 μV, and σ = 55 μV [see here Fig. 3(b)].

Curiously, although the data had been subjected to linear transformations both along the x- and the y-axes, from one histogram to the other, the fitting curves are presented by Korn and co-workers with exactly the same set of parameters n = 11, p = 0.62, and q = 80 μV. Independently of any detailed analysis, it is obvious that at least one of the two fitting theoretical curves cannot be what it is claimed to be.

HISTOGRAM IN KTMF, FIG. 10B (132 EVENTS, 7 BINS). This figure is very confusing. The authors represented a Poisson distribution as a dashed curve and a binomial distribution as a continuous curve. Both curves are very regular and have a single peak, which is higher for the binomial distribution. There are several anomalies here. According to the author’s graduations on the ordinate axis, their histogram culminates around 650 events/mV, whereas according to my simulations [Fig. 3(c)] it culminates around 490 events/mV. The parameters provided by the authors for their binomial distribution are n = 10, p = 0.51, and q = 80 μV. With a p value close to 0.5, the summit should have been at 5q, thus 400 μV. It is instead at 375 μV, which corresponds to q = 75 μV (equal to the bin width). Keeping n = 10, the binomial curve that best matched the published curve had substantially different p and q values: p = 0.547, q = 66.9 μV, and σ = 41 μV [continuous curve in Fig. 3(c)]. The published curve is nearly as well simulated with n = 7, p = 0.641, q = 80 μV, and σ = 48 μV [dashed curve in Fig. 3(c)]. The other curve in Fig. 10B is well described with the Poisson parameters (not provided by the authors) m = 11.7, q = 32 μV, and σ = 58 μV. One also gets a nearly perfect fit to this curve using a binomial model with n = 10, p = 0.51, q = 71.7 μV, and σ = 44 μV [dotted curve in Fig. 3(c)]. More disturbingly, the histogram and the first binomial curve are well approximated by the Poisson distribution m = 12.0, q = 0.031, and σ = 35 μV (the maximum separation between the corresponding cumulated probability curves is <2%). In other words, although the authors had represented a couple of quite distinct curves, one being an optimized binomial curve and the other one being an optimized Poisson curve, each curve of one kind can very well be approximated by a curve of the other kind.

HISTOGRAM IN FK, FIG. 8C (199 EVENTS, 11 BINS). Korn and Faber interpret the histogram with a binomial curve for which they give the parameters n = 13, p = 0.48, and q = 177 μV. Keeping n = 13, and using a σ (not provided by the authors) = 71 μV, one comes near the published curve when p = 0.45 and q = 163 μV (very close to the bin width). However, their own curve is much better simulated with n = 12, p = 0.497, q = 163 μV, and σ = 70 μV [Fig. 3(d)].

Statistical evaluations

Leaving aside the cases, illustrated in Fig. 3, that raise serious problems, the reader may criticize my “eyeball fitting” treatment of the milder cases illustrated in Fig. 2. But how does one evaluate the relative merits of the alternative fitting curves in the absence of the original data? Fortunately, the Kolmogorov criterion used by Korn et al. (1982) makes it possible to evaluate rigorous bounds on the goodness-of-fit indicators, whatever the detailed distribution of events within the bins of the histograms. The Kolmogorov test is applicable to cumulative distributions. The theoretical curves and the histograms of Fig. 2 have been replotted in cumulated form in Fig. 4. Thus instead of representing probability density functions, the theoretical curves now represent the probability that the amplitude of a response is inferior or equal to the value given in the abscissa. The statistical indicator d of the Kolmogorov test is merely the maximum height difference (in absolute value and measured at the experimental points) between the experimental and the theoretical cumulated distributions.

Let us consider three distributions labeled 1, 2, 3, and let d12, d13, and d23 be the indicators related to the three couples. Knowing two of the values allows us to say something about the third, thanks to the triangle inequalities. Suppose we determine the height differences (in absolute values) h12, h13, and h23 between the distributions at a given abscissa, any of the three h values is inferior to the sum of the two others. For instance, h12 ≤ (h13 + h23). By definition, d13 ≥ h13 and d23 ≥ h23, thus h12 ≤ (d13 + d23) everywhere. In particular, because h12 = d12 at the point of maximum height separation between distributions 1 and 2, we are certain that d12 ≤ (d13 + d23). I will use this type of reasoning to find upper bounds on the d values for my alternative fitting curves.

Let us call now 1 and 2 the two theoretical distributions to be compared, and let us use the label e for the distribution derived from the experimental measurements. The experimental results are best represented as a stepwise function, each event giving rise to an additional step of unit height. Due to the regrouping of the events into the bins of histograms, there is some uncertainty concerning the exact shape of this function. However, it is entirely included within the gray-shaded areas that in the panels of Fig. 4 represent the consecutive bins of the histograms in cumulated form. So, we do not have direct access to the experimental distribution, but we have upper and lower bounds on this distribution for the various bins of the histogram.

Let d be the maximum height difference between an experimental distribution, and the theoretical distribution to which it
is compared. The correspondence between the $d$ values and the statistical confidence levels can be found in standard tables. If $N$ is the number of events collected in the experiment, and $n$ is the square root of $N$, then the probability $P$ that $d$ exceeds a value $z$ is 1% for $z/H_110051.63/n$, 5% for $z/H_110051.36/n$, 10% for $z/H_110051.22/n$, and 15% for $z/H_110051.14/n$ (taken from Table A21 in Dixon and Massey 1957). The left black icons in each panel of Fig. 4 indicate the height differences that correspond to the 15, 5, and 1% values of $P$, given the number $N$ of events used to construct the histogram. The cutoff value used by Korn and co-workers for accepting or rejecting a theoretical curve seems to be 5%. So it corresponds to the intermediate level in the icon.

The maximum height separation $d_{12}$ between the two theoretical distributions is easy to compute. It is shown graphically in the panels of Fig. 4 as the central black rectangle, within the group of three vertical rectangles to the right of the confidence levels icon.

The $P$ values between the detailed experimental distributions and the best-fitting binomial distributions proposed by Korn and co-workers were usually provided in the legends to their figures. We have $P > 0.75$ for (a), $P > 0.07$ for (b), and $P > 0.05$ for (d). These values appear to be reliable. The corresponding $d_1$ values are shown graphically as the leftward black bar represents the probability level associated with the first distribution, usually taken from the original publications of Korn and colleagues. Intermediate black rectangle represents the maximum separation between the couple of theoretical distributions. Rightward black rectangle gives the maximum separation between 2 curves derived by incrementing or decrementing $q$ by 5% in the first curve of each panel. Arrows signal 2 bins in which an extreme bias of the events within the bin may create a goodness-of-fit indicator in favor of the first theoretical distribution.
and the fourth, around 1.4 mV. It is this difference that is reproduced exactly in the leftmost black rectangle in (c). It corresponds to $P < 1\%$, indicating that the binomial distribution should have been rejected, according to the criterion of Korn and colleagues.

Knowing $d_{e1}$ and $d_{e2}$, our task is now to evaluate the third term, $d_{e2}$, the statistical indicator for the comparison between the experimental distribution (unknown in detail) and my alternative distribution. We will scan the bins of the histograms, in the four panels, trying to detect configurations that may generate a $d_{e2}$ leading to $P < 0.05$ or a $d_{e2}$ significantly larger than $d_{e1}$.

For all bins located at the beginning or the end of the histogram in the four panels, the height difference between the bottom or the top of a bin and the dashed curve is always smaller than $d_{e1}$. So these bins cannot produce a $d_{e2} > d_{e1}$ and, presumably, $d_{e1}$ is not created there.

For more central bins, although the dashed curve may seem to run ideally across the shaded areas of the bins, it is conceivable that, due to a very uneven distribution of the events within a bin, a large $d_{e2}$ is created at some point there. However, if there is a near coincidence between the two alternative curves, which is often the case in the central bins, the height differences between the experimental distribution and the two theoretical distributions are nearly equal there, so they cannot give rise to a $d_{e2}$ significantly larger than $d_{e1}$.

Therefore a $d_{e2}$ significantly larger than $d_{e1}$ may be created only in the bins in which three conditions are fulfilled: 1) the two theoretical distributions have a significant height difference, 2) the height difference between the top or the bottom of the bin and the dashed curve is larger than $d_{e1}$, and 3) the relative positionings of the three elements are compatible with $d_{e2} > d_{e1}$.

A conjunction of these three necessary conditions occurs on the right side of the seventh bin of the histogram of (c) (signaled by a horizontal arrow), if most of the 65 recorded amplitudes are clustered at the extreme right of the bin, which is extremely unlikely. Otherwise, the situation is to the advantage of the dashed curve. Another conjunction occurs on the left side the 10th bin of the histogram in (a) (signaled by a horizontal arrow), if most of the 20 recorded amplitudes are clustered at the extreme left of the bin, which is unlikely. Otherwise, the situation is also to the advantage of the dashed curve. Therefore excluding highly biased distributions of the recorded events within the 10th bin of the histogram in (a) or the seventh bin of the histogram in (c), there is no way to create a $d_{e2}$ clearly larger than $d_{e1}$.

Moreover, if Korn and co-workers claim that the events within the 10th bin in (a) were really clustered to the left, it is easy to produce an alternative fitting curve that coincides with their curve in the critical region. For instance, the binomial curve $n = 9, p = 0.462, q = 0.0279$, and $\sigma = 0.070$ is interlaced with their $n = 17$ curve, and the maximum separation $d_{i2}$ between the two curves is 1.5%. Even if we do not focus on the critical region, knowing that $d_{e1} \leq 7.6\%$, we deduce that $d_{e2} \leq 9.1\%$ and, because there were 176 events in the histogram, we are certain that $P \geq 15\%$ for this $n = 9$ curve. With respect to (c), I was not able to construct a satisfactory interlacing $n = 11$ curve, due to the pronounced character of the peaks and the troughs of the $n = 13$ curve. At worst, because $P \leq 1\%$ for the reference curve, both the reference curve and the alternative curve are rejected.

It should be noted that although the probability density functions in Fig. 2 are very sensitive to the values taken by $\sigma$, the cumulated distributions in Fig. 4 are relatively insensitive to changes in $\sigma$. A pair of curves, generated from the continuous curves of Fig. 4 by incrementing or decrementing $\sigma$ by 20\%, has a maximum separation of 1.4. [The relative insensitivity to $\sigma$ was noted by Korn et al. (1982).] The cumulated curves are more sensitive to changes in $q$. Incrementing or decrementing $q$ (at constant $pq$) by 5\% creates maximum separations around 3\% (shown as the rightward rectangles in the triplets).

What can be said now about one of the author’s major claim: that Poisson distributions are ruled out? With rare exceptions (e.g., KTMF 10 or KTMF 11B), the histograms cannot be modeled with Poisson distributions because these distributions usually have a substantial extension to the right of the histograms. However, the use of a quantal saturation correction, in which $iq$ is replaced by $iqE/(iq + E)$, would tend to compress the right side of the Poisson distribution and possibly help to improve the fit with the histograms.

Restricting ourselves to binomial distributions, it is now clear that the histograms cannot provide an unambiguous determination of the parameter $n$. The Kolmogorov test has little power to discriminate between the best-fitting curve for binomial $n$ proposed by Korn and co-workers and alternative binomial curves for other values of $n$. However, all my examples of alternative curves shown in Figs. 2 and 3 are still compatible with a rough equality between the binomial $n$ and the anatomical $n$. The result, if correct, would still be very important.

However, it will be noticed that none of the eight histograms in Figs. 2 and 3 allows one to “read” the value of $n$. The histograms are compact. The bin width is often close to $q$ [Figs. 2, (c) and (d) and 3, (c) and (d)] so it is much too wide to make visible the peaks and the troughs of the postulated binomial distribution. How then do the numerical simulations lead to estimates of $n$? Let us take for instance the case of FK Fig. 9B. An $n = 17$ value was proposed by Faber and Korn (1988), and I showed in Figs. 2(d) and 4(d) that $n = 13$ was equally good. I now examine a still wider range of $n$ values. In Fig. 5(a), I show how the histogram may be modeled with $n = 8$ or $n = 34$, and the Kolmogorov analysis in Fig. 5(b) indicates that both binomial curves fit the data at least as well as the original binomial curve of Faber and Korn (1988). The $n = 34$ curve requires $\sigma = 275 \mu V$, which is much larger than the $q/2$ or $q/3$ value most often used in the simulations of Korn and co-workers. So, accepting or excluding $n = 34$ ultimately rests on the trust we place in the Gaussian spreading factors.

An essential assumption made by Korn and co-workers is that there is a unique $\sigma$ in each experiment, whatever the number of released quanta. Mathematically, the $\sigma$ used in Eq. 3 describes the dispersion of the amplitude of the IPSPs recorded in the same experiment, when a well-defined number of quanta are released. Because $i$ cannot be directly measured, Korn et al. use an indirect measure of $\sigma$ derived from recordings of the background noise (Fig. 5 in Korn et al. 1982).
a first article [Fig. 3(a)] appears in a second article shifted and compressed [Fig. 3(b)]. The anamorphosis applied to the data was not justified by the authors. The theoretical curves were redrawn accordingly, yet presented with exactly the same set of parameters.

2) In several cases (Fig. 2) a binomial distribution with a definite n value was proposed as the unique outcome of an optimization procedure. However, as shown here, other binomial distributions with different n values fit the data equally well.

3) In at least one case, the authors presented a binomial curve that was supposed to correspond to a certain value of n, whereas the simulations indicate that it was a binomial curve for another value of n [Fig. 3(d)]. In other cases [Figs. 2(a) and 3(c)] the proposed n value was plausible, but the indicated p or q values could not be correct.

I add here that in contrast to all the numerical problems enumerated earlier, the mathematical developments in Korn et al. (1982) are written with care and reflect a rather good level of mathematical competence. Two reproaches can nevertheless be made: 1) The authors presented their optimization algorithm in a misleading manner. They claimed that they had minimized “the effects of background noise through a deconvolution process.” Actually, they worked the usual way, taking hypothetical binomial or Poisson distributions, multiplying them by a spreading function (this operation is a convolution, not a deconvolution), and comparing the resulting theoretical binomial or Poisson distribution with the experimental histograms. This is what all their figures show. 2) The authors heavily insisted on their use of an essential quantal saturation correction, and it takes the reader a long time to discover that this correction does not play any role in the represented curves.

Because there are often major mathematical blunders in biological publications, see the case of the “theoretical equation,” which turns out to be a vacuous identity, discussed in Ninio (1975), one may be tempted to attribute the numerical problems to various errors, bugs in the computer programs, and experimental uncertainties. However, all errors should have contributed to produce rather noisy results, whereas the central claim of the work was a “striking equivalence” between the binomial term n and the number of stained presynaptic boutons (see Fig. 1).

Beyond the purely numerical problems that were addressed in the simulations of Figs. 2 and 3, there are, I feel, more general problems with the whole approach. The preference given to binomial models over Poisson models, multiplying them by a spreading function (this operation is a convolution, not a deconvolution), and comparing the resulting theoretical binomial or Poisson distribution with the experimental histograms. This is what all their figures show. 2) The authors heavily insisted on their use of an essential quantal saturation correction, and it takes the reader a long time to discover that this correction does not play any role in the represented curves.

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REFERENCES


