Illustrating concepts of quantal analysis with an intuitive classroom model

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The quantal hypothesis is central to the modern understanding of how a neurotransmitter is released from synapses. This hypothesis expresses that a neurotransmitter is packaged together in quanta that are released probabilistically. The experiments that led to the quantal hypothesis are often related in introductory neuroscience textbooks, but these experiments use advanced concepts that can be unfamiliar even to advanced students. To remedy this, I have developed a tangible model of the concepts associated with the quantal hypothesis, suitable for an interactive classroom demonstration.

Katz formulated the quantal hypothesis in the 1950s, for which he shared the Nobel Prize. This conclusion originated in a series of classic experiments with Fatt, studying the neuromuscular junction of the frog (4). They stimulated motorneurons and recorded synaptic potentials [called “end-plate potentials” (EPPs)] in the muscle fiber. EPPs had amplitudes that varied from trial to trial. At low Ca$^{2+}$ concentrations, these amplitudes tended to be at integer multiples of a unit size, i.e., one quantum. This unit size matched the size of spontaneous synaptic potentials that occurred independently of motorneuron stimulation, which were called miniature EPPs (mEPPs). Katz and colleagues used these purely electrophysiological pieces of evidence along with highly insightful interpretations to deduce the quantal nature of neurotransmitter release.

In a followup to these experiments, del Castillo and Katz (3) applied a more mathematically rigorous approach, modeling neurotransmitter release as a random process. Under their experimental conditions using low external Ca$^{2+}$, they were able to make the simplifying assumption that the EPP amplitude followed a Poisson distribution, which is a type of random distribution. They used the number of failures to estimate the average rate of quantal release and used this to predict EPP amplitudes. This approach was replicated in a beautiful experiment by Boyd and Martin (1). They measured the amplitude of hundreds of synaptic potentials and were able to quite precisely duplicate both the expected amplitude and number of synaptic potentials of different sizes (Fig. 1). The power and insight of this approach have led to its inclusion in undergraduate textbooks of neuroscience (e.g., Ref 8), but textbooks typically do not explain what a Poisson distribution is or how to work with it. Therefore, recapitulating these insights can be a significant challenge.

These electrophysiological findings were confirmed later using anatomical approaches when vesicles that contain neurotransmitter were directly imaged using an electron microscope. Remarkably, the first accounts of vesicles at presynaptic terminals lack accompanying images (2, 7), and, indeed, better fixation methods arrived later to make the images interpretable by a wider audience. From a student’s perspective, the anatomic data are more simply understandable as evidence for the quantal hypothesis, but the beauty and elegance of the electrophysiology experiments, as well as the underlying probabilistic nature of neurotransmitter release, warrant significant emphasis.

In the undergraduate, senior-level neurobiology class that I teach, I have found that the physiology experiments of Katz pose a significant challenge for students to understand. There are two fundamental problems. First, the distribution of EPP amplitudes is an indirect measure of the number of vesicles released. This potential source of confusion was anticipated by del Castillo and Katz (3):

To test the applicability of Poisson’s law may seem difficult, because all we can do is measure amplitudes of supposedly composite e.p.p.’s; we cannot count the components directly.

This logical step is evidently difficult on first encounter with this material and could be improved by a tangible analogy. I have developed an analogy that I convey here, by asking students to estimate the number of M&Ms in sealed bags, using weight as an indirect measure.

The second challenge for students is understanding the application of probability theory. Most students of biology have had no course in probability and, therefore, probably only know of coin tossing, die rolling, or the Gaussian distribution (also known as the normal distribution or informally as the bell curve). They are not familiar with the Poisson distribution and when to use it. In addition, students have difficulty with the concept that random events can still be predictable. It seems desirable to provide simplified exposure to these ideas without a full-blown course in probability theory. Therefore, I developed an activity to become more familiar with the Poisson distribution, also using M&Ms.

I explain both these activities here. Ideally, one could do this interactively in a discussion section or laboratory and watch the data develop. Alternatively, the activities can be streamlined and covered during a lecture. To ease the analysis, I have also made available an Excel spreadsheet that has been programmed already, so that the curves take shape as the data are entered (see the Supplemental Material).¹ Also, to help the instructor, a duplicate sheet with sample data already entered is also available (Supplemental Material). These activities will help students appreciate the trial-by-trial nature of the quantal analysis performed by del Castillo and Katz (3) and Boyd and Martin (1).

Activity 1: Using Quantal Analysis to Estimate M&M Weight and Number

Materials. The following materials are needed for activity 1:
Small M&M packages (1 package/student)
Scale with 0.1-g precision
Microsoft Excel (or similar)

¹Supplemental Material for this article is available at the Advances in Physiology Education website.
Procedures. This is a classroom activity that uses an indirect measurement to tell what is not directly observable. Specifically, the goal is to measure how many M&Ms are in a package. Small M&M packages are widely available in October and contain ~20 M&Ms in each package. The packages are opaque, making it extremely difficult to count the M&Ms without opening the package. Students should weigh their individual packages, and the data are entered into a spreadsheet. A histogram of package weights is prepared, ideally as each value comes in during the class if time permits. If classroom time does not permit, packages can be weighed ahead of time and 15.9 g) divided by the single M&M weight (0.8 g) yields the measured weight. The corrected peak weights (13.5, 14.3, 15.1, and 15.9 g) divided by the single M&M weight (0.8 g) yields the number of M&Ms in the package, which in the example ranged from 17 to 20 M&Ms.

To bring the analogy back to the principle being discussed, the weight of a package is analogous to the amplitude of an EPP at the neuromuscular junction, in that the number of M&Ms (quanta) inside is unknown. When reviewing a histogram, the clear separation between peaks indicates that the total weight is composed of individual M&Ms, just as the EPP is composed of individual mEPPs. The students have an intuitive understanding that the difference in weight comes from the different numbers of M&Ms in each package. This should help them to understand the basis for the multiple peaks in the histogram of Boyd and Martin (1).

Furthermore, just as it was possible to estimate the total number of M&Ms in the package, it is possible to estimate the number of neurotransmitter quanta in each EPP by dividing the peak amplitude by the quantal size.

Conclusion 1. At the end of this exercise, students will have a better idea of how an EPP amplitude histogram is generated and how to analyze the data presented in such a manner. This makes the more advanced concepts associated with electrophysiology more accessible.

Activity 2: The Poisson Distribution

Materials. The following materials are needed for activity 2:

- Small M&M packages (1 package/student)
- Microsoft Excel spreadsheet

Fig. 1. Quantal analysis of the neuromuscular junction by Boyd and Martin (1). Bars show a histogram of end-plate potential (EPP) amplitudes recorded in the tenuissimus muscle of the cat after stimulation of a motorneuron. The solid line is the predicted EPP amplitude assuming that neurotransmitter release follows a Poisson process with an average rate of release (m) = 2.33. Roman numerals indicate the expected mean amplitude of release events with 1 quantum, 2 quanta, etc. [Inset: distribution of miniature EPP amplitudes used to construct the hypothetical line. Reproduced with kind permission from John Wiley and Sons.]

Fig. 2. Histogram of weights of 40 M&M packages, prepared in Excel. Note that the weights form four clusters, separated by 0.8 g. These data are used to estimate the weight of a single M&M (0.8 g) and the number of M&Ms in each package (17–20 M&Ms/package). These data have been adjusted for the empty package weight (2 g).
Procedures. Neurotransmitter release is currently thought of as a random process, in which there is a pool of readily releasable vesicles that each has a probability of being released during an EPP (10). If the pool of vesicles is large and the probability of each being released is low, the number of vesicles that is actually released during any individual EPP would follow the Poisson distribution. This was tested by del Castillo and Katz (3) and Boyd and Martin (1).

The Poisson distribution is particularly useful for predicting EPP amplitudes for several reasons. First, the Poisson distribution only depends on a single parameter, the average rate of release \( m \), which is simple to measure experimentally. Second, to use the Poisson distribution, it is not necessary to know how big the total pool is or what the individual release probability is. Third, once \( m \) is measured, it allows you to predict the frequency of observing EPPs with different numbers of quanta and therefore the Poisson distribution, it is not necessary to know how big the total pool is or what the individual release probability is. Third, once \( m \) is measured, it allows you to predict the frequency of observing EPPs with different numbers of quanta and therefore of different amplitudes. The probability that \( k \) vesicles \( (p_k) \) are released in a given EPP is given by the following formula: 

\[
p_k = \frac{m^k e^{-m}}{k!}.
\]

Thus, with the value of \( m \) in hand, it is possible to predict how many EPPs you expect to see in an experiment that reflect the release of one vesicle, or two, or three, as well as how many times a presynaptic action potential results in no EPP at all ("failures").

Most students have little experience with probability theory, so it is helpful to give them more exposure to the idea that there are different probability distributions and that random events can be predicted. M&Ms are a very close analogy to neurotransmitter release and are easily accessible and tangible. In this analogy, a package of M&Ms represents the outcome of a single action potential. All the M&Ms in the package represent the pool of releasable vesicles. The green M&Ms represent the vesicles that are actually released and comprise the EPP (any individual color can, of course, substitute for green). This activity, students replicate the del Castillo and Katz (3) and Boyd and Martin (1) experiments using M&M packages in place of EPPs to demonstrate that the number of green M&Ms in a package (i.e., the number of vesicles released in an EPP) follows a Poisson distribution.

The first step in using the Poisson distribution is to estimate the average number of events observed (i.e., \( m \)), which can be done in two ways. The first is to make a lot of measurements and average the number per trial. Therefore, to predict the number of green M&Ms in each package, every student should count and report the number of green M&Ms in their package, and the average frequency (\( m \)) should be calculated. In a representative experiment, this frequency was \( m = 3.4 \) green M&Ms/package (Fig. 3A).

Applying this analogy back to quantal analysis, this is the same as calculating the average number of quanta released across all the trials. In the experiment shown in Fig. 1, Boyd and Martin (1) measured the average EPP at 0.93 mV. To convert that average voltage to the average number of quanta, an extra step was necessary. They divided the average EPP by the average mEPP (0.40 mV), which represented the amplitude of a single quantum. Therefore, the average number of quanta in each EPP was 2.33.

The second way of estimating \( m \) is to use the method of failures. For events that occur with low frequency, there is a reasonable chance that no events at all will be observed. This is the \( k = 0 \) case, which, according to the Poisson formula, is 

\[
p_0 = m^0 e^{-m}/0! = e^{-m}.
\]

By rearranging this formula, this means that \( m = \ln(1/p_0) \). The value of \( p_0 \) can be measured experimentally as the frequency of failures, which can then be used to calculate the average frequency (\( m \)). In the case of our specific example, there was 1 package of 35 total packages with no green M&Ms, so the measured \( p_0 = 1/35 \approx 0.029 \), giving \( m = 3.5 \).

In quantal analysis, this is the same as counting the number of times a stimulus led to no measurable EPP. For the experiment of Boyd and Martin (1) shown in Fig. 1, they observed 18 failures of 199 stimuli, which yielded \( m = 2.4 \). This number corresponds well to their other estimate of \( m \) (2.33). This approach would be unreliable when the number of failures is small, so large sample sizes are necessary. This caveat is relevant to this exercise: the example M&M data shown here yielded similar estimates of \( m \) using both approaches, but the method of failures is probably too unreliable to use with <100 packages of M&Ms, so we will use the average number of green M&Ms per package for our estimate of \( m \).

Returning to our activity, the value of \( m \) (3.4) can be used to predict how many packages had no green M&Ms \( (p_0 = m^0 e^{-m}/0! = 3.3\%) \), one M&M \( (p_1 = m^1 e^{-m}/1! = 11\%) \), 2 M&Ms \( (p_2 = m^2 e^{-m}/2! = 19\%) \), etc. These predicted values are then compared against the observed values. In the example shown in Fig. 3A, there was no statistical difference between observed and predicted values \((P > 0.5 \text{ by } \chi^2\text{-test})\). For the purposes of this activity, statistical similarity is not as good as visual similarity, which depends on the sample size.

To determine what sample sizes would be best as well as to test whether this exercise would be reliable as a classroom demonstration, two additional data sets were collected (data not shown). The first data set had a sample size of 20, and the frequency of green M&Ms was \( m = 3.4 \). There was no significant difference between the observed counts of green M&Ms and the Poisson distribution \((P > 0.5 \text{ by } \chi^2\text{-test})\). The second data set had a sample size of 42, with \( m = 2.9 \), and showed no significant difference from the Poisson distribution \((P > 0.8 \text{ by } \chi^2\text{-test})\). Thus, this has been a reliable exercise in our hands. A class size of 40 students yields a good visual as well as statistical match. A class size as small as 20 students provided a good statistical match and, in this particular case, a
good visual match but, in general, may require multiple M&M packages per student to increase the sample size.

In quantal analysis, the average number of quanta released on each EPP is measured and then used to estimate the number of times one expects a single vesicle to be released, or two vesicles, or none, etc. Since the size of single quanta could be measured using the mEPP, this made it possible to predict the size of EPPs composed of different numbers of quanta, leading to the predicted, smooth line shown in Fig. 1. Thus, del Castillo and Katz (3), and later Boyd and Martin (1), could conclude that neurotransmitter release is a random process, with a pool of vesicles being released independently of each other.

Conclusion 2. By doing this activity, students will gain better familiarity with the Poisson distribution as well as a better general sense of how probability distributions can be used to predict experimental outcomes. In addition, students will get to see how the Poisson distribution can be used to predict EPP amplitudes, using a very close analogy that requires no electrophysiology.

Discussion

Here, I have presented two activities that help to illustrate two concepts in quantal analysis. Activity 1 illustrates the use of an indirect measurement to determine an underlying mechanism. In the case of quantal analysis, this is the use of EPP amplitude to evaluate how many vesicles are released during an EPP. The validity of this approach in the classic literature is evident using amplitude histograms, but even the generation of such a histogram may be confusing to students, because it involves abstract measurements most undergraduates have no direct contact with. By replacing this with a simple idea, the weight of a package of M&Ms, it may enhance the students’ ability to understand the more complex idea. Furthermore, by following the steps of looking at intervals between peaks on the histogram, they can even make simple calculations (the weight of one M&M and the number of M&Ms per package).

Activity 2 illustrates a more difficult concept: that it is possible to predict random events in the aggregate. Students intuitively understand the randomness of coin tossing, but its relationship to the number of quanta in EPPs may not be immediately obvious. Quantal analysis as discussed in introductory neuroscience textbooks uses the Poisson distribution. The number of green M&Ms in a package also follows the Poisson distribution, so by counting the number of green M&Ms and seeing how different students get different random but predictable results, they can see the Poisson distribution as an effective way of describing random events.

The major advantage of these activities is that they bring abstract ideas down to a tangible level. In addition, they do it in a memorable way. As a student remarked, “I will never think of M&Ms the same way again.” The students are being asked to analyze something quite trivial in a closely quantitative and scientific way, and they can appreciate the humor in that, which makes the concept more memorable.

The second activity can provide additional benefits, as it gives ways for a lecturer to discuss more advanced concepts related to neurotransmitter release. These would include activity-dependent changes in EPPs and excitatory postsynaptic potentials, such as short-term facilitation and depression, as well as neuromodulation. Once the students adopt the basic analogy between vesicles and green M&Ms, one can talk about facilitation as an increase in the frequency of green M&Ms in a package and depression as a reduction in the total number of M&Ms in a package. Extending this analogy to these situations can be quite useful and natural.

There are some drawbacks to these activities. At a practical level, entering and analyzing the data takes time and, therefore, is ideally done in an associated recitation or laboratory. For all-lecture classes such as the one I teach, these activities would be done during lecture time. Therefore, for activity 1, I minimize class time by weighing the packages and entering the data ahead of time. For activity 2, I give each student one M&M package, ask them to e-mail me the data, and then present the data in the next lecture period. I have made an effort to streamline data analysis by providing a supplemental Excel spreadsheet file that assists with building histograms and charts as the data are entered.

At a more scientific level, this activity does differ from the details of quantal analysis in some ways. The number of quanta in an EPP is Poisson distributed. However, the total number of M&Ms in a package appears to be Gaussian distributed, presumably because the Poisson distribution leads to unacceptably high variance in the number of M&Ms in each package. Because of that, activity 1 was insufficient to illustrate the Poisson distribution, so activity 2 was used. As a result, the analogies for the two activities are different. In activity 1, the weight of a package of M&Ms is analogous to the EPP amplitude, whereas in activity 2, the number of green M&Ms within a package is analogous to the EPP amplitude. This should not interfere with students understanding the basic concepts. The activities themselves are distinct enough to provide a clear break between them. Furthermore, these activities are not an end in themselves but rather as a way to develop a more intuitive understanding of electrophysiology experiments concerning quantal analysis.

A second issue is that activity 2 emphasizes only the randomness associated with the number of M&Ms and, by analogy, the number of quanta in an EPP. However, the predicted line shown in Fig. 1 incorporates the additional complexity of the variability of a single quantum. In activity 2, that additional randomness is absent, which allows a simpler introduction of the probabilistic ideas without the added complication of physiological issues. Ideally, that additional complexity can be discussed in the course of the lecture as the analogy is placed back into context. There are many additional complexities that come into play in real quantal analysis, including heterogeneity of vesicle size, release probability, and receptor density, which have reduced the utility of quantal analysis at central synapses (9). Such nuances are beyond the scope of an undergraduate course.

Finally, neurotransmitter release is currently thought of as depending on some number of releasable vesicles, each with a probability of being released, which should follow a different random distribution, the binomial distribution (11). In general, both the number of releasable vesicles and the probability of a vesicle releasing a neurotransmitter are difficult to measure. Katz and del Castillo developed a workaround by assuming the probability is low and the pool of vesicles is large. Under these assumptions, the binomial distribution can be well approximated by the Poisson distribution, which relies on only a single parameter (m), which they could measure. (The derivation of this approximation can be found in Ref 5.) The close fit between theory and measured data provided strong support for the idea that vesicles are released randomly and independently.
The probability question here (how many green M&Ms are in a package?) would also be more accurately modeled using the binomial distribution. For that, we would need to know the total number of M&Ms and the probability that any individual M&M will be green. For activity 2, we adopted the same assumption as del Castillo and Katz (3): that the number is large enough and the probability low enough for the Poisson distribution to be an adequate approximation. Furthermore, the close fit between the predictions of the Poisson distribution and our measured M&M data provide strong support for the idea that the color of each M&M is random and independent.

These activities are intended to help students with the concept that neurotransmitter release is probabilistic in nature. This randomness has a major effect on synaptic physiology. Many synapses in the nervous system have a small pool of vesicles and low probability of release, which means many synapses release no neurotransmitter at all in response to an action potential (for example, Ref. 6). In contrast, the neuromuscular junction has a large pool of vesicles that are normally released with a high probability. The classic experiments shown in Fig. 1 reduced release probability using low Ca\(^{2+}\) and high Mg\(^{2+}\), effectively making the neuromuscular junction more similar to central synapses. The consequences of synaptic unreliability for neuronal function are still being uncovered.

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AUTHOR CONTRIBUTIONS

Author contributions: M.A.X.-F. conception and design of research; M.A.X.-F. performed experiments; M.A.X.-F. analyzed data; M.A.X.-F. interpreted results of experiments; M.A.X.-F. prepared figures; M.A.X.-F. drafted manuscript; M.A.X.-F. edited and revised manuscript; M.A.X.-F. approved final version of manuscript.

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