The critical role of logarithmic transformation in Nernstian equilibrium potential calculations

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The membrane potential, arising from uneven distribution of ions across cell membranes containing selectively permeable ion channels, is of fundamental importance to cell signaling. The necessity of maintaining the membrane potential may be appreciated by expressing Ohm's law as $I = V/R$, and recognizing that no current flows when $V = 0$ i.e. trans-membrane voltage gradients, created by uneven trans-membrane ion concentrations, are an absolute requirement for generation of currents that precipitate the action- and synaptic potentials that consume over 80% of the brain's energy budget, and underlie the electrical activity that defines brain function. The concept of the equilibrium potential is vital to understanding the origins of the membrane potential. The equilibrium potential defines a potential at which there is no net trans-membrane ion flux, where the work created by the concentration gradient is balanced by the trans-membrane voltage difference, and derives from a relationship describing the work done by the diffusion of ions down a concentration gradient. The Nernst equation predicts the equilibrium potential, and as such is fundamental to understanding the interplay between trans-membrane ion concentrations and equilibrium potentials. Logarithmic transformation of the ratio of internal and external ion concentrations lies at the heart of the Nernst equation, but most undergraduate neuroscience students have little understanding of the logarithmic function. To compound this no current undergraduate neuroscience textbooks describe the effect of logarithmic transformation in appreciable detail, leaving the majority of students with little insight into how ion concentrations determine, or how ion perturbations alter, the membrane potential.

Background

The concept of the equilibrium potential, also called the reversal potential, and the role that it plays in determining the membrane potential is of fundamental importance to understanding neural excitability (in this paper Em denotes membrane potential, E_{rev} denotes a general description of the equilibrium/reversal potential, and E_K etc. denote the equilibrium potential for a particular ion). The Nernst equation, which determines E_{rev}, can estimate the Em in astrocytes, a glial cell subtype whose cell membrane is exclusively permeable to K^+ (23). The Goldman Hodgkin Katz voltage equation, an expansion of the Nernst equation that was developed in order to estimate the permeability changes that underlie the action potential (14), also functions to estimate the Em of cells permeable to more than one ion (12).

Students who fail to grasp the Nernst equation are at a disadvantage, as they are inclined to learn rather than understand. The limitations of such a strategy may be readily appreciated by realizing that Em is not a static property, but varies in response to such processes as synaptic input and action potential generation. For example, students should intuitively be able to deduce the effects of altering the trans-membrane concentrations of Na^+, K^+ or Cl^- on E_{rev} or Em, and appreciate that in circumstances where $Em \neq E_K$, that K^+ moves across the membrane in the direction that restores Em towards E_K. However students struggle to master the concept of how selectively permeable ion channels in the cell membrane combined with trans-membrane ion gradients lead to a trans-membrane potential difference, an issue that has been recognized (27) and repeatedly addressed (7, 17, 26, 33).

The Nernst equation may be regarded as comprising a conceptual component and a practical component. The conceptual component was addressed in a recent article (6), where models were proposed that described in an elegant and accessible manner movement of K^+ down a trans-membrane concentration gradient via K^+ permeable ion channels, when $K^+_i > K^+_o$. The work done or energy associated with such diffusion of ions can be quantified as $W_{diff} = RT \ln K_o/K_i$ (see Equation 3 for definitions), implying that the steeper the trans-membrane concentration gradient the greater the energy generated. However this diffusion creates a potential difference across the cell membrane, which drives K^+ from the outside to the inside of the cell; the resulting electrical work quantified as $W_{elec} = E_K F z$. Equilibrium occurs where the diffusional and electrical forces balance and there is no net movement of K^+. This can be expressed as $W_{diff} = W_{elec}$, which can be expanded into the Nernst equation thus:

$$RT \ln \frac{K_o}{K_i} = E_K F z \tag{1}$$

$$E_K = \frac{RT}{zF} \ln \frac{K_o}{K_i} \tag{2}$$

An accessible derivation of the Nernst equation can be found in Bertil Hille's classic textbook (12).

This paper deals with the practical component, the mathematical operations required to solve the Nernst equation, and may be viewed as a companion piece to the conceptual description (6).

In describing E_{rev} most neuroscience textbooks deliver contrived scenarios based on even concentrations of ions dispersed across a membrane that suddenly becomes selectively permeable to a particular ion (3), or describe existing uneven ion distributions (15), with no explanation as to how such a situation arose. These artificial descriptions deprive the student of the fascinating origins of the membrane potential, which date back over 3.7 billion years to the emergence of the first life on earth. Although some aspects of the origins of life on earth remain contentious (18) a consensus opinion may be summarized as follows. The first single celled prokaryotic organisms originated in a sea high in salt content with a Na^+: K^+ ratio of 5:1. A key definition of life is a cellular entity capable of reproduction, thus life consisted of lipid membrane bound sacks of cytoplasm containing RNA (1). An impermeant lipid membrane would have retained the intracellular macromolecules, but would not have permitted entry of nutrients or expulsion of waste products, thus a permeable membrane favored cellular survival. The negatively charged intracellular proteins created an ion gradient favoring entry of positively charged ions into the cell. However the stability of RNA is compromised in high Na^+ environments (8), thus in order to balance the negative charge the membranes evolved a selective permeability to K^+, while remaining relatively impermeable to Na^+ (12). The K^+ accumulation in the cell in excess of its extracellular concentration was sustained by the Na^+-K^+ ATPase, which maintained a low $[Na^+]_i$ and high $[K^+]_i$ (28). Thus, maintenance of a steep trans-membrane potassium gradient has been a feature of cells for billions of years and is fundamental in generating the negatively charged cell membrane potential. The negative membrane potential and high $[Na^+]_o$ was exploited in the evolution of the action potential, the primary mode of cell-to-cell communication in the nervous system.

The equilibrium potential

Complex relationships exist between ion concentrations, fluxes of ions across selectively permeable cell membranes, and the resulting voltage differences across cell membranes. However if certain basic assumptions are accepted, namely if one assumes flow of ions across a cell membrane is one-dimensional, and that the voltage gradient is perpendicular to the membrane, then the relationship between ion fluxes and voltage gradients can be described by the Transport equation (32). This equation can be further simplified to the Nernst equation by assuming that the membrane is permeable to only one ion and that net flux of all ions is zero. The Nernst equation introduces the concept of the equilibrium potential of an ion that is distributed across a semi-permeable membrane, and is defined as the electrical potential at which there is no net ion movement across the membrane, due to trans-membrane concentration gradients being balanced by uneven electric charges across the membrane. The relationship is derived ultimately for an individual ion such as a representative cation A^+, as:

$$E_{rev} = \frac{RT}{zF} \ln \frac{[A^+]_o}{[A^+]_i} \qquad (3)$$

where E_{rev}, the point of zero current flow, is the reversal potential for A^+, measured in Volts, R is the Gas constant ($8.315\ \mathrm{V\ C\ K^{-1}\ mol^{-1}}$), T is the temperature in Kelvin (K), z is the valence of the ion (unitless), and F is the Faraday constant ($96{,}480\ \mathrm{C\ mol^{-1}}$) (12). In the context of ions distributed across a cell membrane, $[A^+]_o$ and $[A^+]_i$ are the extracellular and intracellular concentrations of cation A^+, respectively. As mammals maintain a constant body temperature and laboratory experiments tend to be carried out at fixed temperatures, the Nernstian relationship may be simplified, since RT/F can be expressed as a single number relative to a fixed temperature, e.g. 26.7 mV at 37°C. This value is unaffected when the value of z, the number of elementary charges per ion, is +1, as is the case with Na^+ and K^+. In addition, the constant for converting from the natural logarithm (ln) to $\log_{10}$ is 2.303. Thus, the Nernst equation at 37°C simplifies to

$$E_{rev} = 61.5 \log_{10} \frac{[A^+]_o}{[A^+]_i} \qquad (4)$$

For anions e.g. Cl^-, where z = -1, RT/zF at 37°C is -26.7, thus E_{Cl} is expressed as

$$E_{Cl} = -61.5 \log_{10} \frac{[Cl^-]_o}{[Cl^-]_i} \quad \text{or} \quad E_{Cl} = 61.5 \log_{10} \frac{[Cl^-]_i}{[Cl^-]_o} \qquad (5)$$

The second expression is a result of log(a/b) = -log(b/a) (see later). However this is as far as elementary undergraduate textbooks proceed with the equation (3, 21), leading to students *learning* the equation rather than *understanding* how each of its constituent parts contributes to the reversal potential. According to Equation 3 the reversal potential varies linearly with temperature and logarithmically with the ion concentration ratio, but no information is given regarding how logarithmic transformation affects the ion concentration ratio. This is not a trivial point as the ion concentration ratio can be positive, negative or equal to 1, with each condition responding differently to logarithmic transformation. Thus, in order to understand how ion concentrations affect reversal potentials we must first describe the effects of logarithmic transformation.

Description of the logarithmic function

Detailed accounts of the development and implementation of the logarithmic function by Napier in 1614 can be found in the following reviews (4, 5, 13, 19, 31).

For our purposes the following definitions will suffice.

If $c^x = a$ then $\log_c a = x$ (6)

i.e. the logarithm, to the base c (where $c \neq 1$), of a, is x.

The 1st Law of Logarithms (2) may be expressed as

$\log_c ab = \log_c a + \log_c b$ (7)

In addition, the 2nd Law of Logarithms, whose derivation can be found elsewhere (2), is also fundamental in helping us to understand the effect of logarithmic transformation in Nernstian calculations, where

$\log_c(a/b) = \log_c a - \log_c b$ (8)

The logarithm to the base 10

The logarithm to the base 10 ($\log_{10}$) is a convenient form by which to express numbers since it simplifies calculations based on the decimal numbering system, and clarifies the relationships between numbers separated by orders of magnitude e.g. 3.14 and 31.4. Logarithms, such as 2.871, are composed of the characteristic and the mantissa, the characteristic being the integral part (2) and the mantissa being the fractional or decimal part (.871). From Fig 1A it can be reasonably deduced that the characteristic of 743.2 is 2, since 743.2 lies between 100 and 1,000. Expressing a number in scientific notation (30), i.e. in the form $a \times 10^b$, where $1 < a > 10$, and b is the appropriate exponent or power, clarifies the conversion of a number to $\log_{10}$ since the characteristic can be deduced from Fig 1A as the power or exponent required to express the number in scientific notation, i.e. 7.432×10^2. This is comparable to considering $\log_{10}743.2$ as being equivalent to $\log_{10}7.432 + \log_{10}100$

(Equation 7), by employing the 1st Law of Logarithms. The mantissa of any number expressed in scientific notation is between 0 and 1 since the number, by definition, must lie between 1 and 10 (Fig 1A). In this case the mantissa is 0.871, thus $\log_{10}743.2$ is equal to 2.871 (0.871 + 2). It should be readily apparent that one of the great advantages of the $\log_{10}$ system is that it relates numbers such as 743.2 and 7432, with the characteristic increasing by 1 for each order of magnitude increase in the number, the mantissa unchanged.

Fig 1A reveals that $\log_{10}10 = 1$, $\log_{10}1 = 0$, and that $\log_{10}(> 1)$ is a negative number. Although not immediately apparent such simple relationships govern the association between E_{rev}, Em and trans-membrane ion concentrations. Since $\log_{10}$ of any number less than 1 is negative, the polarity of the E_{rev} can be deduced by a simple rule of thumb: if the extra- and intracellular concentrations of the ion are known, for cations, if the extracellular concentration is greater than the intracellular concentration, E_{rev} is positive, but if the reverse is the case then E_{rev} is negative. Similar reasoning can be applied to anions. For example under normal conditions $[K^+]_o$ in the brain is about 5 mM, with $[K^+]_i$ 150 mM (3). Since the ratio of $[K^+]_o/[K^+]_i$ is less than 1, E_{rev} for K^+ is negative. Similarly, for Na^+ where the extracellular concentration is 150 mM, and the intracellular concentration is 15 mM, the E_{rev} for Na^+ is positive (3).

Logarithmic graphing

In disciplines related to neuroscience, such as physiology and pharmacology, students will frequently encounter data plotted on logarithmic scales, with an understanding of such plotting required in order to comprehend the underlying scientific principles (22). This is especially true of plots of Em versus $[K^+]_o$, where $[K^+]_o$ is conventionally plotted on a $\log_{10}$ scale (14). Plotting data on a $\log_{10}$ scale clarifies the logarithmic relationship, since on a linear scale moving a fixed distance along the axis moves the data along the axis by adding that fixed amount, whereas on a logarithmic scale moving a fixed distance involves multiplication by a fixed factor. Thus, the major ticks on a log scale increase by orders of magnitude when base 10 is used, such that the major ticks progress in the following sequence: 1, 10, 100, 1,000 i.e. each major increment is 10 times larger that the previous value, and the distances between 1 and 10, and 10 and 100, are equal. This is illustrated by examining Fig 1B, which shows the logarithmic scale from 10 to 100. It is apparent that the positions of the minor tick intervals are unevenly spaced, due to the scale being based on the $\log_{10}$ of the number rather than the number itself as occurs on a linear scale. The simple way to understand logarithmic scaling is to realize that the distance between 10 and 20 i.e. 0.301, is the distance gained along the x-axis by multiplying by 2. This is shown in Fig 1Ca, which illustrates that the product of multiplying 10 by 2 (i.e. 20) results in a movement of 0.301 along the x-axis. Similarly, the product of 20 and 2 (40) gains 0.301 along the axis, as does the product of 40 and 2 (80) (i.e. $\log_{10}40 = \log_{10}20 + \log_{10}2 = 1.301 + 0.301 = 1.602$). Thus, the distances between 10 and 20, 20 and 40, and 40 and 80, are equal, the product of each calculation being the result of multiplication by 2. In a similar fashion the distance from 10 to 30, and from 30 to 90 (0.477) represents multiplication by 3 (Fig 1Cb). The value of 60 can be calculated by adding the distance between 10 to 30 (i.e. multiplication by 3) and 30 to 60 (multiplication by 2, Fig 1Cc), and finally the position of 50 on the axis can be deduced by dividing 100 by 2 i.e. expressing $\log_{10}50$

as $\log_{10}(100/2)$ and using the 2nd Law of Logarithms to expand the expression, ultimately subtracting the distance between 10 to 20 from 100 (i.e. 2 - 0.301 = 1.699, Fig 2Cd). The conversion of multiplication into the movement of fixed distances along an axis is the basis of slide rule operation (29).

The membrane potential and $[K^+]_o$

The Nernst equation was applied in two classic papers from 1966, published by the American Physiological Society, in which Steven Kuffler and co-workers described the response of the Em of glial cells (equivalent to mammalian astrocytes) in the optic nerve of the mud puppy *Necturus* to alterations in $[K^+]_o$ (16) and to electrical stimulus (20). These two experimental paradigms offer an ideal opportunity with which to illuminate how logarithmic transformation of trans-membrane ion ratios determine Em, and how stimulus-induced, identical increases in $[K^+]_o$ result in attenuating Em depolarizations. These were landmark studies as they showed for the first time, using an equation based on logarithms, that the glial cell membrane was exclusively permeable to K^+. Appreciation of these papers can be enhanced by calculating how the Em of a hypothetical cell, exclusively permeable to K^+, would respond to changes in $[K^+]_o$ by applying the appropriate values to the Nernst equation. Note that Kuffler's experiments were carried out at 23°C, thus RT/F is 59 mV, and since the cell is exclusively permeable to K^+, it follows that Em is equal to E_K.

$$\mathrm{Em} = E_K = 59 \log_{10} \frac{[K^+]_o}{[K^-]_i} \tag{9}$$

The first step is to assume a reasonable estimate of $[K^+]_i$ of 75 mM (12), and that this value remains constant. The Em for a range of $[K^+]_o$ from 1 to

100 mM is shown in Fig 2A, where plotting $[K^+]_o$ on a $\log_{10}$ scale reveals a linear relationship between $[K^+]_o$ and Em, with the slope increasing by 59 mV for each order of magnitude increase in $[K^+]_o$. How can we explain this 59 mV shift in Em in a simple, logical and easy to understand manner?

We start by calculating the Em where $[K^+]_o$ is either 3 mM or 30 mM, with a constant $[K^+]_i$ of 75 mM, and employing the 1st and 2nd Laws of Logarithms.

3 mM $[K^+]_o$	**30 mM $[K^+]_o$**	
$Em = 59 \log_{10} (3/75)$	$Em = 59 \log_{10} (30/75)$	(10)
$Em = 59 * [\log_{10}3 - \log_{10}75]$	$Em = 59 * [\log_{10}30 - \log_{10}75]$	(11)
	$Em = 59 * [\log_{10}10+\log_{10}3-\log_{10}75]$	(12)
$Em = 59 * [0.477 - 1.875]$	$Em = 59 * [1 + 0.477 - 1.875]$	(13)
$Em = 28.1-110.6 = -82.5$ mV	$Em=59+28.1-110.6 = -23.5$ mV	(14)

Note the only difference occurs in Equation 12 where the 1st Law of Logarithms is used to expand $\log_{10}30$ into $\log_{10}10 + \log_{10}3$. The subsequent multiplication by 59 of the logarithmically transformed fragments reveals why Em increases by 59 mV for each decade increase in $[K^+]_o$.

In the experiments carried out by Kuffler, the *Necturus* optic nerve was placed in a perfusion chamber; the composition of the artificial cerebrospinal fluid perfusing the tissue could be altered as desired. Glial cells were impaled with a sharp microelectrode and the Em continuously recorded. Kuffler found that the glial cell Em of *Necturus* behaved in the same way as the hypothetical cell, with a 59 mV shift in Em for a decade increase in $[K^+]_o$ (Fig 8 of 16), which we have reproduced as Fig 2B. This data strongly indicated that extracellular K^+ controlled the Em of the glial cell to the extent that increases in $[K^+]_o$ depolarised the Em in a manner predicted by the Nernst relationship, validating the assumption that in *Necturus* optic nerve glial cell Em was equal to E_K.

Estimating the value for $[K^+]_i$

Kuffler then used the Nernst equation to estimate the value of $[K^+]_i$ in the following way. As illustrated (Fig 1A) $\log_{10}1 = 0$, thus where the concentrations of $[K^+]_o$ and $[K^+]_i$ are equal, $\log_{10}[K^+]_o/[K^+]_i = 0$, and therefore Em = 0 (Fig 2B). Kuffler showed experimentally that where Em = 0 mV, $[K^+]_o$ = 99 mM, thus $[K^+]_i$ = 99 mM also, illustrating how understanding the Nernst equation allows estimates of intracellular concentrations of ions, measurements that were beyond the technical capabilities of the day.

Stimulus induced changes in Em: individual stimuli

The effect of axon impulses on the Em of glial cells was investigated by stimulating the intact nerve with 10 pulses at a frequency of 10 Hz, while recording the Em of glial cells with sharp electrodes (Fig 4 of 20), redrawn as Fig 3A. Frankenhauser and Hodgkin provided clear evidence of K^+ efflux during the repolarising phase of an action potential (9), data since confirmed with K^+-sensitive microelectrodes (25). Given the low resting $[K^+]_o$, long duration of K^+ channel opening and the small interstitial space, there is a measurable rise in $[K^+]_o$ resulting from action potential conduction, with repetitive firing resulting in significant and prolonged $[K^+]_o$ elevations (25). The stimulus-induced transient glial depolarisations are shown when nerves were bathed in $[K^+]_o$ of 3 mM, 4.5 mM and 1.5 mM. As previously calculated (Equation 9) the Em of a *Necturus* glial cell perfused with 3 mM $[K^+]_o$ and 99 mM $[K^+]_i$ is -89 mV. The increase in $[K^+]_o$ that underlies the stimulus-induced transient 12 mV depolarisation (Fig 3A, left trace) can be calculated as:

$$(-89 - 12) = 59 \log_{10} \frac{[K^+]_o - 3}{99} \tag{15}$$

$$-1.305 = \log_{10} \frac{[K^+]_o - 3}{99} \tag{16}$$

which can be rearranged according to Equation 6 as

$$10^{-1.305} = \frac{[K^+]_o - 3}{99} \tag{17}$$

$$[K^+]_o = 1.9 \text{ mM} \tag{18}$$

thus the stimulus causes release of sufficient K^+ from the axons into the interstitial space to increase $[K^+]_o$ by 1.9 mM. Similar reasoning was used by Hodgkin and Katz to calculate the attenuation in action potential amplitude when $[Na^+]_o$ was decreased in squid giant axon (Fig 6 of 14).

In *Necturus* nerves bathed in 4.5 mM $[K^+]_o$ the Em can be calculated, as previously demonstrated in Equation 9, as

$$Em = 59 \log_{10} \frac{4.5}{99} = -79 \text{ mV} \tag{19}$$

At a baseline value of 4.5 mM $[K^+]_o$ stimulus will augment this to (4.5 + 1.9 mM) 6.4 mM, and hence the stimulus-induced transient depolarisation can be calculated as

$$Em = 59 \log_{10} \frac{6.4}{99} = -70 \text{mV} \tag{20}$$

Similarly, for 1.5 mM baseline $[K^+]_o$ the Em is -107 mV, and the stimulus causes a depolarisation to -86 mV.

The depolarisation induced by identical stimuli to those imposed during the experiment (i.e. stimuli that will release sufficient K^+ from axons to increase $[K^+]_o$ by 1.9 mM) over a range of $[K^+]_o$ from 1.5 to 4.5 mM can be calculated by subtracting the Em calculated with an additional 1.9 mM $[K^+]$ in the interstitial fluid, from the Em calculated as previously described (Equation 9).

$$\text{Depolarisation (mV)} = 59\log_{10}\frac{[K^+]_o + 1.9}{99} - 59\log_{10}\frac{[K^+]_o}{99} \qquad (21)$$

Note that the lower the $[K^+]_o$, and hence the more hyperpolarised the Em, the greater the amplitude of the stimulus-induced depolarisation (Fig 3B).

Stimulus induced changes in Em: sequential stimuli

In subsequent experiments Kuffler measured the Em of the *Necturus* optic nerve glial cells in response to a train of 9 stimuli at a frequency of 10 Hz, 1 second apart. The response recorded offers an ideal opportunity to relate the effect of altering $[K^+]_o$ on Em/E_K. If we adhere to the principal that identical stimuli increase $[K^+]_o$ by identical amounts, then we can assume that each of the 9 stimuli increases $[K^+]_o$ by identical amounts. Under such circumstances a simple way to understand the effects of $[K^+]_o$ on Em is to visually relate the two properties. This can be done by plotting the $\log_{10}[K^+]_o$ versus the recording of Em. The Nernst relationship in these conditions can be reduced to Em ~ $\log_{10}[K^+]_o$ since RT/zF and $[K^+]_i$ remain constant (Equation 9). For the purposes of clarity we assume that each stimulus increases $[K^+]_o$ by 1 minor tick (right axis, Fig 3C). By plotting $\log_{10}[K^+]_o$ on a vertical axis and aligning with Em, it can readily be seen that, although not a perfect match, the increases in $[K^+]_o$ result in successive glial Em depolarizations of diminishing amplitude. Such a relationship is succinctly expressed by the authors of similar experimental results, when they stated "According to the K^+ theory each depolarizing step in the response to one of these equal K^+ increments and the diminishing amplitude of successive steps is simply a consequence of the logarithmic relationship between $[K]_o$ and membrane potential (i.e. the efficacy of a given K^+ increment in producing depolarization decreases as the ambient $[K]_o$ increases)." (24).

Workshops

In our interactions with students entering the 1st year Neuroscience undergraduate course at the University of Nottingham it has become apparent that they are unprepared for the rigours of the types of calculations using logarithmic transformation illustrated in this paper, despite the fact that many of these students possess A level passes in Maths. This is particularly concerning as the A level syllabus has the following goals regarding logarithms (10).

1. Understand the relationship between logarithms and indices, and use the laws of logarithms (excluding change of base);
2. Understand the definition and properties of e^x and $\ln x$, including their relationship as inverse functions and their graphs;
3. Use logarithms to solve equations of the form $a^x = b$, and similar inequalities;
4. Use logarithms to transform a given relationship to linear form, and hence determine unknown constants by considering the slope and/or y-intercept.

The most likely explanation for students' deficits in understanding logarithms is the perceived redundancy of the subject in the age of desktop computers/calculators/smartphones, which can easily carry out logarithmic transformation without the need to understand or navigate log tables, which only a generation ago was an absolute requirement for all students. In informal conversations with students logarithms were viewed as antiquated and irrelevant reinforcing the impression that most students regarded Maths as a difficult and inaccessible subject.

In order to reacquaint 2nd year Neuroscience students with the logarithmic function we carried out revision workshops designed to identify deficiencies in students' knowledge, followed by a 2-hour workshop that covered the content of this paper, with a post-workshop assessment to determine improvement in performance. At this stage of the course, students have had a 1 hour introductory lecture on membrane potentials and the Nernst equation. As the first part of the workshop students were given an exercise (Appendix 1) that was designed to test their knowledge of multiple areas related to logarithms (nomenclature, laws of logarithms, simple calculations, Nernst equation related calculations and complex calculations). Students were required to complete the test in 30 minutes; no calculators were allowed and students were not forewarned about the test. The test was carried out anonymously to protect student privacy. Students were asked to write on the front of the test paper their mathematical qualifications and grade (e.g. B grade at A level). Sixty-five students contributed, and of those students 21 had passes at A level Maths whereas the other 44 had achieved a GCSE in Maths, a lower qualification (for a description of the A level system in England see (11)). Figure 4A shows the performance of students either possessing or lacking A level Maths. The results are dismal, as out of 1105 questions only 75 were answered correctly (6.6%). There was no significant difference in the performance of those students possessing or lacking A level Maths (p =

0.47; Fisher's exact test). Within a week of carrying out the test students were given a workshop covering the contents of this paper. Students were then reassessed within a few days with a new exercise (Appendix 2), which was similar in structure and content to the initial test, in order to allow quantitative comparison of any improvement in performance. Post-workshop performance (Fig 4B) showed a considerable improvement ($p < 0.0001$; Wilcoxon's matched pairs single ranked test). The data collected in the first workshop determined the extent of the problem that confronts students. Academic staff that were taught logarithms expect present day students to possess an equivalent degree of understanding, but this is not the case. In circumstances such as these students are reluctant to admit to such gaps in knowledge, and thus these deficits are not remedied. The workshop described in this paper, or equivalent variations, appear an effective means by which to address this issue.

The objective of this workshop was to determine if students *understood* the logarithmic transformation by applying the rules described in this paper. These results strongly suggest that no assumption of competency in logarithms can be made in incoming undergraduate Life Sciences students in the UK, the possession of A level Maths no indicator of understanding in the subject. Students clearly benefit from remedial workshops that reinforce basic logarithmic descriptions and calculations. This is likely due to the fact that in the workshop we reinforced the role of the logarithm as integral to key processes (logarithmic transformation underlies the graphing of dose-response relationships that are ubiquitous in pharmacology, pH and Nernst equation calculations relating to membrane potentials) that must be mastered by students wishing to prosper in a Life Sciences degree. We would thus recommend a revision session is given to all incoming undergraduate Life Sciences students entering UK universities on topics contained in this paper to bring all students to an equivalent level of understanding.

Discussion

Examination of classic physiology textbooks shows an attention to detail with regard to elementary physico-chemical relationships that has not survived into the 21st century. It could reasonably be argued that the 45 pages devoted by J Walter Woodbury (32) to the genesis of the membrane potential is excessive, but in his defence all the information that an undergraduate is ever likely to need on the subject is present and can be skipped by the disinterested student, whereas to omit such information leaves the student in a state of ignorance. The general level of knowledge assumed by authors of previous generations with regard to the reversal/membrane potential can be appreciated when considering statements such as "the reversed potential difference which could be obtained by a mechanism of this kind might be as great as 60 mV, in a nerve with an internal sodium concentration equal to one tenth that of the outside (14)" and "As would be expected ... equilibrium potentials change sign if the charge of the ion is reversed or if the direction of the gradient is reversed, and they fall to zero when there is no gradient (12)".

The omission of the rules of physics and physical chemistry that govern physiological processes in current textbooks is compounded by recent advances in technology that have rendered the requirements to understand the logarithmic function obsolete for everyday purposes. However these technical advances have come at a cost, namely an ill-conceived acceptance of redundancy of aspects of mathematics that are vital to students' understanding of fundamental physiological principles. In this paper we have identified deficits in student understanding of the logarithmic function, which were remedied with a revision workshop, covering the contents of this paper. We sought to impress upon students the benefits of mastering the logarithmic function, and reference two classic papers, whose conclusions can only be fully appreciated if the application of the logarithmic function in the Nernst equation to membrane potential calculations is fully understood.

Disclosure statement.

The authors declare no conflict of interests

Figure Legends

Figure 1

Representation of data on a logarithmic scale. A. Linear representation of the relationship between geometrical progression (top) of numbers (n) and the arithmetic progression (bottom) of the corresponding logarithms to the base 10 of the numbers ($\log_{10}n$). B. A logarithmic scale between 10 and 100 displays uneven intervals between numbers. C. The underlying concept that multiplication by a fixed amount moves data along the axis by a fixed distance on a logarithmic scale

Figure 2

Nernstian relationship between Em and $[K^+]_o$. A. A hypothetical cell permeable only to K^+, with a constant $[K^+]_i$ of 75 mM shows a linear relationship (bold diagonal line) with $[K^+]_o$ plotted on a $\log_{10}$ scale. Em depolarised 59 mV for each order of magnitude increase in $[K^+]_o$, e.g. raising $[K^+]_o$ from 3 to 30 mM depolarised the Em from -82 mV to -23 mV, a 59 mV shift (horizontal dotted lines). B. The $[K^+]_i$ can be estimated by extrapolating from 0 mV (dotted horizontal line: see text).

Figure 3

Stimulus induced changes in glial Em A. Redrawn Fig 4 from (20) demonstrating the stimulus evoked transient depolarisation of glial cell membrane in *Necturus* optic nerves bathed in 3 mM (left trace), 4.5 mM (middle trace) and 1.5 mM (right trace) $[K^+]_o$. B. Calculated stimulus evoked depolarisation that would occur in glial cells bathed in $[K^+]_o$ over the range 1 to 6 mM. The dotted lines border the $[K^+]_o$ employed experimentally (20). Note that this relationship is what one would obtain by subtracting the open circle data points from the corresponding resting Em in Fig 5 of (20). C. Diminished amplitude of *Necturus* glial Em depolarisation in response to sequential stimuli. The vertical $\log_{10}$ scale illustrates the attenuation of fixed increases in $[K^+]_o$. Experimental trace redrawn from Fig 6 of (20) to show attenuation of Em depolarization in response to 9 sequential stimuli. Horizontal scale bar is 5 seconds, vertical axis (mV) omitted due to qualitative nature of our description.

Figure 4

A summary of student performance pre- and post-workshop. A. Comparison of performance in the 17 questions of Assessment 1. Results are normalised based on 65 student responses and the students were categorised as lacking (open columns) or possessing (grey columns) A level Maths. There was no significant difference between the two groups. B. Post-workshop analysis showed a significant improvement in student performance post-workshop (light grey columns) compared to pre-workshop performance (dark grey columns), based on the 17 questions of Assessment 2.

Assessment 1 (answers in parentheses)

Nomenclature of the logarithm

1. What is the characteristic of 2.4? (2)
2. What is the mantissa of 3.6? (.6)

Laws

3. How else can logab be expressed? (loga + logb)
4. How else can log(a/b) be expressed? -(log(b/a))

Simple calculations

5. $\log_{10}100$ = (2)
6. $\log_{10}0.1$ = (-1)
7. $\log_{10}10000$ = (5)

Understanding the logarithmic scale

8. On a $\log_{10}$ scale what is the ratio between the distance from 10 and 20, and 20 and 80? (0.5)
9. If $\log_{10}30 = 1.477$ what is $\log_{10}900$? (2.954)
10. If $\log_{10}2 = 0.301$ what is $\log_{10}5$? (0.699)

Nernst type calculations

11. For anion A, if $[A]_o$ = 100 mM and $[A]_i$ = 10 mM what is polarity of E_A ? (-ve)
12. For cation B, if $[B]_o = [B]_i$ what is the value of E_B ? (0 mV)
13. For cation A, if $[A]_o$ = 100 mM and $[A]_i$ = 10 mM, and for cation B, if $[B]_o$ = 1 M and $[B]_i$ = 1 mM, what is the ratio of E_A/E_B ? (0.33)

Complex calculations

14. What $[H^+]$ equates to pH 2.301? (0.005)
15. $\log_{10}0.0003$ = (-3.523)
16. $\log_{10}0.3 + \log_{10}30$ = (0.954)

Assessment 2

Nomenclature of the logarithm

1. What is the characteristic of 1.0? (1)

2. What is the mantissa of 4.9? (.9)

Laws

3. How else can $\log_a b$ be expressed? (logb/loga)

4. How else can -log(a/b) be expressed? (log(b/a)

Simple calculations

5. $\log_{10}1$ = (0)

6. $\log_{10}1000$ = (3)

7. $\log_{10}0.01$ = (-2)

Understanding the logarithmic scale

8. On a $\log_{10}$ scale what is the ratio of the distance from 10 and 30, and 30 and 90? (1)

9. If $\log_{10}2 = 0.301$ what is $\log_{10}400$? (2.602)

10. If $\log_{10}50 = 1.699$, roughly what is $\log_{10}7$? (0.85)

Nernst type calculations

11. For anion A, if $[A]_i$ = 100 mM and $[A]_o$ = 10 mM, what is polarity of E_A? (-ve)

12. For cation B, if $[B]_o = 10[B]_i$ what is the polarity of E_B(+ve)

13. For monovalent cation A the value of E_A = +60 mV. What would the value of E_A be for a divalent cation if the value of $[A]_o$ was doubled and $[A]_i$ was unchanged? (+40 mV)

Complex calculations

14. What $[H^+]$ equates to pH 3.301? (0.0005)

15. $\log_{10}0.02$ = (-1.699)

16. If $\log_{10}2 = 0.301$ what is $\ln_e 2$? (0.693)

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