Models and Mechanistic Insight

"The mission of the *Journal of General Physiology* is to publish original work of the highest quality that elucidates basic biological, chemical, or physical mechanisms of broad physiological significance. This expectation—*that the articles provide mechanistic insight*—has served the Journal well; it also has been cause for much uncertainty, mostly pertaining to what actually constitutes mechanistic insight? ... The Journal has a proud tradition of providing a venue where authors are able to convey their work, its significance, and its mechanistic implications, in full. The emphasis on mechanistic insight and the associated scientific rigor also means that articles published in the Journal tend to have a very long shelf life, a factor not currently considered in the calculus of 'impact'" (Andersen, 2008).

The January 2008 editorial (re)emphasized the importance of mechanistic insight in the evaluation of submissions. In this editorial, written jointly at the transition of our editorships, we try to flesh out by example and analysis the nature and the role of models in conveying mechanistic insight. We do so because the question "what constitutes mechanistic insight" continues to come up in communications with authors and reviewers.

To structure the editorial, we selected from the wealth of possibilities in the Journal's archives four publications that present diverse examples of models of physiological phenomena—and which continue to be cited: Hecht, Schlaer, and Pirenne (1942); Hille (1973); Armstrong and Bezanilla (1974); Baylor, Hollingworth, and Chandler (2002), hereafter HSP('42), H('73), AB('74) and BHC('02). After briefly discussing each of the four articles we proceed to discuss the nature and roles of models in general physiology.

1. Hecht, Schlaer, and Pirenne (1942): Single Photon Signaling by Rods

HSP('42) was an experimental effort to determine whether the quantal nature of light energy transfer to visual pigment molecules had implications for vision. They developed an experimental model of a human observer detecting a small, briefly flashed target imaged under night vision conditions: the target was flashed for 1 ms on a circular patch of the peripheral retina subtending ~500 rod photoreceptors. In the model, an observer is hypothesized to detect the target if some criterion minimum number of photons is captured. For 7 observers in 25 different sessions, the average threshold (arbitrarily defined as the light intensity giving rise to 60% detection) was 92 quanta. Physics dictates that the actual number of photons captured on any given trial using a flash of nominally fixed intensity will be a Poisson random variable. The "frequency of seeing" curve, which plots the fraction of trials in which light was seen at a given light intensity in a series of flashes, therefore can be derived from the Poisson distribution. To fit the model to an observer's frequency of seeing curve, two parameters must be estimated: (1) the criterion minimum number of photons required for the observer to say "I see it"; and (2) the fraction of light lost in transmission from the cornea to the retina. HSP('42) estimate the latter parameter two ways, first from known transmission losses, and second by the fit of the model curve. Based on fitting of the model and the parameter estimates, HSP('42) conclude that between 5 and 14 quanta are absorbed at threshold. The key conclusion of the study, which has stood the test of time, is that under the experimental conditions of a target comprising ~500 photoreceptors, no individual rod could possibly be capturing more than one photon at threshold—and thus that rods were able to reliably detect and signal single photons.

The HSP('42) model is now considered oversimplified, and has been superseded by more complex models that include measurements of the light energy transfer through the optical media, additional details about light guiding in rods and the photoisomerization of rhodopsin in the disc membrane, and the consequences of the spontaneous noise arising in photoreceptors and other neurons in the visual pathway for visual threshold (Barlow, 1956; Hallett, 1969). Nonetheless, the model has had an enduring importance because it brought to physiology the insight that the quantal nature of light has implications for vision and focused attention on the molecular mechanisms that underlie reliable single photon responses. In the late 1970s Baylor et al. (1979a,b) developed the suction pipette method for recording rod outer segment membrane currents and in elegant, landmark experiments measured the single-photon responses of rods isolated from toads. Indeed, through the 1980s on until the present, a great body of physiological research has continued to explore and establish the molecular mechanisms by which rods of all species perform the stunning feat of reliable single-photon detection, first inferred by HSP('42).

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Abbreviations used in this paper: GHK, Goldman-Hodgkin-Katz; PSF, point-spread function; TTX, tetrodotoxin.

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Hille (1973): Mechanisms of Ion Channel Permeation and Selectivity

A cardinal issue in electrophysiology has long been the mechanism(s) underlying the ion selectivity of different types of channels. The relative permeability of a channel to different ions provides a basis for reasoning about the dimensions and nature of the selectivity filter. In the early 1970s, Bertil Hille published a series of articles in which he undertook a systematic analysis of the ion selectivity of voltage-dependent sodium and potassium channels in myelinated sciatic nerve fibers from *Rana pipiens*. For measurements on sodium channels, the potassium channels were blocked using TEA^+; for measurements on potassium channels, the sodium channels were blocked using tetrodotoxin (TTX). The currents were measured under voltage-clamp conditions, with the critical measurement being the current reversal potential with different ionic concentrations in the extracellular solution. The series of articles culminated with H('73), which reported the most extensive measurements to date on the relative permeability of voltage-activated potassium channels to a series of metallic and organic cations.

The experimental approach employed by Hille allowed for the functional isolation of the different channel types. The model used to estimate the relative ion permeabilities was the Goldman-Hodgkin-Katz (GHK) description of the membrane potential, which remains an important tool for evaluating the selectivity of ion channels. H('73), along with its predecessors on voltage-activated sodium channels, provided a quantitative, empirical basis for a sketch of the size of the channels' pores and reasoning about their selectivity filters. The mechanistic interpretation of the results identified the key issues that continue to be central in discussions of ion selectivity: the relative importance of steric fit, flexibility, and ion solvation by the pore-lining groups. Many subsequent investigations—most notably the production and analysis of potassium channel crystal structures (Doyle et al., 1998), which in turn provided the foundation for detailed molecular dynamics studies (Noskov and Roux, 2007)—have greatly refined the understanding of the nature of ion channel permeability and selectivity. Yet, the key elements were clearly identified in Hille's work, which laid a broad, firm foundation for one wing of the great edifice of modern ion channel physiology.

Armstrong and Bezanilla (1974): Mechanism of Voltage Gating

The landmark study of Hodgkin, Huxley, and Katz, which culminated in the reconstruction of the action potential based on analysis of the voltage-clamp currents (Hodgkin and Huxley, 1952), laid the groundwork for understanding the mechanisms underlying electrical excitability. But a key piece of experimental evidence was missing: detecting and measuring the charge movement predicted by Hodgkin and Huxley to be associated with the voltage-dependent changes in membrane conductance, that is, the so-called gating currents. The problem was resolved by AB('74), who unequivocally characterized the gating charge movement in voltage-gated sodium channels. The preparation was the giant axon of the squid, perfused externally and internally, which then was voltage clamped using a circuit with a time resolution of 10 μs. Ionic currents were suppressed using impermeant ions, TEA^+ and TTX.

A key insight underlying the success of AB('74) was that the gating charge movement should be nonlinear—being measureable only over a voltage range where the sodium channels could be activated. In contrast, charge movement not involved in gating would be expected to be linear (be of equal magnitude with hyperpolarizing and depolarizing voltage steps of equal magnitude), and could thus be removed by (post-hoc) subtraction of capacitive currents measured with equal and opposite voltage steps. Moreover, the gating currents would be expected to have distinct kinetics during activating and deactivating voltage steps, but the total charge movement should be conserved. The measured gating currents satisfied these expectations; there also were distinct differences between the kinetics of measured gating currents and the predictions of Hodgkin and Huxley (1952), differences that inspired many further investigations.

Baylor, Hollingworth, and Chandler (2002)

BHC('02) reported a model of the release and diffusional dissipation of Ca^{2+} in the myoplasm of intact frog skeletal muscle fibers. The model was used to interpret a large body of previously published calcium spark measurements (Hollingworth et al., 2001). Sparks are caused by small, brief bursts of Ca^{2+} released from the SR through RYRs. These noisy signals are observed as changes in fluorescence of a Ca^{2+}-sensitive dye, measured with a high resolution confocal microscope. The basic model, which was developed by BHC over the previous decade, describes the diffusion of Ca^{2+} released in brief, stereotypic openings of one or more RYRs, and includes the binding and unbinding of Ca^{2+} to the known myoplasm Ca^{2+} buffers, including ATP, parvalbumin, troponin, the Ca^{2+}-sensing dye fluo3, the diffusion of the buffers, and the reuptake of Ca^{2+} by the SR Ca^{2+}-ATPase. The kinetic scheme describing these reactions is complex, involving >30 parameters as well as the resting concentrations of Ca^{2+} and its buffers. Most of the parameters were reasonably well known from biochemical literature and prior physiological experiments.

Important features of the BHC('02) spark model are the inclusion of photon noise and explicit treatment of the effect of the sampling by the confocal microscope

point-spread function (PSF) because the precise location of the centroid of the PSF relative to the RYR source is unknown. A fully adequate model therefore must consider explicitly the location of the PSF relative to the spark source. By carefully processing hundreds of thousands of theoretical sparks with the same selection criteria as was applied to experimentally measured sparks, BHC('02) were able to address the mechanistic basis underlying several small but robust discrepancies between theoretical and experimental sparks. Rejecting a number of potential mechanisms that might underlie the discrepancies, BHC('02) reached the conclusion that the simplest modification of the basic model that could bring theory into concert with the spark results is to allow for the two Ca^{2+} binding sites on troponin (the major myoplasmic Ca^{2+} buffer) to have distinct affinities and be cooperative.

Characteristics of Good Models and their Role in Conveying Mechanistic Insight

We now return to the question: what are the characteristics of a "good" model, and what is the role of models in conveying mechanistic insight? Before listing the important characteristics, however, we note a useful criterion for whether an article provides mechanistic insight, namely that the reader thinks differently about the problem after having read the article. We further emphasize that in the review of scientific articles "mechanistic insight" is taken to mean novel mechanistic insight. A study may provide mechanistic insight but, if the results were predictable from previous studies in related systems, the study might be considered an incremental advance.

1. Embody the Critical Features of the Physiological System being Investigated

Each of the articles discussed above focuses on a specific physiological question and presents a model of the phenomenon, which in turn guides the experimental design and the data analysis and interpretation. In HSP('42) the critical feature is the predicted fluctuation in photon capture from a flash of nominally fixed intensity, as dictated by the quantal nature of light. In H('73) the critical feature is that the reversal potential of an ion channel under any specified set of permeant ion concentrations is a condition in which diffusional ion fluxes are balanced by the electromigrative fluxes such that net charge movement through the channel is zero. In AB('74) the critical feature is the separation of the ionic and capacitive current components and the "isolation" of the nonlinear capacitative currents. In BHC('02) the critical feature is the articulation of all the binding interactions and spatial features of the SR myoplasm expected to determine the measured spatiotemporal characteristics of the dissipation of Ca^{2+} released from brief openings of RYR(s).

2. Incorporate Relevant, Up-to-Date Physical and Chemical Principles and Parameters

The models developed in the chosen articles incorporate well-established physico-chemical principles that apply to the specific preparation. A good model needs to be realistic in its characterization of the preparation and be framed in such a way that the parameters to be estimated are physically reasonable and interpretable. Whether or not a model satisfies this criterion may be difficult to ascertain, as it will depend on advances in other fields—and it is a frequent topic of debate among authors, reviewers, and editors. Models that include parameters with no clear physical interpretation, which are developed using concepts that do not incorporate recent advances or are out of date, are problematic. In investigations of ion channels (or any other proteins), for example, if there is a relevant crystal structure it should be used, keeping in mind that PDB coordinate files depict static structures devoid of the molecular motions that underlie the proteins' physiological functions.

3. Link Measured Quantities with the Underlying Mechanisms

In physiology, as in other arenas of science, there are the measurables and there are physical (cellular and molecular in this case) events hypothesized to underlie the measurements. Moreover, the results critical for probing a mechanism often have been extracted from measurements that have intrinsic fluctuations and contamination from systematic noise. A feature of a good model therefore is that it helps to identify what is "signal" and what is "noise," and to guide attempts to extract the signal, either by experimental design or by post-processing.

In HSP('42) the subjective variability in the detection of a flash of a given intensity was ascribed to fluctuations in photon delivery to and capture by rods, and the fitting of the frequency of seeing curve links the measured independent and dependent quantities (percent correct vs. average quanta at the cornea) to mechanism. In Hille('73) the reversal potential measured with a specified set of concentration differences is linked via the GHK equation to the permeability ratios. In AB('74) the capacitive current extracted after all other predicted "contaminant currents" were eliminated experimentally or by post-processing was compared with expectations based on the kinetics, magnitude, and other properties (such as correlation with reversible manipulations of the sodium current) of the hypothesized/predicted gating charge movement. In BHC('02) a meticulous analysis of the effects of the PSF of the confocal imaging system was needed to bring the predictions of a dynamic model of Ca^{2+} release from a point source into concert with the measurements.

In general, then, a good model should incorporate not only a description of the underlying physiological system and mechanism but also relevant features of the

entire measurement system. Moreover, the link between measurements and mechanism usually is quantitative: a proposed model may predict the correct sign of an effect, but may yet be rejected because it does not predict the correct magnitude or because the residuals show systematic deviation from theoretical curves generated with the model. This quantitative link between measurements and mechanism, in turn, becomes important for a model's ability to predict new phenomena and, eventually, to be supplanted by a more complete model.

4. Reduce a Complex System to the Features Essential for Describing the Mechanism under Investigation

In each of the articles discussed, a complex system was reduced to a few features that were considered to be the most relevant for the system and mechanism under investigation. This conceptual reduction is, in part, an issue of scale. It would not have been much help to the goal of AB('74) to have included a detailed model of the charged phospholipids, which were known to flip flop across lipid bilayers at a time scale much too slow to be relevant for sodium channel voltage activation. The goal of H('73) to extract permeability ratios did not consider the current magnitudes, except to the extent they affect reliability of the reversal potential measurements. A good model frames the mechanism while at the same time conceptualizing for the experimenter (and reader) those aspects of the system that are considered to be irrelevant to the investigation. A good model does not neglect complexity but informs us how to extract the relevant components from the measurements. This principle can be thought of as a variant of "Occam's Razor," the interdiction against postulating more entities than are needed: keep the model as simple as possible, but as complex as needed to describe the critical features of the system and resultant data.

5. Provide a Framework for Summarizing the Results Succinctly in Terms of Physiologically Interpretable Parameters

Most models are expressed in terms of parameters or manipulated variables that are independently known or measured, and parameters that are estimated from the results. As presented in an article, an ideal model should be framed in a manner that focuses attention on important, system-specific parameters to be estimated. In HSP('42) the physiologically meaningful parameter is the number of photoisomerizations triggered in a region of the retina subtending ~500 rods that are needed for the observer to say "Yes, I see it" 60% of the time. In Hille('73) the critical estimated parameters are the permeability ratios of the potassium channels to a series of permeant cations. In AB('74) key parameters include the conservation of the outward- and inward-moving gating charge and the time course of the gating currents relative to the time course of sodium current activation and deactivation. In BHC('02) the critical features of the measured calcium sparks include rise time, peak amplitude, decay time, full width at half maximum, and mass, features that are compared quantitatively with the properties of simulated sparks generated with several parametrically distinct models.

Because models serve as frameworks for summarizing the results, they also become mnemonic devices. The model of HSP('42), for example, can be summarized as "single photon detection"; of H('73) as "selectivity filter," of AB('74) as "gating charge movement," etc.

6. Focus Attention on Poorly Understood Features of the System, the Features for which New Experimental Results or Expanded Theoretical Analysis Are Needed.

The development and testing of a model almost invariably brings out limitations and inadequacies that need to be addressed—by the development of new experimental methods and preparations, by further theoretical elaborations, or by a combination of the two. Providing a reasonable "fit" to the results is a necessary, but not a sufficient, basis for accepting a model as an adequate description of a mechanism. Subsequent to HSP('42), for example, two important developments occurred that required modification of their model of detection. First, it became broadly accepted after the work of H. B. Barlow (1956) that the visual system had intrinsic noise of retinal origin that would necessarily limit threshold detection. Second, the development of signal detection theory led to the insight that false positives were inevitable at the limit of signal detection, and that by performing a detection task so as to keep the false positive rate low, observers were inevitably not reporting instances in which targets were reliably detected (Hallett, 1969). This in turn led to investigations on the nature of the intrinsic noise in the visual system, including the photon-like "shot noise" in rods (e.g., Baylor et al., 1980).

The GHK model employed by H('73) incorporated the assumption that different ions move independently of each other through the channels, an assumption that was known to be incorrect in detail, and that turned out to be unnecessarily restrictive. Today, a channel's ion selectivity continues to be characterized in terms of permeability ratios deduced using the GHK equation, but the theory of permeation and selectivity is focused on the microscopic landscape of the channel pore as visualized in crystallographic studies (Doyle et al., 1998) and further elaborated using computational methods. This modern focus was foreshadowed by H('73) in the discussion of the physical mechanism(s) underlying a channel's ion selectivity.

7. Provide a Framework to Guide the Design of Experiments on Related Systems

The experimental design and analyses in each of the selected articles provide paradigms for similar analysis of

related systems. HSP('42) laid the foundation for behavioral experiments that demonstrated single-photon sensitivity of rods in nonhuman species, and showed that the retina of vertebrate species with rods is organized to process single-photon signals and yet able to guide visual behavior over many log units of light intensity. H('73) described a paradigm for characterizing a channel's ion selectivity. Selectivity remains a central issue in ion channel research, and the "selectivity series" for an appropriate set of permeant ions constitute canonical data that serve as reference targets that molecular models of selectivity (based on the channel ultrastructure) must explain. The physical mechanisms underlying the gating charge movement has been investigated extensively since AB('74), and has been associated with a movement of the S4 helix relative to the electric field (Bezanilla, 2008). The combination of structural (Long et al., 2007) and functional studies have revolutionized our understanding of voltage-dependent gating, but the measurements of AB('74) laid the foundation for understanding voltage dependence in the superfamily of voltage-gated channels. Calcium sparks in muscle fibers may seem esoteric to many, but they are paradigmatic for the characterization of calcium release from internal stores, and the framework developed by BHK('02) sets a gold standard for the quantitative understanding of the internal dynamics of calcium signaling in a vast variety of cells and tissues.

Conclusion

Models are sketches of physical reality that scientists construct in an effort to understand and manipulate the system under investigation—and to characterize and summarize the mechanisms that give rise to the system behavior manifest in the measured quantities. As knowledge about a physiological system accumulates, good models tend to require increasingly rich detail and become expressed in increasingly complex formulations. Models need to be faithful to the relevant physical principles and laws at the given moment in the evolution of a field, and yet need not (especially early in a field's evolution) be expressed in complex mathematics. It is noteworthy that the articles selected for discussion demonstrate that model development need not require a significant mathematical superstructure, and therefore belie the notion that the *JGP* favors articles that present models having a lengthy list of equations. Indeed, HSP('42), H('73), and AB('74) together contain only two explicit equations. But neither does the *JGP* shy away from formal complexity when it is needed for an adequate description, as is illustrated by the elegant and powerful model of BHC('02), which characterizes the quantitative nature of a calcium spark in the intact frog skeletal muscle fiber.

In closing, our goal here has been to provide guidance to and provoke discussion among the authors, reviewers, and readers of the *JGP*. We look forward to an ongoing dialog about the characteristics of articles that yield novel mechanistic insight.

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