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COMMENTARY

Voltage-Gated Na Channels

Mind the midpoints: Rethinking variability in cardiac I_{Na}

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The function of the heart depends critically on the precise timing and coordination of electrical signals generated by ion channels in cardiac cells. The voltage-gated sodium current (I_{Na}) plays a pivotal role in initiating the rapid depolarization that drives each heartbeat. Two important descriptive properties of cardiac I_{Na} are its *activation* and *inactivation* midpoints, which describe the membrane voltages at which there is a 50% probability of the channel being open or unavailable, respectively. These midpoints determine the voltage range over which sodium channels contribute to the action potential and influence how easily the heart can initiate and propagate electrical signals. Because even small shifts in these kinetic parameters can affect excitability, conduction, and arrhythmia risk, they are commonly used to characterize the effects of drugs, mutations, and disease states.

While cardiac ion channel kinetics are often regarded as stable and predictable, a closer examination via meta-analysis by Clerx et al. (2025) reveals a startling degree of inconsistency across studies and systems. Their analysis underscores the overarching reality that cardiac electrophysiological research is marked by substantial heterogeneity in cellular morphology, maturation states, and electrophysiological measurements. Clerx et al. (2025) undertook a systematic analysis that yields a striking reality: broad ranges in the voltage midpoints of activation and inactivation for the cardiac sodium current (I_{Na}). This is despite the perception that activation and inactivation curves are generally viewed as stable and that small changes in the voltage dependence or slopes in these curves, resulting from natural (genetic) or applied (drug) perturbations, can affect human physiology. The comprehensive meta-analysis from Clerx and coauthors challenges long-standing assumptions about data interpretation, data comparison, and electrophysiological data used to develop models.

Using a dataset of 157 activation and 165 inactivation midpoint values from 117 studies, Clerx and colleagues dissect the variability across and within the studies. Their most striking observation is not just the range of variability, spanning 39 mV for activation and 51 mV for inactivation, but the notably strong correlation between these parameters across experiments. The correlation cannot be accounted for by known experimental variables such as α-subunit type, β1 co-expression, or cell line. Their decomposition of variance into correlated and uncorrelated components reveals that variability is highly correlated, suggesting shared biases or systematic influences that affect both midpoints similarly.

This revelation has two important implications. First, it raises the possibility that I_{Na} measurements reflect technical artifacts more than intrinsic biological variability. Second, it exposes the vulnerability of mechanistic and statistical models that rely on datasets assuming they reflect canonical I_{Na} values. The authors provide a catalogue of possible underlying culprits, from liquid junction potential miscalculations and redox drift to variation in voltage control, protocol design, and culture conditions. Despite this careful consideration, much of the variance remains unexplained, even within the same study. The residual unknowns may reflect a need to reassess how electrophysiological data are gathered, validated, and reported.

On the other hand, given the value that decades of measurements have given to deep understanding of the mechanistic underpinnings of cardiac electrophysiology, perhaps *absolute quantitative midpoints* are not the metric we need to focus on the most. Most measurements of I_{Na} kinetics are performed in heterologous expression systems, far removed from the cardiac

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myocyte native context. In the living heart, sodium channels are embedded in a tightly regulated environment of accessory proteins, lipid domains, posttranslational modifications, and cell type–specific architecture. Measuring I_{Na} in HEK or CHO cells *by design* strips away the intrinsic biological complexity of the cardiac myocyte, yielding better experimental control.

It may well be that the scientific and clinical value of I_{Na} measurements lies less in the *absolute voltage* at which channels activate or inactivate, and more in the *relative changes* these parameters undergo in response to drugs, mutations, or environmental shifts. A +5 mV depolarizing shift might reveal something meaningful in one experimental context and be meaningless in another. The Δ may be the key metric required to reveal fundamental physiology. Even still, if a small shift in a kinetic parameter falls within the natural range of experimental variability, how do we interpret its linkage to normal physiology or pathophysiology in disease models? Current experimental approaches make it difficult to determine what constitutes meaningful change versus methodological noise.

From a technological standpoint, Clerx et al. (2025) offer a constructive path forward. Newer data acquisition systems with integrated metadata that include voltage protocols, compensation settings, and series resistance could overcome or enhance reproducibility. But more importantly the authors advocate for rigorous and complete reporting standards (e.g., Minimum Information about a Cardiac Electrophysiology Experiment) and embrace of open data practices that could transform patch-clamp data into reusable, high-resolution resources for future modeling and analysis. Strong data standards might include converting existing noncurated datasets to allow for direct testing of the hypotheses regarding protocol details or solution compositions. Incorporating such analyses accessible through metadata could further identify starting point protocol standardization and actionable guidance to the field.

To identify true physiological ranges for ion channel kinetics, there are in situ proxies in native myocytes. The upstroke velocity of the cardiac action potential is one such indicator Na^+ channel as a steep, rapid upstroke suggests robust I_{Na} availability and conductance, while a blunted upstroke can reflect impaired channel activation or excessive inactivation. Under appropriate experimental conditions, the peak membrane potential reached during the upstroke can offer additional insight into the activation midpoint, especially when computational models are used to deconvolve the contribution of I_{Na} from other inward and outward currents. Inactivation dynamics, while more elusive, may be inferred through recovery protocols, where the time course of current reactivation after a depolarizing pulse gives clues about channel availability. Whether these can be directly translated into a "midpoint of inactivation" remains debated, but such approaches at least anchor measurements in physiological relevance.

Computational modeling, simulation, and prediction are essential tools to link in vitro measurements to in vivo mechanisms. The large variability seen in patch-clamp experiments underscores the challenge of defining a single canonical voltage dependence for I_{Na}. But computational models, particularly those constrained by physiologically observed action potentials, can allow inference into plausible physiological ranges for activation and inactivation midpoints for specific cell types. Simulations can be readily performed to test how shifts in gating properties affect emergent behavior such as conduction velocity, excitability, or arrhythmia susceptibility, and can guide experimentalists toward the most physiologically relevant regimes. Moreover, models offer a sage virtual environment, or *sandbox*, in which to probe questions not easily accessible by experiments such as: What happens when activation and inactivation shift in concert versus independently? How sensitive is the action potential to changes in I_{Na} gating under different load conditions or pacing rates? Such in silico investigations are not replacements for experiment, but essentially coupled companions, especially when experimental data are noisy, heterogeneous, or incomplete.

The work from Clerx et al. (2025) is both pedagogically impactful and practically important and should be incorporated into basic electrophysiology training to teach early-career scientists not just how to measure I_{Na}, but how to interpret the potential impacts of variability on emergent physiology in cells and tissues. Moreover, the implications extend into translational domains such as drug testing and precision medicine. Studies that do not rigorously control or report experimental confounders may lead to misleading conclusions about ion channel modulators or mutations associated with disease phenotypes. A principal contribution of Clerx et al. (2025) is philosophical, as their analysis makes clear that quantitative measurement does not inherently confer meaning and that interpretive analysis must be situated within a robust understanding of contextual frameworks.

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Reference

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