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The surprising heart revisited: an early history of the funny current with modern lessons

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ABSTRACT

40 years ago a single experiment upset a decade of painstaking research on the mechanisms of rhythm generation in the heart. It did so by turning a theory of pacemaker activity upside down. Instead of attributing rhythm to decaying potassium current carrying outward current, it attributed the pacemaker depolarization to the slow activation of a channel conducting sodium ions *into* cardiac cells. But this was no standard upset of a theory. Like the replacement of Newtonian mechanics by relativity theory, the new theory explained every minute detail of the experimental observations that had established the theory it was replacing. Computational modelling of the heart achieved one of the major successes, the complete mapping of one theory onto another without challenging the correctness of any of the experimental findings. This review details the way in which this transition occurred and draws some important lessons for modelling of biological processes today.

1. Introduction

This article is a contribution to a Special Issue of *Progress in Biophysics and Molecular Biology* dedicated to Dario DiFrancesco on his retirement in 2019 from his chair at the University of Milan. Dario has been at the forefront of the biophysics and molecular biology of heart rhythm ever since he left my laboratory in Oxford 40 years ago to continue his work in Milan. It was from there that I received a fateful telephone call. In a commissioned prize review article called *The Surprising Heart* in 1984 (Noble, 1984: page 218) I reported this call:

> "He telephoned me from Milano in January 1980 to tell me this result and the same night I was able to use a computer program he and I had developed together to show that his new interpretation of i_{K2} as a non-specific inward current i_f could give a full and accurate theoretical account of the i_{K2} results." *The Surprising Heart*, 1984, page 10.

What was "this result" and why was it so significant? People do not usually reference telephone calls in scientific papers! It was so important and surprising that it was to cause turmoil worldwide in cardiac electrophysiology for several years. And the discovery was itself the outcome of turmoil in my own laboratory a few years earlier. When the dust settled it turned out to be the discovery of a new class of cardiac ion channels, and the story of its discovery was itself surprising, almost as though nature had set an obscure mathematical trap for cardiac electrophysiologists to fall into. The reason for referencing the telephone call is that there was no other way to tell the full story and its surprises. It was a key. Nowadays we might cite a highly significant email.

My article will be not only a historical account, it will also draw lessons on modelling biological processes that are relevant today.

2. Intercellular ion concentration changes

The story began with the discovery that some components of electrical current change recorded from multi-cellular heart tissue arise from changes in the concentrations of sodium, potassium and calcium ions rather than just from the opening and closing of protein channels. Up to that time, we had all assumed that the electrical properties of the heart would be largely attributable to the opening and closing of membrane ion channels and exchangers. This was the assumption made in the earliest models from my own lab, including the Noble (1960) model (Noble 1960, 1962), and its much more extensive successor, the McAllister, Noble and Tsien (MNT) model (McAllister et al., 1975).

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That assumption began to look shaky when Dick Tsien and I found that the analysis of the slow potassium ion channels, i_{x1} and i_{x2}, (now called i_{Kr} and i_{Ks}, when referring to ion channels, or hERG and K_{VLQT1} when referring to proteins/genes) had also revealed some "minor exceptions" in the ionic current traces (Noble and Tsien, 1969: p 218). These exceptions were even slower components that Dick and I were reluctant to attribute directly to ion channel activity. We preferred the idea that they may have resulted from slow changes in potassium ion concentrations, perhaps in the spaces between the heart cells. Hilary Brown and Susan Noble subsequently worked on these changes in frog atrial muscle, resulting in a series of papers (Brown et al., 1976a; Brown et al., 1976a,b; Noble, 1976) that showed that their interpretation as ion concentration changes was very plausible. The changes seemed to be more dependent on the total charge moved than on any classical voltage-dependent ion channel kinetics, as would be expected if the spaces concerned were accumulating ions.

These developments posed a theoretical dilemma. The analysis of the kinetics and voltage-dependence of ionic channels depends on knowing where the electrical and chemical energy gradients balance each other at what is called the reversal potential. If the relevant ion concentrations are changing, so also is this potential. How could one possibly separate out changes attributable to that process from the real kinetics of the channels? Some critics of our work said it was impossible and that the results obtained from voltage clamp work on multi-cellular tissue of the heart were, quite simply, an inextricable mess. In fact, Johnson and Lieberman (1971) had, even a decade earlier, written a long review saying precisely that.

Later, DiFrancesco and I worked on the equations for this kind of problem. Using the mathematics of perturbation theory we showed how to dissect out the channel gating kinetics from other slower components (DiFrancesco and Noble, 1980), while Susan Noble and Wayne Giles used the Provencher (1976) DISCRETE program to confirm the accuracy of Susan's hand analysis of the multi-exponential changes seen experimentally. These two mathematical analyses formed the background research that ensured that I had a computer program ready for that fateful telephone call in early 1980. I already had the equations and programming to hand to deal with the problem that Dario's discovery created.

3. Discovery of the 'funny' current

But before we come to what that call revealed there is another important discovery to note. In the 1970s, we developed a method to study induced pacemaker rhythm in strips of frog atrial muscle (Brown et al., 1976a; Brown et al., 1976b) using a sucrose gap method pioneered by Hiroshi Irisawa, (1972a, 1972b). With Wayne Giles we extended this work to spontaneously beating strips of frog sinus venosus (the natural pacemaker of the frog heart) (Brown et al., 1977). When the electrical potential was made very negative we recorded a slowly developing inward current (also observed by Seyama (1976) in the rabbit sinus node at about the same time). We called it the 'additional current', without at that time realising its importance. That was the first clue that there might be something special about the natural pacemaker mechanism. But why should it be special? After all the differences between the natural SA node and Purkinje fibres were crystal clear and could not be ignored.

Dario had come from Cambridge to join my group 3 years earlier in 1977. He was keen to investigate pacemaking in *mammalian* tissue rather than the easier amphibian tissue, and to use the so-called 'small preparation' of rabbit sinoatrial node tissue recently pioneered by Akinori Noma and Hiroshi Irisawa (Noma and Irisawa, 1976). It was technically challenging to use this preparation so that the tiny 200 μm diameter ball of tissue continued its spontaneous beating, and it was extremely difficult to impale it with two microelectrodes and obtain a uniform control of voltage. Dario was the lead experimenter in this work and his persistence and skill was rewarded by recordings of a remarkable (as it seemed to us at the time) inward current which appeared in the potential range of the pacemaker, over precisely the same range as i_{K2} in the Purkinje fibres. But, unlike i_{k2}, it did *not* show reversal at the potassium equilibrium potential: all of these features are clearly echoes of the "additional current". Instead of reversing it continued to increase even beyond the expected reversal potential. "There's that funny current" we would say. So, i_f it became (Brown and DiFrancesco, 1980), and was later identified as an HCN1−HCN4 heteromeric channel (Altomare et al., 2003). A paper we published in *Nature* showed that it was reversibly increased by adrenaline (Brown et al., 1979), so contributing to the acceleration of heart rhythm by adrenaline. Dick Tsien had previously shown the same adrenaline effect in Purkinje fibres) before the reinterpretation of i_{K2} (Hauswirth et al., 1968).

In 1979, towards the end of Dario's period in Oxford, he performed experiments with Mitsuyoshi Ohba and Carlos Ojeda (DiFrancesco et al., 1979) that brought the ion accumulation-depletion issue right up against the interpretation of the reversal potential for i_{K2}. They showed that the reversal potential depended on "the degree of activation of i_{K2} at the start of the test hyperpolarization", and concluded that "changes in the level of $[K]_c$ [cleft potassium] induced by pre-pulses must therefore also affect the E_{rev} determination." Dario and I wrote an appendix to that paper in which we showed that a possible confusing factor, voltage non-uniformity, would not explain the magnitude of the effects. In retrospect, that work can be seen to have been very important. If the reversal potential could be shifted to a substantial extent by the degree of activation of the channel, it would be reasonable to doubt the i_{K2} theory. In a comment on an early draft of this paper, Dario has confirmed to me that he was already having increased doubts.

4. The critical experiment

On his return to Milan, DiFrancesco carried out the critical experiment. Reluctant to accept that i_f in the sinus node and i_{K2} in Purkinje cells really were two different channels that happened to share a lot of characteristics, he wondered whether the ion concentration changes my group had already investigated could account for the difference. But the problem was that in Purkinje cells there is a very clear reversal potential dependent on the potassium ion gradient. How could it be possible that this could be observed in Purkinje tissue but not in sinus node tissue? He then reasoned that Purkinje cells have many i_{K1} channels; the sinus node cells have few or none. Could that channel be carrying the ions producing the intercellular concentration changes? Suppose one blocked i_{K1} in the Purkinje tissue? He used barium ions, which were known to do this (Yanigahara and Irisawa, 1980). The result was dramatic. The reversal potential that had identified the channel as a pure potassium channel simply disappeared! (DiFrancesco, 1981). The Purkinje tissue then resembled that of the sinus node. You couldn't invent a better conjuring trick that nature had given us!

At this stage the investment of time and mathematical modelling of ion accumulation paid off. After he told me this result in that phone call, I immediately turned to the computer program that we had been using to analyse the effects of ion concentration changes. With a few relatively simple tweaks it was ready to address an audacious question. The reversal potential results in Purkinje fibres looked clean, and the dependence on extracellular potassium ion concentration followed the Nernst equation faithfully, with a 60 mV change resulting from a 10 fold change in ion concentration. Similar

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results had also been obtained by Shrier and Clay (1982) using embryonic chick hearts. In fact, their reversal potential recordings were perhaps even cleaner and more convincing than ours. How could all of those firmly established results be illusory?

5. Mapping the two theories

The audacious question was how could this possibly arise if the channels were really not pure potassium ion channels? Could Nature have set a cruel trap for electrophysiologists? This was an even greater challenge for computational modelling than anything I had tackled this far (see Fig. 1). The question to be settled was this: could the current variations attributable to ion concentration changes have kinetics so similar to the channel kinetics that they could cancel each other out cleanly? And not only do that, they also had to do so in a way that created an accurate illusion of a Nernstian reversal potential. With the relevant mathematics and computational programming already done, I worked on the mainframe computer (no desktops or laptops in those days) all night and by the next morning I was able to tell Dario that it had all worked out like a dream. The trap had become an almost magical dream. If one replaced i_{K2} in the MNT model with a mixed (sodium and potassium) current, i_f, like that in the sinus node, and included the accumulation and depletion of potassium ions in the spaces between the cells, the resulting mixture of ionic current changes behaved just like i_{K2} (Fig. 2, see also (Noble, 1984), Fig. 5).

Not only did this explain the Nernstian behaviour of the false reversal potential, it also explained the, as yet unexplained, result that the current disappeared when one removed sodium ions (McAllister and Noble, 1966) and why the apparent reversal was always a few mV negative to the expected reversal. Even the accurate 60 mV slope was fully explained. We set to work to analyse this, initially very strange, result mathematically and published it the same year (DiFrancesco and Noble, 1980a,b). The full details were published two years later (DiFrancesco and Noble, 1982).

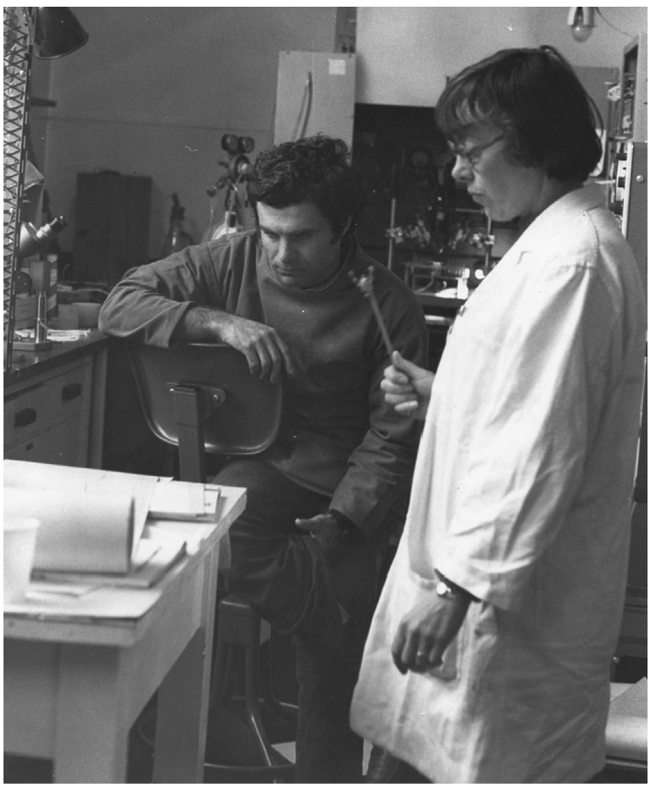

Fig. 1. "What a funny current!" Dario DiFrancesco and Hilary Brown contemplate an experimental problem in the Oxford lab in late 1970s. It might have been the first recordings of the 'funny' current.

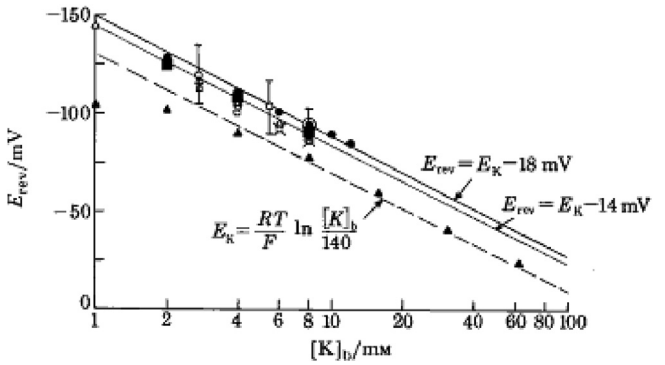

Fig. 2. One of the results of the extensive computational mapping of the original and revised theories. The experimental data on reversal potentials for "i_{K2}" as a function of $[K]_o$ are open squares (Noble and Tsien, 1968); Open triangles (Peper and Trautwein, 1969); Open circles (Cohen et al., 1976). The model results from DiFrancesco and Noble (1985) are shown as filled squares, and those from DiFrancesco and Noble, 1980a,b) are shown as filled circles. The filled triangles are resting potential measurements from (Gadsby and Cranefield, 1977). Above 4 mM [K]o the resting potential follows the Nernst equation assuming $[K]_i$ is 140 mM. All the experimental results show the same slope as the Nernst equation but with a negative displacement between 14 and 18 mV. In DiFrancesco and Noble, 1980a,b) it was shown that these displacements are well predicted by setting the extracellular space to between 7% (18 mV shift) and 28% (14 mV shift). The results are only moderately sensitive to the size of the intercellular space.

I finish this section with a note on nomenclature. The results on the slow plateau currents i_{x1} and i_{x2} are clearly the first analyses of i_{Kr} and i_{Ks} in today's nomenclature. So, why did Dick and I use the nomenclature we did? The reason was simple. If E_K really was as negative as the i_{K2} reversal potentials suggested then the plateau K current channels must have been less specific. The fast component reversed at −85 mV while the slow component reverses at an even less negative potential (Noble and Tsien, 1969 Figure 9). We concluded "the pathways conduct mainly $K+$ ions … with some leakage to other ions." Of course, all ion channels do that to some degree. Our caution, though, meant that we were not credited as much as we might have been with the discovery and kinetic analysis of the plateau-level K^+ channels.

6. Modelling lesson 1

I believe it is still quite rare in computational biology to map two opposing theories onto each other so completely. This can be important and worth the effort. Just as Einstein's relativity equations explain Newton's mechanics under the right conditions, so we need to know when there can be totally different fundamental interpretations of the same reliable experimental results. The lesson gained in this case was that much of the previous hard experimental work and mathematical modelling of the heart could be carried over as the basic theory changed. The utility of that mapping will become clear later.

7. Why was it shocking?

If you are not an ion channel electrophysiologist, it is hard to appreciate the full nature of the shock this result produced and why it was so important to do the mapping. The Nernst equation is, after all, the gold standard for identifying the ionic composition of a channel current. Yet we had shown that it could 'lie'. So

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unbelievable was the result that I had many rounds of correspondence with those who had also identified the i_{K2} mechanism in other species and tissues of the heart. My lab was far from being alone in producing results supporting the i_{K2} theory. It took some time for the significance to sink in. Was it just a coincidence? If so, why should it occur so widely? In fact, the mathematics showed that it was far from a coincidence, and it only required very moderate (10%) changes in intercellular ion concentration to produce the effects. Yet again in the work of my laboratory, not only was mathematical modelling necessary to reveal the relevant insights, mathematical *analysis* was also required; the apparently obscure work on perturbation theory had borne fruit. Once again, analytical mathematics had complemented computation to produce results of complete generality and which could provide powerful explanations of counterintuitive experimental results. Numerical computation alone would not have been so convincing.

It was shocking also because the MNT 1975 model was at that time, in its own way, another gold standard. It was a Titanic model, explaining many difficult and even counter-intuitive experimental results, most particularly on the influence of electrical and chemical factors on heart rhythm (electrical: Figure 14 of (McAllister et al., 1975), chemical: Figure 15 of that paper). It was painful to contemplate that the 'Titanic', representing a whole decade of research, had been 'holed' by a simple passive process of ion accumulation and depletion. As I show later, the context of all of this careful and accurate work could actually be preserved as a framework within which to develop the new model. The major differences will be identified below (see section 13. The Complete Model).

8. Modelling lesson 2

Computer modelling of biological systems has become much more widely implemented, and of great practical use in the pharmaceutical industry (e.g.(Mirams et al., 2012) since the 1960s (when it was extremely rare) and 1980s (when it started to take off as what led to the Physiome project). One of the problems with differential equation models is how to be sure that the solutions are general and therefore of wide application. There are many approaches to solving this problem, sensitivity analysis being one: run many simulations with different parameters to determine how sensitive the solutions are to parameters for which experimental data may be weak or very variable.

My laboratory has recently used this approach successfully in extending a model of skeletal muscle (Noble et al., 2020; Tasaki et al., 2020), showing its robustness to parameter variations. A general introduction to these methods is found in (Saltelli et al., 2008). Computers were too slow in the 1960s and 1980s for such analysis to be done effectively. Instead, we attempted to find analytical solutions where possible. The use of perturbation theory (DiFrancesco and Noble, 1980) in the analysis of ion accumulation changes was a good example. So also are many of the analytical approaches used in the book that Dick Tsien and I wrote with Julian Jack, *Electric Current Flow in Excitable Cells* (Jack et al., 1975). Even the challenging sodium channel cubic activation dynamics of Hodgkin and Huxley, (1952) yielded to a formal closed solution ((Hunter et al., 1975), equation (2.28), p 124). Analytical approaches have become less frequently used since computers have become so much faster, but they are worth exploring since the results can then be very general indeed. That is why we need good mathematicians as well as good computational modellers. Analytical approaches can also yield insights on general mechanisms, such as the spiral forms that arise in phenomena on spatial scales as different as galaxies, weather systems and heart arrhythmia (Noble, 2016, Fig. 3.4).

9. A literary digression

When Dario and I wrote all of this up for an article published in 1982 (DiFrancesco and Noble, 1982), we looked for an appropriate piece of literature that could reflect the painful, yet joyful, nature of this journey of discovery, and which would also reflect his and my side interests. I had already been exploring the medieval Troubadour poets, and had found that Dante Alighieri had praised one of the Occitan Troubadour poets, Arnaud Daniel (circa 1180) in the *Purgatorio* of *La Divina Commedia* (circa 1308–1321). Not only did he praise Arnaud as the best craftsman (*il miglior fabbro*) of poetry in the language of the people, in his case Occitan, he also wrote these verses of his great work in Occitan rather than Italian as his tribute. The verse fully expresses the pain of discovery, yet how easy it is for others to follow where the discoverer has led:

Ara vos prec, per aquella valor
que vos guida al som de l'escalina,
sovenha vos a temps de ma dolor
(Purg, XXVI, 140–147)

Which I have translated as:

Therefore do I implore you, by that virtue
Which guides you to the summit of the stairway,
Remember in due course my suffering.

The stairs of the poem were, of course, the stairs of mount Purgatory in the *Divina Commedia*. I rather like to think of them as the difficulty the researcher experiences in blazing a path up mount Discovery. Others coming later can climb readily what he found difficult. 40 years later, no-one today finds the i_f story difficult at all. But its transformation from the apparently secure i_{K2} story was far from easy. The switchover led to the DiFrancesco-Noble model in 1985.

10. Towards the DiFrancesco and Noble (1985) model

The obvious next step was to develop the MNT 1975 model to replace i_{K2} by i_f. But that was much easier said than done. It took a full 5 years of development. The reason was that it was not just a matter of replacing one ionic channel mechanism by another. It also involved modelling global ion concentration changes for the first time in an electrophysiological model of the heart, including the intracellular calcium signalling. Dario and I did that because it was necessary to explore fully what we had discovered. We did not know then that we would be creating the seminal model from which virtually all subsequent cardiac cell models would be developed. There are now many such models for various parts of the heart and many different species to be found (downloadable) on the cellml website (www.cellml.org).

I want here to also pay tribute to Dick Tsien. The model with Dario could not have been developed on the basis only of the reinterpretation of i_{K2}. Both through his experimental and modelling work on the other K^+ channels and in his mathematical skills, Dick's work was seminal in the creation of the MNT model. Since there was also no reason to doubt any of the earlier experimental results, except the reversal potential of the pacemaker current, the MNT model was therefore the initial framework within which we developed the new model: "In formulating the equations … we have used the McAllister et al. (1975) model where appropriate for all currents except for the new inward current, i_f." (DiFrancesco and Noble, 1985). Dario and I naturally wished to inherit as much as we could from the MNT model. But as soon as we started incorporating variations in ion concentrations almost everything else had to

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adapt to that new development. So, the extension was not easy. In a book dealing with my own development in cardiac electrophysiology (Noble et al., 2012) I wrote.

"Extending biological models is often like tumbling a row of dominoes. Once one has fallen, many others do too. The reason is that all models are necessarily partial representations of reality. The influences of the parts that are not modelled must either be assumed to be negligible or to be represented, invisibly as it were, in the assumed boundary conditions and other fixed parameters of the model. Once one of those boundaries is removed, by extending out to a different boundary, other boundaries become deformed too. In this case, modelling external potassium changes required modelling of the influence of those changes not only on the ion channels already in the model, but also on exchange mechanisms, like Na–K-ATPase (sodium pump) and the Na–Ca exchanger. That in turn required the model to extend to modelling internal sodium concentration changes, in turn requiring modelling of intracellular calcium changes, which then required modelling of the sarcoplasmic reticulum uptake and release mechanisms. For a year or two it was hard to know where to stop, where to stake out the new boundaries."

The boundaries between levels of biological organisation form a relatively neglected area of modern biology, largely because the hard forms of reductionism do not even recognise the existence and, even less, the causal role of higher levels. In a recent study of this problem, my colleagues and I have attempted to put this neglect right (Noble et al., 2019). An important conclusion of that study is that the forms of causation between different levels, acting across the boundaries, are complementary, not identical. It is mathematical modelling that revealed that insight, which I believe is going to be very important as multi-level studies develop further. The implications extend way beyond biology itself (Lee et al., 2019; Noble and Noble, 2020).

11. Predictions on sodium-calcium exchange

It was the boundary issue that uncovered a major new insight. To simplify the story I will focus on just one of the new mechanisms: the sodium-calcium exchanger. This had been discovered in the heart by Harald Reuter (Reuter and Seitz, 1968), who also discovered cardiac calcium currents (Reuter, 1967). In the 60s and 70s Harald and I complemented each other experimentally, with his focus being on calcium ion transport, while mine had been on potassium ion transport.

Until the work with Dario, Harald and I had kept off each other's territory. But now Dario and I were forced to enter his area. But in doing so, we were also forced to change it. Harald's experimental work strongly suggested that the sodium-calcium exchanger was electrically neutral. To extrude one calcium ion carrying two positive charges, the exchanger would transport two sodium ions into the cell. It was using the sodium gradient to maintain the calcium gradient, and doing so in an assumed 2:1 ratio. Sodium ions carry one charge, calcium carries two, so the charge balance would be neutral.

We put this ratio into the model. It didn't work. Instead of driving the intracellular calcium down to around 100 nM, a level at which the mechanics of the cell would be quiescent, it barely achieved 10 times that level, i.e. around 1 μM, at which level the cell would be in a permanent state of contraction! Clearly, during each heartbeat the levels of intracellular calcium must oscillate between these extremes, but not be permanently at the high end of the range. This discovery forced us to abandon the assumption of neutrality. But that was to fly in the face of the best experimental evidence at that time. Later experiments (Kimura et al., 1987) confirmed the 3:1 stoichiometry. The mathematical modelling was ahead of the game at that time. It was a huge pleasure for Dario and me to see the published experimental recordings of the voltage-dependence and ion concentration-dependence of the exchanger (Fig. 3).

Those experimental and computational discoveries led to many others, including explaining slow inward currents recorded in many cardiac cells that could now be attributed to current carried by the sodium-calcium exchanger, and most importantly, its involvement in rhythmic and arrhythmic forms of activity.

12. Modelling lesson 3

Modelling cellular biological processes using differential equations is usually modular. Specific equations are used for the various protein and other components. They are then assumed to behave well when incorporated together in a cell model. But that is not always true. Context always matters in biology. In fact this is a general characteristic of living systems. For example proteins behave differently in different contexts dependent on how they

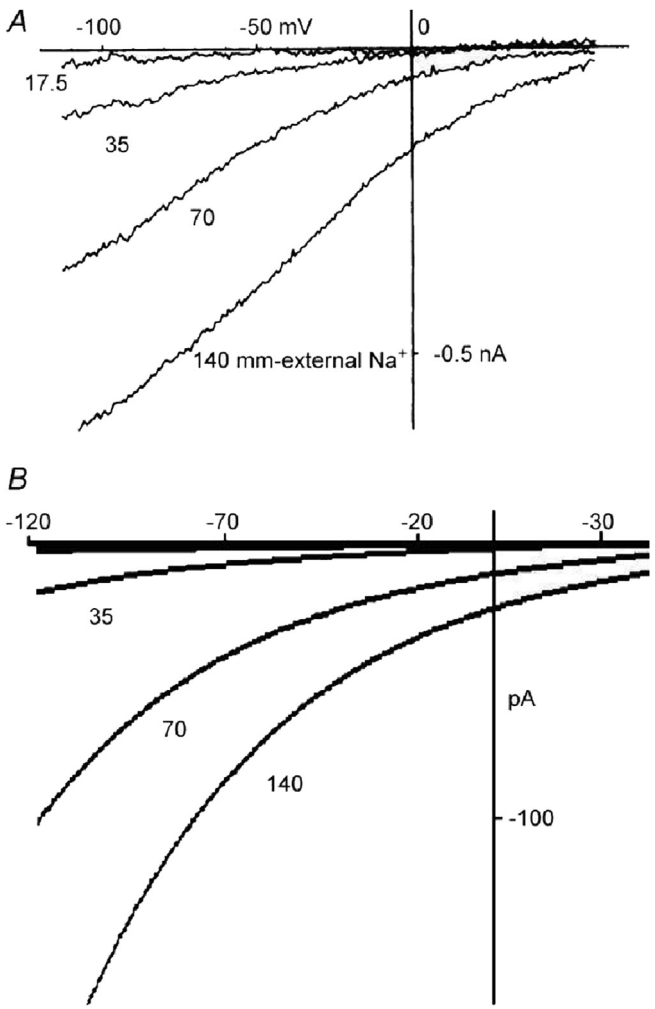

Fig. 3. Comparison between the experimental results (top) on the sodium-calcium exchange current (Kimura et al., 1987) with those given by the equations used in the DiFrancesco and Noble, 1985 model (bottom). The curves were obtained at different levels of external sodium ions between 17 and 140 mM. The graphs are remarkably similar. The main difference is that the exponential growth of the current continues negative to −70 mV in the model, whereas the experimental curve does not. This could readily be explained by an incorporating a maximum rate of the exchanger. From (Noble et al., 2010).

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fold and interact (Baverstock, 2019).

In modelling the sodium-calcium exchange Dario and I used the equations already developed by Lorin Mullins, (1981). Lorin himself had favoured a 4:1 stoichiometry. The modelling work led to us also rejecting this stoichiometry. Plugging it into the model would have meant that heart cells might never contract. It would drive the free intracellular calcium concentration far too low, and very rapidly. This lesson introduces another boundary problem. The various modules also interact across boundaries, that between the modules. One of the aims of the Physiome project and its new journal, *Physiome*, is to make the plugging and unplugging of modular representations easier by ensuring that published models satisfy the criteria of reproducibility, reusability and discoverability (https://journal.physiomeproject.org/about.html). Amongst other advantages, these criteria make it possible to envisage a linking between genomics and quantitative physiology (Noble and Hunter, 2020).

The lesson from the sodium-calcium exchange story is that by modelling in a context where the interaction between components is important, insights can be generated that constrain parameters in the molecular details of the components themselves. Fig. 3 must be one of the most remarkable of modelling predictions with such confirmation in beautiful experiments. Remember that Mullins formulated his model in 1981, 6 years before Kimura et al. (1987) published their experiments. The DiFrancesco-Noble model (1985) also predated the experiments.

13. The complete model

For this article I re-ran the action and pacemaker potential simulation using the CellML coding of the model, partly to demonstrate how reliable CellML and the Physiome project tools are and how easy to use. The results are shown in Fig. 4 and are numerically identical to those published in 1985.

The diagram is arranged to illustrate the main differences from the MNT 1975 model. Those are highlighted by the two red graphs. The inward current, i_f, shown in the middle panel replaces the role of i_{K2} in the MNT model. During the slow pacemaker depolarization activation of i_f replaces the deactivation of i_{K2}. The bottom panel highlights the role of the sodium-calcium exchange, which was absent from the MNT model. Its time course during the action potential plateau replaces the slower components of inward current in the MNT model.

14. Accolades

Dario's discovery has received many accolades.

The French pharmaceutical company, Servier, successfully developed a blocker of the HCN4 receptor, ivabradine (DiFrancesco and Camm, 2004), which is now used in patients for the treatment of heart rhythm problems.

The French Academy of Sciences awarded its prestigious Lefoulon-Delalande prize to Dario in 2008 (Fig. 5).

The DiFrancesco and Noble, 1985 model paper was published in the oldest scientific journal in the world, *The Philosophical Transactions of The Royal Society*. Founded by the first secretary of the Society Henry Oldenburg in 1665, the journal celebrated 350 years of publication in 2015. It did so by publishing special issues of the physical (A) and biological science (B) sides. The editors selected a very few articles over the whole 350 year run to be republished with commentaries from distinguished scientists today. The selected authors included Leeuwenhoek (1677) (Lane, 2015) on seeing unicellular animals for the first time with a light microscope, Alan Turing on the chemical basis of morphogenesis (Ball, 2015), Peter Medawar on tissue transplantation (Simpson, 2015), all on the biological side, and Isaac Newton (1672) on a new theory about light and colours (Fara, 2015), Joseph Priestley on different kinds of air (McEvoy, 2015), Caroline Herschel (1787) on an account of a new comet (Winterburn, 2015), Humphrey Davy on explosive mixtures in coal mines (Thomas, 2015), Michael Faraday on experimental researches in electricity (La-Khalili, 2015), James Prescott Joule on the mechanical equivalent of heat (Young, 2015), James Clark Maxwell on a dynamical theory of the electromagnetic field (Longair, 2015a), Osborne Reynolds on Reynolds number and incompressible viscous fluids (Launder, 2015), Dyson, Eddington and Davidson on bending space-time (Longair, 2015b), Ronald Fisher on the mathematical foundations of theoretical statistics (Hand, 2015), on the physical science side.

Because our article had been selected I was present at the launch presentation and reception in London when the celebratory journal issues first appeared. We were invited because our article was one of those selected. I think I may have been the only living author celebrated who was actually present (Dario was unable to accompany me). I kept as quiet as a mouse in the audience. It was deeply humbling to have been put in such extraordinarily distinguished company. It would have been inappropriate to disturb such an august, reverential event with a living author speaking up; a bit like a mouse pretending to be a massive elephant.

I also wish to pay a tribute to the article written by Dibb, Trafford, Zhang and Eisner (Dibb et al., 2015) in the celebratory issue of *The Philosophical Transactions of The Royal Society*. I don't need to update readers on recent developments. That article does it all better than I could.

15. Biological Relativity

Modelling the heart was one of the experiences that led me to formulate what I call the principle of Biological Relativity (Noble 2012, 2016), which is that all biological levels are causal, there isn't just one privileged level. That idea also goes back to the founding of *The Philosophical Transactions of The Royal Society* in 1665. Henry Oldenburg was at that time in correspondence with the philosopher Spinoza. The journal might well have become the publisher of one of his great works. That did not happen. There was a war between England and Holland, when Oldenburg (who was Dutch) was imprisoned, and by the time he resumed his work on the journal the opportunity seems to have passed. I also suspect that Oldenberg was disturbed by Spinoza's views on religion. However, what does survive is that The Royal Society still possesses the correspondence between Spinoza and Oldenburg, which was all conducted in Latin. A few years ago I discovered that the correspondence (Fig. 6) contains one of the key foundations of the principle of Biological Relativity. Spinoza imagined an observer (a minute worm) living amongst the 'particles' of blood, who "would be unable to determine, how all the parts are modified by the general nature of blood, and are compelled by it to adapt themselves, so as to stand in a fixed relation to one another". The other foundations of the principle are outlined in Noble, (2016) (chapter 6).

When Dario and I incorporated the sodium-calcium exchanger into our model, it was the general context of the model itself that told us that the stoichiometry of the exchanger had to adapt so that all the components of the model could "stand in a fixed relation to one another." For a while the experimental evidence had to be put aside. It is a function of modelling to indicate when the interpretation of experimental results needs to be reassessed.

16. Final modelling lesson

The final lesson is not computational, it is conceptual, and it concerns the way science develops. Broadly speaking, there are two

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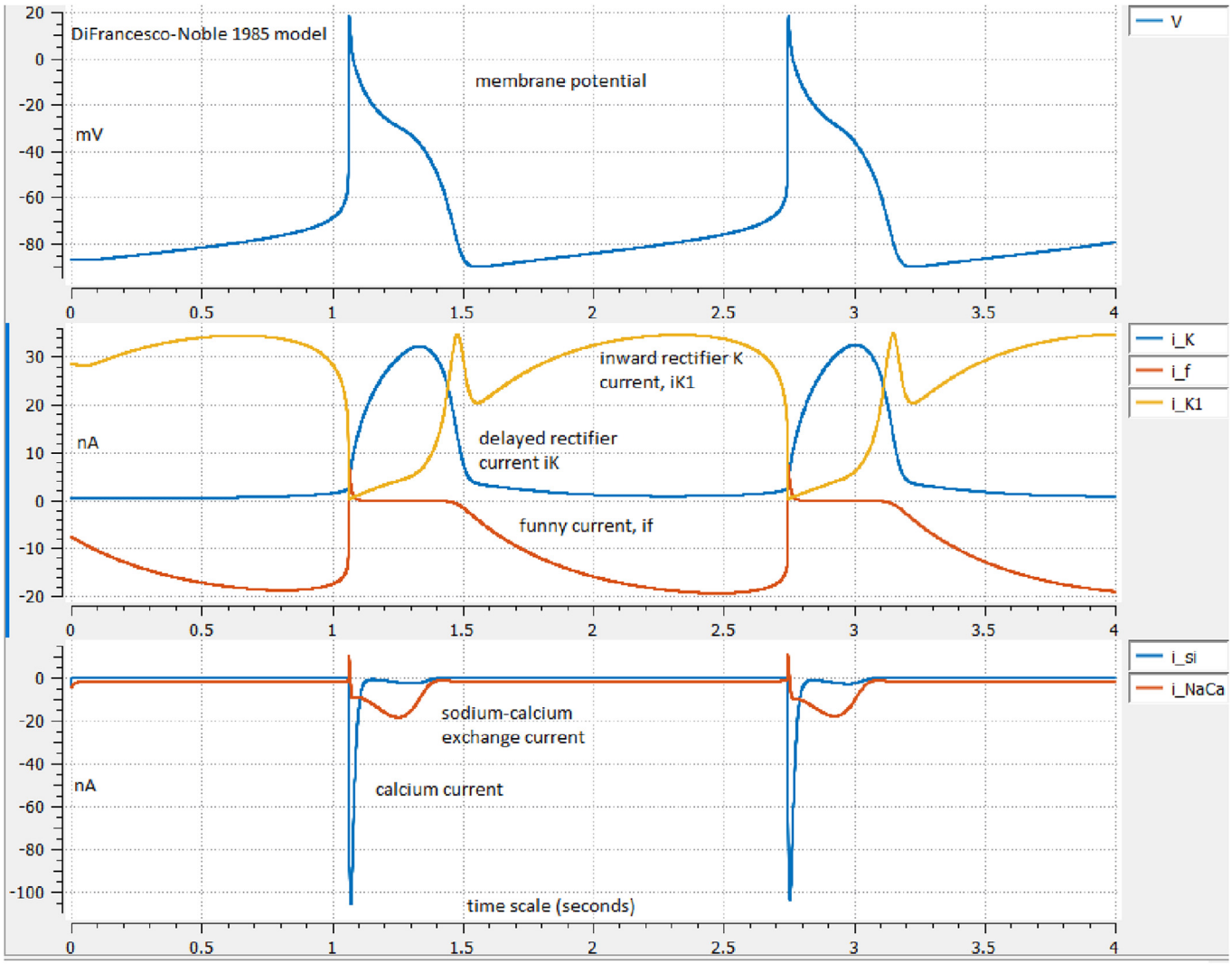

Fig. 4. The DiFrancesco and Noble, 1985 Model. This figure was made using OPENCOR (https://opencor.ws/) and the cellml coding of the model. The top panel shows the computed membrane potential (blue). The second panel shows the delayed rectifier current, i_K, (blue), the inward rectifier current i_{K1}, (yellow), and the funny current, i_f, (red). The bottom panel shows the temporal relationship between activation of the L-type calcium current (blue) and the almost immediately following activation of the sodium-calcium exchange current (red). In this diagram, the red graphs (i_f and i_{NaCa}) represent the main changes from the MNT 1975 model.

Fig. 5. At the Institut de France in Paris in 2008. From left to right: Professor Alain Carpentier, President of the Committee that chooses the laureate, Dario DiFrancesco, Denis Noble, Paris. The prize was awarded for his discovery of the i_f channel.

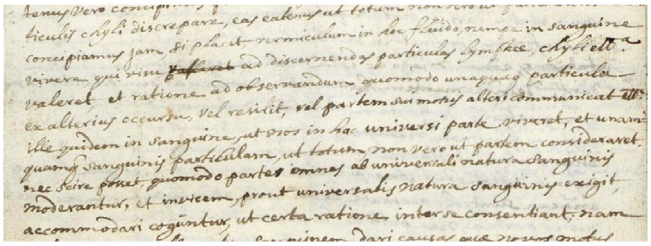

Fig. 6. Part of Spinoza's letter to The Royal Society secretary, 1665. The third line of this extract begins "concipiamus jam, si placet, …" (Let us imagine, with your permission, ….).

conceptual models of science: the incremental and the paradigmatic. They are often presented as alternatives. I think they are complementary. The history of the funny current illustrates that complementarity. The 1980 telephone call from Dario can be seen as a sudden reversal of a well-established theory. It literally turned the interpretation of the pacemaker current upside down, and

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apparently as a consequence of one crucial experiment. Although that is true, my review article shows that there was also an underlying incremental process, all the way from the first signs of ion accumulation-depletion phenomena in work with Dick Tsien in 1969 through to the successful incorporation of such processes in the 1985 DiFrancesco-Noble model.

In the transition between the two models, there was both a paradigm shift and an incremental building on previous work. As I have shown in this review all the successful reconstructions of the electrical and chemical influences on rhythmic activity achieved in the 1975 model reappeared in the 1985 model. In that sense the 1985 model was incremental. But it was also paradigmatic since, following that paper, it was no longer plausible to ignore ion accumulation-depletion phenomena in cardiac electrophysiology. 1980 was the tipping point at which an incremental set of advances became an avalanche that immediately changed the paradigm.

Author statement

Denis Noble, carried out all actions in relation to this article.

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