Sounds Physiological

A well-channeled journey from heart to brain: An interview with Dick Tsien

Transcript of a conversation between Elizabeth M. Adler and Richard W. Tsien[1]

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Transcript condensed and edited by EMA

EMA: Hi, this is Liz Adler, Executive Editor of *The Journal of General Physiology*; I'm in Big Sky, Montana, at the FASEB Summer Research Conference on ion channel regulation, speaking with Professor Richard Tsien, the Director of the NYU Neuroscience Institute.

Dick, thanks so much for taking the time to speak with me.

RWT: Thank you, Liz.

EMA: As the introduction implied, your research has mainly focused on various aspects of neuroscience recently. But, as I understand it, you actually started out in cardiac physiology. Can you tell me a little bit about how you got started in cardiac physiology and how you came to change?

RWT: It was a series of interesting and lucky accidents.

I was a student at MIT, and majored in electrical engineering, and, after I was lucky enough to win a Rhodes Scholarship, I was trying to decide what to do with three years of paid graduate education in any subject that I might choose. And a friend of mine, named Robert Macdonald, saw this as an opportunity to win a convert to the new area of neuroscience that *he* had fallen in love with. He wouldn't take no for an answer, and insisted that I meet a famous auditory physiologist named Nelson Kiang, and eventually he beat down my resistance; I met Kiang, and I actually worked in the Eaton Peabody Lab of Auditory Physiology.

So, as a result of all those interactions, I learned about a bright young scientist at Oxford named Denis Noble who had published a review on the Hodgkin–Huxley equations and its application to different excitable cells. I wrote to Noble, and, after a long delay, finally got a response accepting me as a potential graduate student. And, although I had intended to work on the brain, Noble was actually not doing any experiments on neurons or nervous tissue; he was working on the basis of the heartbeat. So I had to choose, then, between working on the area that I *thought* I was going to work on, or joining with Noble in trying to unravel the basis of the cardiac action potential. And, since I knew no biology, *everything* seemed interesting. So I joined Noble, published a series of papers on electrical activity in the heart, and co-authored a book on electric current flow and excitable cells with him [and Julian Jack], all sort of going with the flow of what was interesting and what was available at the time.

In retrospect, I look back on that period as one of the most wonderful of my life, because studying electrical activity at the cellular level provided an excellent platform for the things I'm doing now, including modulation of ion channels and their regulation. And I could hardly think of a better back-

ground for the goals that I am actually trying to accomplish in science than to work directly with Noble.

EMA: You actually stayed in cardiac physiology for quite a while.

RWT: The problems that we were working on were so fascinating that I couldn't resist the idea of staying there. How, for example, a hormone can regulate not only the opening and closing of a channel, but also its voltage dependence of gating. Or how calcium is important for an excitable cell. And people who work nowadays in neuroscience tend to be a little bit neuro-chauvinistic. I think it's part of the water we drink, or the food we eat, that it almost becomes a bit of a religion, that if something is happening in the brain or in a nerve cell, it's just bound to be more interesting than if it were happening in a pancreatic β cell.

Having been on both sides of that border, working in heart on one hand, and having people rediscover things that we worked on, and basically claiming they were totally new, or being in the nervous system, and seeing how *we* were rediscovering things that other people had found in other tissues, I am acutely aware of that kind of neuro-chauvinism. It doesn't bother me very much; I think it's kind of amusing. It's almost quaint. But, if possible, I think neuroscientists would do to learn a *little* bit more from the lessons that people have learned in other systems.

EMA: Did you always have in the back of your mind that you'd *get* to neuroscience, having fallen in love with it on the basis of your friend's lobbying? Or did you just happen to find a problem you were interested in and make the shift?

RWT: I think I stayed interested in the brain, and in learning and memory.

But I have to credit Martha Nowycky and Aaron Fox for bringing me to the point where experimentally it was possible to have an entry back into the field of neuroscience. That came in the middle 1980s, when we began patch-clamping dorsal root ganglion neurons—sensory neurons—and studying them with the newly invented patch-clamp technique. And that led us to the discovery of multiple types of calcium channels. We started off expecting that a sensory neuron wouldn't be that much different than a heart cell. But Martha, to her credit, noticed things that just didn't fit with that pattern, and so she had to convince Aaron and I, first of all, that there really *was* another type.

Now, the rumor that it was called the N-type, after Martha Nowycky, is actually *not* true.

But that leads to another interesting story. Suffice it to say that studying ion channels in sensory neurons provided a very fast way for us to make a contribution to a growing field, and that was the area of calcium handling by nerve cells, which had been worked on by many other people, but could be approached more powerfully with patch-clamp methods, and we found ourselves in the middle of a hot controversy, and controversy usually breeds interest, and the interest proved to be very favorable. And the discovery of the N-type channel led to synaptic transmission, synaptic transmission led to synaptic plasticity, synaptic plasticity and LTP led to learning and memory. So within the space of a half-dozen years, we had gone from being a cardiac lab to a lab that was working on LTP.

To be fair, we weren't the first to say that there was not just one calcium channel. Hagiwara, for example, thought that was the case, and Clay Armstrong did as well. I think what made that particular area most interesting is the fact that we found a type of calcium channel that *wasn't* found in the heart, that seemed to be neuron specific. And, to this day, everybody agrees that that subfamily of channels is *not* expressed in heart or most other excitable tissues. And, as luck would have it, that fam-

ily of calcium channels that are characterized as Ca_V2 or N-type, P/Q-type, and R-type, are critically involved in synaptic transmission in almost all excitable tissues in the brain and spinal cord.

So our luck was to be trying to be systematic in understanding the phenomena in front of us, and what we stumbled upon, and stuck up for, and eventually exploited was the subclass of channels that is most important for rapid synaptic communication in the brain. That's a very fortunate thing to be working on in your very first foray into a new area that you may have *wanted* to work on for 18 years, but you hadn't actually done so. So I owe those postdocs [Aaron, Martha, and Ed McCleskey] and Richard Miller and all the colleagues that we worked with for that. Because, shortly after we found the channels in the dorsal root ganglion cells, we went to sympathetic neurons, found N-type channels there, and showed with Miller that they were involved in synaptic transmitter release.

Then, almost like a *deus ex machina*, Doju Yoshikama and Baldomero Olivera started up a collaboration with us, using a newly discovered form of toxin, omega-conotoxin. Back then, it wasn't known as "GVIA." It was *the* omega-conotoxin. And it blocked the N-type channel, [and in so doing] blocked transmitter release. And so, really, between 1985 and 1990, we not only were able to describe the type of channel, but also show its role in synaptic transmission. I think that's what really captured everyone's imagination.

EMA: It sounds like you both enjoy participating in controversy and also observing other people participating in controversies.

RWT: Well, it can be uncomfortable if you're right in the middle of it. It really hurts when you overhear conversations where people are dismissing your work because one of your scientific adversaries has dissed it. That I feel acutely. But, all in all, you can judge a person's taste by the way they behave, and I think over the years I've engaged in more than my share of hot topics, and, you know, I'm happy to have worked on them because I think the controversy reflected a genuine curiosity that the field had about the outcome.

Having been involved in a number of very hot controversies over the years, some of which are still going on, my advice to students is: "Don't always run for the hills when there's something controversial going on. If you think it's a really important problem, stick to your guns and *work* on it. And if it turns out that you're wrong, try to be the first person to prove that that's the case. It's not *bad* to be involved in a controversy as long as you keep your wits about you, you're trying to solve the problem for its own sake, you're not just trying to win the contest in order to be top dog, but you're genuinely interested in finding the truth.

Oftentimes, what's controversial is also really interesting. So don't shy away from that."

I noticed that, in some of your interviews, you have interviewed people who take the opposite point of view to mine. Chris Miller, who I greatly admire, is very happy to work on something that other people don't necessarily care about. And I think that's admirable. We need scientists who are willing to follow their curiosity wherever it takes them. But I think it's also okay to have people like *me* who don't want to work on a problem unless it's of general interest, because I feel that solving those really well is the fastest way of making the field advance as a whole, and it's also a good way of getting papers published and getting your trainees to get jobs, which is part of our profession.

So I don't really mind controversy, and I am willing to take my lumps and to be proven wrong some of the time.

EMA: What are some of the controversial areas you're exploring now?

RWT: We're working on something that has been spicy for many years, and that is the idea that voltage-gated channels don't just provide a flux of ions, but actually can signal conformationally.

Now, that has been shown without any doubt for activation of skeletal muscle contraction in the classic work of Clay Armstrong; [then,] Tanabe, Beam, Numa, etc., showed that, in skeletal muscle, the motion and the conformational change in a voltage-gated calcium channel doesn't need to support calcium flux, but it transmits that signal directly to the SR membrane, probably by a protein–protein interaction; it still hasn't been totally figured out. And my friends Martin Schneider and Knox Chandler have contributed mightily to that. But that's the only area that I know of where a voltage signal seems to be independent of *that* protein supporting a calcium flux.

Now, it was proposed for neurotransmission by Itzchak and Hanna Parnas that, in fact, excitation-secretion coupling also involved a combination of a calcium signal, a la Bernard Katz, *and* a signal mediated by conformation. But in later years, before Itzchak passed away, that [hypothesis] became very much attenuated, and they argued that the voltage dependence was due to a G protein–coupled receptor, not due to the calcium channel itself. And, nowadays, hardly anyone puts much stock in a direct voltage-dependent signal. So we're now engaged in how L-type channels, Ca_V1 channels, signal and send on signals, and we have evidence, some of which is unpublished work of Michael Tadross and Boxing Li, that they do both.

They let calcium in, but they also, independent of the calcium flux they support, transmit information conformationally. And we *think* that this is actually going to be very important for the way [the] nervous system processes information, that not only is a calcium flux necessary (and it can be provided not only by the L-type channel, but by an NMDA receptor or a calcium-permeable AMPA receptor) [but] that that, combined with the conformational change mediated by the Ca_V1 channel, is the dynamite combination that you need.

We're used to the idea of combinatorial signaling, coincidence detection; the thing that distinguishes *this* type of information flow is that the optimal signal is not simultaneous calcium and voltage [-dependent change in] conformation, but that these two need to be staggered somewhat. It's almost like the NMDA receptor, where the arrival of ligand, glutamate, needs to be followed a few milliseconds later by the depolarization that drives the magnesium out of the open channel.

In our case, the optimal Δt for giving the biggest signal is not 2 or 3 ms; it's about 10 or 15 *seconds*: in other words, three orders of magnitude slower. And I think the field is having trouble getting their mind around that, because they kind of think that everything should happen simultaneously. But what they're forgetting is that many of the protocols that are used to induce long-lasting plasticity involve multiple activations. So you could imagine that *one* activation is providing the calcium signal, and then a *second* activation is providing the voltage signal.

EMA: 10 or 15 seconds sounds like an extraordinarily long time between the two signals.

RWT: It *is* a very long time if you're only thinking on milliseconds. But if you're studying the synaptic plasticity, you are interested in events—or stimuli—that can spread over several minutes. A typical LTP-inducing protocol may consist of two or three or four bouts of stimulation; and they're often separated by almost exactly the optimal period that we find.

EMA: So the calcium signal comes first, and then the conformational signal?

RWT: Well, our argument is that the calcium signal can be provided by the first theta-burst stimulation, and then the second theta-burst stimulation, which mysteriously seems to work *much* more effectively than

just one, is providing the voltage signal, and then the next calcium signal, and then so on and so forth. So what we still need to do is to *prove* this at the level of an LTP-inducing protocol, rather than simply study it in a biophysically clean situation, which is what we've done up to now.

So, this is an example of an ongoing project, where we're not shying away from saying something that surprises people a little bit.

EMA: Do you think it's the same population of channels? Or do you think one population might get turned on the first bout, and another the second bout? Or do you reserve hypothesizing about that right now?

RWT: We think that they could well be separate populations of channels, and that NMDA receptors could provide the calcium flux that is then capitalized upon by the L-type channel conformational change. In the small environment of a dendritic spine, you know, only a micron or so across, lots of interesting magic can happen in a relatively small space.

EMA: Do you have a favorite project?

RWT: That's a good question.

I have a series of nostalgic periods where things happened that I just felt fascinated by and loved.

The most obvious one is a recent one where we worked out a mechanism by which the surface of the cell, and in particular, synaptic spines, send information to the nucleus, which, on biological scale of distance, is *very* far away. And, we discovered that there is actually a shuttle mechanism which transports calmodulin from near the channel all the way into the nucleus, where the calmodulin is dropped off and activates an enzyme cascade there.

And people who hear this story usually bifurcate into two camps: those who think it's intricate, but wonderfully well-organized, and who are not fazed by complexity, and others who throw up their hands and say, "*What*? You have four or five different calmodulin-sensitive kinases and a calmodulin-sensitive phosphatase, and all five or six need to conspire in order to make a signal go to the nucleus? Why don't you just raise calcium in the nucleus?"

And we give our explanations, but, in the end, they are unwilling to suspend their disbelief because they have a preconceived notion about how simple it should all be.

I would argue that almost any biological event that's really interesting, whether it be propagation of an action potential, or generation of pacemaker activity, or secretion of transmitter from a nerve terminal, or let's say the opening of a ligand-gated channel to make a synaptic potential, that *all* of these phenomena are, once you examine them closely, both simple and complicated, all at the same time. I feel that this one I'm describing has elements of both; it evolved from the worm—or from an antecedent of the worm—and you can see all the seeds there. So, I'm willing to try to simplify as much as possible, but not beyond the point where we no longer really understand the phenomenon. I would argue that even oxidative phosphorylation, the MAP kinase cycle, almost any piece of biology that's really important, will seem befuddlingly complex to someone.

So that's one type of nostalgic period; another period that I am very mindful of, here at a meeting where Catterall, Hille, [Lily] Jan, Bean, Siegelbaum are all present, is a wonderful period where single-channel recordings revealed the existence of modes of gating. Now, this was a phenomenon where channels not only go from closed to open, but they show *bursts* of openings, and they can show different *kinds* of openings, and the openings can occur over many, many seconds, or even minutes, where

a channel will behave in one particular state, and then suddenly, in midstream, change its personality and show a thousand more openings in a different type of pattern. [We (including David Armstrong, a fellow modal enthusiast)] still haven't found the [precise] molecular basis for modes of gating, particularly the long mode of gating that Peter Hess and I worked on, where an L-type calcium channel, instead of opening for a millisecond, like normal activity, would suddenly find itself switched open for 10 ms—an *enormous* length of time. That proved to be very valuable to us in our studies of permeation, but it also provided a wonderful example of how a single molecule could change right in midstream and behave differently for a long, long period of time.

One would think that, in the period of time between 1984 and today—30 years—that someone would have actually studied this at the molecular level. But no one has. So I feel like I'm a hiker, a climber who has left a cache of pork-and-beans, something under a rock that I can always go back to, and if someone else finds that can and wants to open it and have a meal—great. But if you don't get around to it, I know where that object is, and I know that there's going to be something really tasty there. And so it's something that sustains me as I'm imagining how to wind down and what I want do when I have only a precious small amount of time left—what I want to work on.

EMA: Are there any ideas that you detest?

RWT: I can't think of an idea that I'm not open to.

Another controversial area that I work on is the idea that vesicles can release their transmitter with a fleetingly [brief] open fusion pore, and then, without fully collapsing into the membrane, reseal and then reform a vesicle without ever losing its identity—that it can refill with transmitter and be used again. And we've worked hard on using different optical and other methods to establish that this is the case. So, I feel that this is just another idea. And it doesn't necessarily need to happen all the time. I think it really happens, and I am willing to stake a claim on that and eventually be proven wrong. But I don't really find that there's anything I dislike as much as opponents of kiss-and-run dislike it.

It's always amazing to me how much people can feel emotionally involved in proving that a "crazy" idea is wrong.

EMA: So, is there anything that you'd like to say that it hasn't occurred to me to ask you?

RWT: I want to express how fortunate I feel to have been in the UK at the time that Hodgkin, Huxley, and Katz were all alive, doing experiments, engaged in their work. Or how much of a pleasure it was to meet Stephen Kuffler, who gave me some excellent advice, and that those individuals set a very, very high standard for us in terms of their appreciation of the wonders of neuroscience and the phenomena that we're studying, and how each of them had their own flavorful, distinctive personality. But what I loved about Hodgkin, Huxley, and Katz [and Kuffler] is how unassuming they were. They didn't think that we would be noting down every word, and they were *very* engaged in the phenomena that they were trying to study at the moment. So I vividly remember meeting Hodgkin at various times in his life, when he was working on the kinetics of the sodium pump, or calcium entry into nerve terminals, or the topic of how skeletal muscle gets activated. And I remember being the young upstart student, cornering him in an Oxford garden, near where he grew up, and he gave me 40 minutes of his time.

And I repaid him by not only asking him questions about cable theory of the spread of excitation in muscle, which was my official reason for meeting with him, but actually giving him a little bit of a questioning about what he knew about how signals got from the transverse tubules to the sarcoplasmic

reticulum, and didn't there have to be some kind of special communication between the two? And were they ever electrically connected? And how did he *know* they weren't electrically connected? And where was the calcium? And so on and so forth.

And I think he treated me *very* kindly—smiled—but squirmed a little bit. Because I was asking him questions that he didn't have an answer to.

And, finally, in the most memorable part of the conversation, I asked him a question about his classic paper with Paul Horowicz. And he looked at me, sort of blank, and said, "You don't happen to have a reprint of that paper along, do you?"

And being the naive graduate student that I was, having had only one or two years of experience, I said, "But Professor Hodgkin" (and I was polite enough to address him as "professor"), "you wrote the paper; surely you must know what's in it."

And he turned to me, and with a sort of slight trace of a smile said, and I don't think he said "young chap" or "young man" or anything like that. But he said, "You know, the reason we write these papers is so that we don't have to keep them in memory."

I sort of remember that as I get older and older and realize that there are lots of things about my own work that I can barely remember. And I'm just thankful that we have memories (coming back to memory) that allow for a reboot every once in a while, that you can put things off your short-term memory or your portable hard-drive–you can put them into a long-term storage and go back to them. That's why it's a pleasure to read your own papers and to realize, "Gosh, did I write that? That's not too bad." I think that's the way Hodgkin felt about his work.

So, you know, we have an interesting relationship to the senior people in our field. What's always fun about it is to realize that one of the things that energized them and fueled them is this youthful zest, this combination of competitive spirit and philosophicalness. You have to have some sense of self-worth. But the most important thing is to not be afraid to be wrong every once in a while, and not take yourself too terribly seriously.

EMA: Dick, thank you so much for taking the time to speak with me. I really appreciated it.

RWT: Well, I look forward to the sort of fantasy of someone riding on the London Underground with a small phone and a pair of earbuds in their ears, listening to that, and getting to Gower Street and entering their lab and saying, "That was fun; now I'm ready to do my experiment."

ARTICLES CITED AND SUGGESTIONS FOR FURTHER READING

Noble, D. 1966. Applications of Hodgkin-Huxley equations to excitable tissues. *Physiol. Rev.* 46:1–50.

Noble, D., and R.W. Tsien. 1968. The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. *J. Physiol.* 195:185–214. http://dx.doi.org/10.1113/jphysiol.1968.sp008454

Hauswirth, O., D. Noble, and R.W. Tsien. 1968. Adrenaline: Mechanism of action on the pacemaker potential in cardiac Purkinje fibers. *Science.* 162:916–917. http://dx.doi.org/10.1126/science.162.3856.916

Noble, D., and R.W. Tsien. 1969. Outward membrane currents activated in the plateau range of potentials in cardiac Purkinje fibres. *J. Physiol.* 200:205–231. http://dx.doi.org/10.1113/jphysiol.1969.sp008689

Noble, D., and R.W. Tsien. 1969. Reconstruction of the repolarization process in cardiac Purkinje fibres based on voltage clamp measurements of membrane current. *J. Physiol.* 200:233–254. http://dx.doi.org/10.1113/jphysiol.1969.sp008690

Hauswirth, O., D. Noble, and R.W. Tsien. 1969. The mechanism of oscillatory activity at low membrane potentials in cardiac Purkinje fibres. *J. Physiol.* 200:255–265. http://dx.doi.org/10.1113/jphysiol.1969.sp008691

Tsien, R.W., and D. Noble. 1969. A transition state theory approach to the kinetics of conductance changes in excitable membranes. *J. Membr. Biol.* 1:248–273. http://dx.doi.org/10.1007/BF01869785

Hauswirth, O., D. Noble, and R.W. Tsien. 1972. The dependence of plateau currents in cardiac Purkinje fibres on the interval between action potentials. *J. Physiol.* 222:27–49. http://dx.doi.org/10.1113/jphysiol.1972.sp009786

Hauswirth, O., D. Noble, and R.W. Tsien. 1972. Separation of the pace-maker and plateau components of delayed rectification in cardiac Purkinje fibres. *J. Physiol.* 225:211–235. http://dx.doi.org/10.1113/jphysiol.1972.sp009934

Armstrong, C.M., F.M. Bezanilla, and P. Horowicz. 1972. Twitches in the presence of ethylene glycol bis(β-aminoethyl ether)-N,N′-tetraacetic acid. *Biochim. Biophys. Acta.* 267:605–608. http://dx.doi.org/10.1016/0005-2728(72)90194-6

Schneider, M.F., and W.K. Chandler. 1973. Voltage dependent charge movement of skeletal muscle: a possible step in excitation-contraction coupling. *Nature.* 242:244–246. http://dx.doi.org/10.1038/242244a0

Tsien, R.W. 1974. Mode of action of chronotropic agents in cardiac Purkinje fibers. Does epinephrine act by directly modifying the external surface charge? *J. Gen. Physiol.* 64:320–342. http://dx.doi.org/10.1085/jgp.64.3.320

Tsien, R.W. 1974. Effects of epinephrine on the pacemaker potassium current of cardiac Purkinje fibers. *J. Gen. Physiol.* 64:293–319. http://dx.doi.org/10.1085/jgp.64.3.293

Jack, J.J.B., D. Noble, and R.W. Tsien. 1975. Electric Current Flow in Excitable Cells. Clarendon Press, Oxford. 518 pp.

Kass, R.S., and R.W. Tsien. 1975. Multiple effects of calcium antagonists on plateau currents in cardiac Purkinje fibers. *J. Gen. Physiol.* 66:169–192. http://dx.doi.org/10.1085/jgp.66.2.169

Hagiwara, S., S. Ozawa, and O. Sand. 1975. Voltage clamp analysis of two inward current mechanisms in the egg cell membrane of a starfish. *J. Gen. Physiol.* 65:617–644. http://dx.doi.org/10.1085/jgp.65.5.617

Kass, R.S., and R.W. Tsien. 1976. Control of action potential duration by calcium ions in cardiac Purkinje fibers. *J. Gen. Physiol.* 67:599–617. http://dx.doi.org/10.1085/jgp.67.5.599

Siegelbaum, S.A., R.W. Tsien, and R.S. Kass. 1977. Role of intracellular calcium in the transient outward current of calf Purkinje fibres. *Nature.* 269:611–613. http://dx.doi.org/10.1038/269611a0

Colatsky, J.J., and R.W. Tsien. 1979. Sodium channels in rabbit cardiac Purkinje fibres. *Nature.* 278:265–268. http://dx.doi.org/10.1038/278265a0

Marban, E., T.J. Rink, R.W. Tsien, and R.Y. Tsien. 1980. Free calcium in heart muscle at rest and during contraction measured with Ca^{2+}-sensitive microelectrodes. *Nature.* 286:845–850. http://dx.doi.org/10.1038/286845a0

Cohen, C.J., B.P. Bean, T.J. Colatsky, and R.W. Tsien. 1981. Tetrodotoxin block of sodium channels in rabbit Purkinje fibers. Interactions between toxin binding and channel gating. *J. Gen. Physiol.* 78:383–411. http://dx.doi.org/10.1085/jgp.78.4.383

Reuter, H., C.F. Stevens, R.W. Tsien, and G. Yellen. 1982. Properties of single calcium channels in cardiac cell culture. *Nature.* 297:501–504. http://dx.doi.org/10.1038/297501a0

Bean, B.P., C.J. Cohen, and R.W. Tsien. 1983. Lidocaine block of cardiac sodium channels. *J. Gen. Physiol.* 81:613–642. http://dx.doi.org/10.1085/jgp.81.5.613

Bean, B.P., M.C. Nowycky, and R.W. Tsien. 1984. β-adrenergic modulation of calcium channels in frog ventricular heart cells. *Nature.* 307:371–375. http://dx.doi.org/10.1038/307371a0

Hess, P., and R.W. Tsien. 1984. Mechanism of ion permeation through calcium channels. *Nature.* 309:453–456. http://dx.doi.org/10.1038/309453a0

Hess, P., J.B. Lansman, and R.W. Tsien. 1984. Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature*. 311:538–544. http://dx.doi.org/10.1038/311538a0

Armstrong, C.M., and D.R. Matteson. 1985. Two distinct populations of calcium channels in a clonal line of pituitary cells. *Science*. 227:65–67. http://dx.doi.org/10.1126/science.2578071

Nowycky, M.C., A.P. Fox, and R.W. Tsien. 1985. Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature*. 316:440–443. http://dx.doi.org/10.1038/316440a0

Nowycky, M.C., A.P. Fox, and R.W. Tsien. 1985. Long-opening mode of gating of neuronal calcium channels and its promotion by the dihydropyridine calcium agonist Bay K 8644. *Proc. Natl. Acad. Sci. USA*. 82:2178–2182. http://dx.doi.org/10.1073/pnas.82.7.2178

Matteson, D.R., and C.M. Armstrong. 1986. Properties of two types of calcium channels in clonal pituitary cells. *J. Gen. Physiol.* 87:161–182. http://dx.doi.org/10.1085/jgp.87.1.161

Rosenberg, R.L., P. Hess, J.P. Reeves, H. Smilowitz, and R.W. Tsien. 1986. Calcium channels in planar lipid bilayers: insights into mechanisms of ion permeation and gating. *Science*. 231:1564–1566. http://dx.doi.org/10.1126/science.2420007

Hess, P., J.B. Lansman, and R.W. Tsien. 1986. Calcium channel selectivity for divalent and monovalent cations. Voltage and concentration dependence of single channel current in ventricular heart cells. *J. Gen. Physiol.* 88:293–319. http://dx.doi.org/10.1085/jgp.88.3.293

Lansman, J.B., P. Hess, and R.W. Tsien. 1986. Blockade of current through single calcium channels by Cd^{2+}, Mg^{2+}, and Ca^{2+}. Voltage and concentration dependence of calcium entry into the pore. *J. Gen. Physiol.* 88:321–347. http://dx.doi.org/10.1085/jgp.88.3.321

Parnas, I., and H. Parnas. 1986. Calcium is essential but insufficient for neurotransmitter release: the calcium-voltage hypothesis. *J. Physiol. (Paris)*. 81:289–305.

McCleskey, E.W., A.P. Fox, D.H. Feldman, L.J. Cruz, B.M. Olivera, R.W. Tsien, and D. Yoshikami. 1987. ω-conotoxin: direct and persistent blockade of specific types of calcium channels in neurons but not muscle. *Proc. Natl. Acad. Sci. USA*. 84:4327–4331. http://dx.doi.org/10.1073/pnas.84.12.4327

Fox, A.P., M.C. Nowycky, and R.W. Tsien. 1987. Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *J. Physiol.* 394:149–172. http://dx.doi.org/10.1113/jphysiol.1987.sp016864

Fox, A.P., M.C. Nowycky, and R.W. Tsien. 1987. Single-channel recordings of three types of calcium channels in chick sensory neurones. *J. Physiol.* 394:173–200. http://dx.doi.org/10.1113/jphysiol.1987.sp016865

Hirning, L.D., A.P. Fox, E.W. McCleskey, B.M. Olivera, S.A. Thayer, R.J. Miller, and R.W. Tsien. 1988. Dominant role of N-type Ca^{2+} channels in evoked release of norepinephrine from sympathetic neurons. *Science*. 239:57–61. http://dx.doi.org/10.1126/science.2447647

Miller, R.J., D.A. Ewald, A.P. Fox, L.D. Hirning, E.W. McCleskey, T.M. Perney, M. Sturek, S.A. Thayer, R.W. Tsien, and M.W. Walker. 1988. The effect of calcium channel antagonists on peripheral neurones. *Ann. NY Acad. Sci.* 522:269–277. http://dx.doi.org/10.1111/j.1749-6632.1988.tb33364.x

Rosenberg, R.L., P. Hess, and R.W. Tsien. 1988. Cardiac calcium channels in planar lipid bilayers. L-type channels and calcium-permeable channels open at negative membrane potentials. *J. Gen. Physiol.* 92:27–54. http://dx.doi.org/10.1085/jgp.92.1.27

Tanabe, T., K.G. Beam, J.A. Powell, and S. Numa. 1988. Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA. *Nature*. 336:134–139. http://dx.doi.org/10.1038/336134a0

Malinow, R., D.V. Madison, and R.W. Tsien. 1988. Persistent protein kinase activity underlying long-term potentiation. *Nature*. 335:820–824. http://dx.doi.org/10.1038/335820a0

Lipscombe, D., S. Kongsamut, and R.W. Tsien. 1989. α-adrenergic inhibition of sympathetic neurotransmitter release mediated by modulation of N-type calcium-channel gating. *Nature*. 340:639–642. http://dx.doi.org/10.1038/340639a0

Malinow, R., H. Schulman, and R.W. Tsien. 1989. Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science*. 245:862–866. http://dx.doi.org/10.1126/science.2549638

Tanabe, T., K.G. Beam, B.A. Adams, T. Niidome, and S. Numa. 1990. Regions of the skeletal muscle dihydropyridine receptor critical for excitation-contraction coupling. *Nature*. 346:567–569. http://dx.doi.org/10.1038/346567a0

Parnas, I., H. Parnas, and B. Hochner. 1991. Amount and time-course of release. The calcium hypothesis and the calcium-voltage hypothesis. *Ann. NY Acad. Sci.* 635:177–190. http://dx.doi.org/10.1111/j.1749-6632.1991.tb36491.x

Delcour, A.H., and R.W. Tsien. 1993. Altered prevalence of gating modes in neurotransmitter inhibition of N-type calcium channels. *Science*. 259:980–984. http://dx.doi.org/10.1126/science.8094902

Wheeler, D.B., A. Randall, and R.W. Tsien. 1994. Roles of N-type and Q-type Ca^{2+} channels in supporting hippocampal synaptic transmission. *Science*. 264:107–111. http://dx.doi.org/10.1126/science.7832825

Tsien, R.W., D. Lipscombe, D. Madison, K. Bley, and A. Fox. 1995. Reflections on Ca^{2+}-channel diversity, 1988-1994. *Trends Neurosci.* 18:52–54.

Deisseroth, K., E.K. Heist, and R.W. Tsien. 1998. Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. *Nature*. 392:198–202. http://dx.doi.org/10.1038/32448

Zühlke, R.D., G.S. Pitt, K. Deisseroth, R.W. Tsien, and H. Reuter. 1999. Calmodulin supports both inactivation and facilitation of L-type calcium channels. *Nature*. 399:159–162. http://dx.doi.org/10.1038/20200

Choi, S., J. Klingauf, and R.W. Tsien. 2000. Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses'. *Nat. Neurosci.* 3:330–336. http://dx.doi.org/10.1038/73895

Choi, S., J. Klingauf, and R.W. Tsien. 2003. Fusion pore modulation as a presynaptic mechanism contributing to expression of long-term potentiation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358:695–705. http://dx.doi.org/10.1098/rstb.2002.1249

Aravanis, A.M., J.L. Pyle, and R.W. Tsien. 2003. Single synaptic vesicles fusing transiently and successively without loss of identity. *Nature*. 423:643–647. http://dx.doi.org/10.1038/nature01686

Aravanis, A.M., J.L. Pyle, N.C. Harata, and R.W. Tsien. 2003. Imaging single synaptic vesicles undergoing repeated fusion events: kissing, running, and kissing again. *Neuropharmacology*. 45:797–813. http://dx.doi.org/10.1016/S0028-3908(03)00310-1

Hudmon, A., H. Schulman, J. Kim, J.M. Maltez, R.W. Tsien, and G.S. Pitt. 2005. CaMKII tethers to L-type Ca^{2+} channels, establishing a local and dedicated integrator of Ca^{2+} signals for facilitation. *J. Cell Biol.* 171:537–547. http://dx.doi.org/10.1083/jcb.200505155

Wheeler, D.G., C.F. Barrett, R.D. Groth, P. Safa, and R.W. Tsien. 2008. CaMKII locally encodes L-type channel activity to signal to nuclear CREB in excitation-transcription coupling. *J. Cell Biol.* 183:849–863.

Zhang, Q., Y. Li, and R.W. Tsien. 2009. The dynamic control of kiss-and-run and vesicular reuse probed with single nanoparticles. *Science*. 323:1448–1453. http://dx.doi.org/10.1126/science.1167373

Parnas, I., and H. Parnas. 2010. Control of neurotransmitter release: From Ca^{2+} to voltage dependent G-protein coupled receptors. *Pflugers Arch.* 460:975–990. http://dx.doi.org/10.1007/s00424-010-0872-7

Wheeler, D.G., R.D. Groth, H. Ma, C.F. Barrett, S.F. Owen, P. Safa, and R.W. Tsien. 2012. Ca_V1 and Ca_V2 channels engage distinct modes of Ca^{2+} signaling to control CREB-dependent gene expression. *Cell*. 149:1112–1124. http://dx.doi.org/10.1016/j.cell.2012.03.041

Tadross, M.R., R.W. Tsien, and D.T. Yue. 2013. Ca^{2+} channel nanodomains boost local Ca^{2+} amplitude. *Proc. Natl. Acad. Sci. USA*. 110:15794–15799. http://dx.doi.org/10.1073/pnas.1313898110

Owen, S.F., S.N. Tuncdemir, P.L. Bader, N.N. Tirko, G. Fishell, and R.W. Tsien. 2013. Oxytocin enhances hippocampal spike transmission by modulating fast-spiking interneurons. *Nature*. 500:458–462. http://dx.doi.org/10.1038/nature12330

Ma, H., R.D. Groth, S.M. Cohen, J.F. Emery, B. Li, E. Hoedt, G. Zhang, T.A. Neubert, and R.W. Tsien. 2014. γCaMKII shuttles Ca^{2+}/CaM to the nucleus to trigger CREB phosphorylation and gene expression. *Cell*. 159:281–294. http://dx.doi.org/10.1016/j.cell.2014.09.019

Ma, H., B. Li, and R.W. Tsien. 2015. Distinct roles of multiple isoforms of CaMKII in signaling to the nucleus. *Biochim. Biophys. Acta*. 1853:1953–1957.