Sounds Physiological

Why we don't twitch when we smoke: An interview with Henry Lester

Transcript of a conversation between Elizabeth M. Adler and Henry A. Lester[1]

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Transcript condensed and edited by EMA

EMA: Hi, this is Liz Adler; I'm Executive Editor of *The Journal of General Physiology*. I'm in Big Sky, Montana, at the FASEB meeting on Ion Channel Regulation, speaking with Henry Lester of Caltech.

Henry, thanks for talking to me.

HAL: Liz, it's delightful to be with you, especially in this marvelous ambiance, and it's a very interesting and stimulating meeting.

EMA: Oh, it's a great meeting.

If I had to characterize you, I'd call you a neuroscientist with a particular interest in channels and receptors and transporters, particularly the nicotinic acetylcholine receptor.

HAL: That's quite true, Liz. Except that the *wonderful* aspect of neuroscience is that it's probably the most accretive field of the 21st century. It accretes people from mathematics, from chemistry, from physics, obviously from physiology and from molecular biology, genetics . . . and so to call oneself a neuroscientist, I think, is a bit pretentious. I contribute to neuroscience, and we'll leave it at that.

EMA: How did you get interested in the nicotinic acetylcholine receptor? I'll avoid saying how did you get interested in neuroscience? [*laughs*]

HAL: [*laughs*] It started with my PhD thesis at Rockefeller in the laboratory of H.K. Hartline and my official advisor, Floyd Ratliff, and my unofficial advisor, Fred Dodge. Fred had made acquaintances with a former Rockefeller graduate student, [David Eaker], who had [purified] toxins that were derived from cobras and kraits, and these would be good probes, we thought, for acetylcholine receptors. In fact, these were toxins that bound tightly to the muscle receptor, and would allow, eventually, in other labs, purification of the receptor, and of course that led to cloning.

But, in fact, if you think back to, let's say, for instance, when Columbus' crew sampled tobacco, the nicotine and nicotinic system has really been the touchstone and leader for pharmacology and neuroscience for many, many years. And so, it is in that tradition that I like to work.

And it's been a natural progression from working on the nerve-muscle synapse to the question of what happens in nicotine addiction. Such questions as, for instance: "Well, why don't we twitch when we smoke? If nicotinic receptors are in the muscle and in the brain, what's the difference?"

So my research partner, Dennis Dougherty, and I spent several years recently trying to understand how brain nicotine receptors, or nicotinic receptors, are *so* much more sensitive to nicotine than muscle nicotinic receptors—that is, why we don't twitch when we smoke.

EMA: So it's just a matter of sensitivity.

HAL: It is, yes. And we've been having a wonderful time tracking down the particular amino acids in brain receptors that make them more sensitive than muscle receptors.

EMA: I heard a story that you managed to unpack equipment and put it together and complete a study in a week that formed the basis for a paper in *Nature*. Is that true?

HAL: Oh, that one. Yes. This was back in the days when I was trying to understand how to apply agonists to receptors much more quickly than we had done before.

I hate changing solutions because I am not a very good plumber, and so we thought of the idea at that time of using photoisomerizable drugs like nicotine. Hai Won Chang and [independently] Bernie Erlanger and their colleagues [both groups were] at Columbia had made azobenzene derivatives of nicotine, and my challenge was to do the photoisomerizations in a millisecond or so.

In those days before pulsed lasers, flash lamps were the way to do it, so I borrowed a piece of equipment from a local Southern California manufacturer that used flash lamps, and used these lamps in a repetitive fashion. Of course, the Southern California aircraft industry needed to take pictures of parts of propellers and parts of jet engines very quickly, using flashes [stroboscopes]. So we modified that to give a very *large* flash to *Electrophorus* electroplaques that were *very* sensitive to acetylcholine.

And so, yeah, we did make that experiment work in a week with a borrowed flash lamp. But that was lucky.

EMA: [*laughs*] Well, you can say that, having gotten it to work.

HAL: [*laughs*]

EMA: Did you have a favorite experiment or series of experiments?

HAL: I think, as a physiologist, one's favorite experiment is the one that you tinker several months to do, and then the experiment itself lasts a couple of milliseconds, and you get the data. Several times that has occurred with me, and it's been quite wonderful. The flash lamp experiment is one; and then the decaging of calcium, and of other molecules, is another.

Of course, as a physiologist, you have the privilege of observing your data as you gather them in real time. You don't have to wait for the counts to come off a counter or for the software to analyze an x-ray diffraction pattern. You actually see the data as you're getting them, and that's always a privilege. But along with that comes the responsibility—because the data are so hard to get in physiology—along with that comes the responsibility of looking at the data as you're gathering them, and then modifying the experiment to improve it.

EMA: Was there a particular experiment that you liked?

HAL: Oh, everybody, I think, is seduced by their first single-channel experiment, because you can watch a molecule—a single molecule—changing in real time. That's certainly happened to me. People are also just delighted at their first recording of an action potential, for the same reasons.

One of the longer-term experiments that pleased me a great deal was one in which we went to great pains to build mice with fluorescent nicotinic receptors, and exposed these mice for 2 weeks or so to mini-pumps containing nicotine, and then cut slices from the brains and asked: "Where did we up-regulate nicotinic receptors? Where were there more nicotinic receptors than previously?"

And so this was a way of getting at the famous experiment that had been done many years ago, that when you give an animal or a person nicotine for weeks, that animal or person grows new nicotinic receptors. And one of the surprises in that series of experiments, then, was that we did not see up-regulated nicotine receptors in dopamine neurons.

Now, I'll remind you that, in the central nervous system, the dopamine neurons have the shape of a handlebar moustache; that the handlebars are the substantia nigra (that's the part that degenerates in Parkinson's disease); the upper lip is the ventral tegmental area (that's the part that gives a feeling of well-being when one smokes). And so we thought we would see nice, *new* nicotinic receptors in both the handlebars and in the upper lip. Actually, we didn't see new nicotinic receptors in either the handlebars *or* in the upper lip, not in the dopamine neurons themselves. Instead we saw new nicotinic receptors in the GABAergic neurons that inhibit the dopaminergic neurons.

EMA: ***Oh . . .***

HAL: And so Raad Nashmi, my postdoc at the time, said, "Oh, you know we may have discovered how it is that the nervous system changes the sign of up-regulation from positive to negative, because the up-regulation is occurring in GABAergic neurons."

And I said, "Oh, Raad, that's very good. You know, we may also have discovered how it is that people who smoke have a lower incidence of Parkinson's disease, because we are making the handlebars work less strongly."

Well, we're still working through both of those insights, but it was fun to have those insights.

EMA: That seems unusual; I usually think about receptors getting *down-regulated* when they're chronically exposed to their agonist. Is that unique or am I looking at it superficially?

HAL: Liz, it *is* the neuroscience model that receptors get down-regulated, and that that explains tolerance. But the sign change that we observed also explains tolerance.

As it happens, we've been now exploring a field that we call "inside-out pharmacology," and in inside-out pharmacology it is much more likely that a drug will lead to *more* receptors than to *less* receptors.

That's because nicotine actually enters neurons.

Now, that's a topic that neuroscientists are surprised by, but in fact, it's been known for about 50 years, and in fact, there's an experiment that one can do, and is done about 150 billion times a day. That's when a person takes a puff on a cigarette: nicotine in its neutral form enters the lungs, goes through the membranes of the alveolar epithelium, gets to the brain, goes through the endothelium in the capillary, goes through the glial endfeet, and enters the brain. So nicotine has actually crossed six cell membranes in about the time it took us to describe this.

EMA: ***Really.***

HAL: Yes.

EMA: That fast.

HAL: That is why people have a sense of well-being as soon as they take a puff on a cigarette.

EMA: I guess it would have to be fast or you wouldn't feel it when you were smoking.

HAL: That's correct; it's one reason why so many people derive a sense of well-being from smoking.

It's been known now for about 50 years that, in addition to the *very* important aspect of activating nicotinic receptors on the surface of the neuron, nicotine keeps going, enters neurons themselves, and enters the endoplasmic reticulum of neurons—and *that* has been known for about 10 years. It is, in fact, in the endoplasmic reticulum that inside-out pharmacology begins to have its effect.

EMA: Before you go on with that, what exactly is "inside-out pharmacology"?

HAL: Inside-out pharmacology is the concept that, rather than acting from outside-in—that is on the plasma membrane, opening ion channels, depolarizing neurons, allowing calcium to flow in, and eventually the signals reach the nucleus, change gene activation, change the physiology of organelles—in *inside-out* pharmacology, the drugs do *indeed* enter the neurons, or another cell, enter the ER, and have their primary effect—especially chronic effects—by binding to their classical targets still (ion channels, receptors, transporters), but while they're being made in the endoplasmic reticulum.

EMA: So it's the same target.

HAL: With one exception—and that is ketamine—the same target, while it's in the endoplasmic reticulum. Totally *different* downstream mechanisms. I'll remind you that nicotine has no natural pharmacology; the process of nicotine activating its receptors and the process of nicotine exerting effects in the endoplasmic reticulum is a purely pathopharmacological process. There is no selective advantage to the tobacco plant to have nicotine addiction; no selective advantage to us to have nicotine addiction. So, this pathopharmacological process, which is not available to acetylcholine, *is* available to nicotine, and that's where nicotine binds to some nicotinic receptors in the ER, in *addition* to the binding on the plasma membrane. So pharmacological chaperoning occurs, decreases in endoplasmic reticulum stress, decreases in the unfolded protein response. Some of these processes underlie nicotine addiction. Others may underlie the apparent neuroprotection that seems to cause people who smoke to have less Parkinson's disease.

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EMA: Tell me about your MOOC. Did you enjoy putting that together?

HAL: I teach a MOOC, a massive open online course, called "Drugs and the Brain." It's enjoyable to teach a MOOC in several ways. You know, serving on the Caltech faculty is a rather rarefied experience. A typical course at Caltech has between 10 and 40 students; it's quite wonderful teaching them, but it's not what I would call mass communication. So there's been a history among Caltech faculty of trying to take the same course or the same ideas, and extending it to a larger audience with whatever technology was available at the time.

So Feynman, of course, published his books, and they got wide circulation; during the 80s, the dominant technology for teaching on a wider basis was videotapes, and distributed movies— David Goodstein came up with this wonderful series of lectures on physics that he called "The Mechanical Universe." During the 90s, more computer animations were done for chemistry. And so, here in the 21st century, the way to reach a larger audience is via the online course.

About 20 years ago, we decided that every Caltech student needed to take biology. That was an easy decision to make and hard to implement because the Caltech students think that biology is a soft science, and not really worthy of their expertise at equations and physics.

EMA: [*laughs*]

HAL: I was serving at the time on the Council of the NIMH, the National Institute of Mental Health, and I said, "Well, you know the neuroscience diseases are going to be the great challenge of the 21st century, and so what I will do will be to convince this group of Caltech freshmen to become neuroscientists and solve the neuroscience diseases."

I went home that night and told my family about my plan, and my children, who were at that time in high school, said, "You know, Dad, this is not going to work, because Caltech freshmen are late-stage teenagers. We teenagers think that we're immortal; we're not interested in diseases. So this course is going to flop. Come up with a better idea."

And my wife, Margaret, said, "Yeah, they're right."

The next night, at dinner, I announced my revised plan, and that revised plan was going to be to teach a course called "Drugs and the Brain." Everybody around the dinner table put two thumbs up, and so that's how I got to teach about neuropharmacology to Caltech freshmen.

So I was faced with a room full of 180 kids, all of whom had received 800s on the College Boards in Math, and thought that Biology was a topic that you could derive from first principles. Well, I taught them a lot of biophysics, a lot of single molecule stuff, and *gently* edged into more complex topics, such as nerve cells, and then into the neuroscience diseases. It was the first course at Caltech taught by PowerPoint.

And a few years ago, the Vice Provost at Caltech said, "We have just signed an agreement with Coursera, the online course people; how about taking your old Biology-1 lectures and making them into a MOOC?"

I innocently said, "Oh, it should not be much trouble." Well, it turned out to be a great deal of trouble, but *very* worth it. And so I've been able to influence people from 75 different countries and lots of different professions. I think one of the most gratifying aspects of teaching an online course is that something like 7% of the students [in my course] are, in fact, MDs; 2% are psychiatrists; another 7% or so are pharmacists—and they would really like to know the rigorous underpinnings of their profession.

And, you know, one of the other aspects of the tradition at Caltech, teaching to a wider audience, is that Caltech students who help with these courses become very interested in teaching, and so some people go off on teaching tracks and have a very rewarding career in scientific communication.

EMA: That's interesting. It wasn't my picture of Caltech, so it's nice to get that view of it, the interest in science communication.

HAL: Yes, indeed.

EMA: I also heard that, when you first got there, you had to take over Richard Feynman's course?

HAL: Let me tell you the actual story.

During my first week at Caltech I went to see Max Delbruck, who had helped to hire me, and who had, incidentally, invented a lot of molecular genetics. Max said, "Well, Henry, what are you going to do?"

I said, "Gee, Max, I'd sort of like to measure single ion channels."

Max said, "Well, I don't understand how to do this. But you know, it's getting to be noon. Let's go over to the cafeteria; I have lunch there with Feynman every day, and he'll tell us how to do it."

Max and I walked over to the cafeteria; the Caltech cafeteria at that time looked like your high school cafeteria—sort of linoleum on the floor, real Naugahyde chairs, Formica tables, not very special. But there was Feynman and Delbruck, who had brought me, and also a Caltech professor named Carver Mead, and Delbruck said, "Feynman, this is Lester; Lester, this is Feynman. Dick, Henry wants to measure single channels—tell him how to do it."

So Feynman smiled and he said, "Well, tell me about the characteristics of these neurons."

I said, "Well, you know, they have a membrane resistance of about [10] $k\Omega \cdot cm^2$, and a capacitance of about a $\mu F/cm^2$ . . ."

He said, "Well, what's the conductance of these channels?"

I said, "Well, there's this person named Hille who estimates that the conductance of a single ion channel might be about, oh, 10 or 100 pS."

Feynman said, "And you're trying to stick your electrodes into those cells and measure them."

And I said, "That's right."

And he said, "No. The way to do it is to measure the current rather than the voltage. That's because if you multiply that membrane capacitance times that membrane resistance, it's going to turn out to be an enormous filter, and so it will filter all of the signals due to single-channel openings, and you won't record anything. But if you can record the current directly, why, then, you can actually put it through a sensitive ammeter, and that will be more sensible."

Now, the key is, of course, getting that pipette up very close to the channel, so you can record the current directly. So Feynman said, "How close do you think you can get it?"

And I said, "Gee, Dick, I'd never thought of it that way, but maybe a tenth of a μm."

So Feynman said, "Nah, that's not close enough; come back when you can get closer." A few years later, when Erwin Neher and Bert Sakmann had invented the GΩ seal, and you could get closer, Feynman said to me, "Well, it's been done, but I would have said it was impossible anyway."

Now, all was not lost that afternoon, when I first came to Caltech, because the other person at the lunch table was Carver Mead, and Carver had invented very large-scale integration and was a great semiconductor physicist. Carver said, "Well, I don't really understand a *lot* of this, but there's a Caltech undergraduate who is an excellent electrical engineer. He's been working in my lab, and, I bet, when you get closer, you could probably measure the signals using this stuff that he's building. His name is Sigworth."

So it all worked out very well, because the next day or the day after, Fred Sigworth showed up at my lab, and he built me my first voltage clamp, and he's done very well.

EMA: Is there anything I haven't thought to ask you that you'd like to talk about?

HAL: I think that's pretty much it.

EMA: Well, thank you again for agreeing to talk with me.

HAL: It's a pleasure to speak with you, Liz.

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