

A LECTURE COMPANION

**Michael Levin talk on bioelectricity at
Stanford Chemical Engineering
Colloquium**

Michael Levin

Recorded on January 27, 2023

About this document

This document is a companion to the recorded lecture *Michael Levin talk on bioelectricity at Stanford Chemical Engineering Colloquium*, recorded on January 27, 2023. You can watch the original lecture or listen in your favorite podcast feeds — all links are on the page [here](#).

This document pairs each slide with the aligned spoken transcript from the lecture. At the top of each slide, there is a “Watch at” timestamp. Clicking it will take you directly to that point in the lecture on YouTube.

Lecture description

Michael Levin joins the Stanford Chemical Engineering Colloquium to explain how non-neural bioelectricity lets cell networks make decisions about body shape and regeneration. He describes an “anatomical compiler” for telling cells what to build, shows real multi-headed and multi-limbed animals as proof of concept, and explores how electroceuticals could transform birth defect repair, limb regrowth, cancer control, and synthetic bioengineering.

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Transcript note

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Main Points

- Significant knowledge gaps remain about control of large-scale **anatomical homeostasis** = design challenge

- Fundamental advances and new biomedicine will require **understanding decision-making** by cells and tissues.

- A key medium for computation in vivo is **non-neural bioelectricity - a new kind of epigenetics**; we developed **techniques to manipulate this physiological software layer**.

- **Cracking the bioelectric code** will enable electroceuticals for novel applications in birth defects, regenerative medicine, cancer, and synthetic bioengineering.



Michael Levin

Thank you for the opportunity to speak to all of you and to share some work and get your feedback. If anyone would like more details or to get in touch with me, all the papers and everything else are at these websites.

There are four basic points that I would like to get across today. The first is that there are really some very significant knowledge gaps about the control of large-scale anatomical homeostasis. It encompasses our understanding of the relationship between the genome and the anatomy. I'll show you why things are quite puzzling there. I will claim that fundamental advances in new biomedicine are going to require not only understanding the molecular mechanisms for this process, but also the decision-making by cellular collectives that is sufficient for it to happen. That a key medium for computation in living tissue is non-neural bioelectricity. We've developed some techniques to manipulate this layer of physiological software, and I'll show you why I say that. Fundamentally, looking towards the future, I will argue that cracking this bioelectric code will enable a novel approach using electroceuticals for applications in birth defects, regenerative medicine, cancer, and synthetic bioengineering.

Like the brain, somatic tissues form bioelectric networks that make decisions (about dynamic anatomy). We can now target this system for control of large-scale pattern editing to over-ride genomic defaults, with many advantages for regenerative medicine and synthetic bioengineering.



Boiling down the whole talk into two sentences, what I'm going to tell you is that your body tissues form electrical networks that make decisions. These are decisions about dynamic anatomy, and we now have the ability to target the system to control large-scale editing that can even override genomic default states with advantages and opportunities for regenerative medicine and synthetic bioengineering. I show you one of our five-legged frogs to point out that you're going to see weird creatures today. None of this is Photoshop. These are all actual living things that represent our attempt to test some of the models we have.

Let's look at these knowledge gaps.

Endgame: anatomical compiler

• Birth defects
• Traumatic injury
• Cancer
• Aging

Problems of information processing

how to control emergence and scaling of purposive activity toward desirable complex, system-level outcomes.

Daniel Lobo

Michael Levin

If we think about what is the end game of our field, where are we going? At what point can we all go home? What we would like to have is something that we call the anatomical compiler.

The deal is that you ought to be able to sit down and draw the animal or plant that you would like to have at the level of the anatomy. Not at the level of pathways, but at the level of final anatomy, the way that we do with machine parts and things like this. You would be able to draw this three-headed worm. If we knew what we were doing, we would have the capability of having the software, which would then compile this anatomical description down into a set of stimuli that would have to be given to cells that would cause them to produce whatever it was that you just drew.

Here's this three-headed flatworm. Now, the reason that this is fundamentally important is that almost all of the problems of biomedicine — except for infectious disease, pretty much everything else: birth defects, traumatic injury, cancer, aging — would be solved if we had the ability to tell cells what to build. We would need the knowledge to control what cellular collectives cooperate towards building.

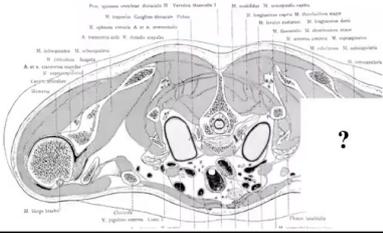
All of these things are in some important sense problems of information processing. How is it that cells work together to build these things? We are very far from having anything like this, and only in a few very special cases do we know anything about how to make specific shapes come out. Let's think about why that is.

Where is Pattern Specified?

stem cell
embryonic
blastomeres



self-assembly



?

- DNA specifies proteins; whence Anatomy?
- how do cell groups know what to make and when to stop?
- how far can we push shape change? Engineers ask: what's possible to build given default genome?

How to repair
(edit) it?



Michael Levin

Where is anatomy specified?

We all start life roughly like this. This is a collection of embryonic blastomeres. Shortly thereafter, you get something like this. This is a cross-section through a human torso.

Look at this incredible invariant order.

All of these organs are the correct shape, size, position, orientation.

Where is Pattern Specified?

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- how far can we push shape change? Engineers ask: what's possible to build given default genome?

How to repair (edit) it?

Michael Levin

Everything is exactly the right stuff. It's absolutely a staggering amount of order. We would like to know, where is this information coming from? We're tempted to say DNA, but of course we know what genomes specify. The DNA specifies proteins. There's nothing directly in there that specifies any of this. We would like to know how cells use the genomically specified hardware that they have to know what to make and when to stop. In regenerative medicine, if a piece of this is missing, how do we get the cells to rebuild it?

As engineers, we ask what's actually possible to build given any particular default genome. The reason that in my group we frame this thing as a collective intelligence problem is that individual cells are extremely competent.

Individual Cells are Highly Competent at pursuing single cell-scale goals



video by Charles Krebs

how can they form collectives to pursue organ-scale anatomical goals?

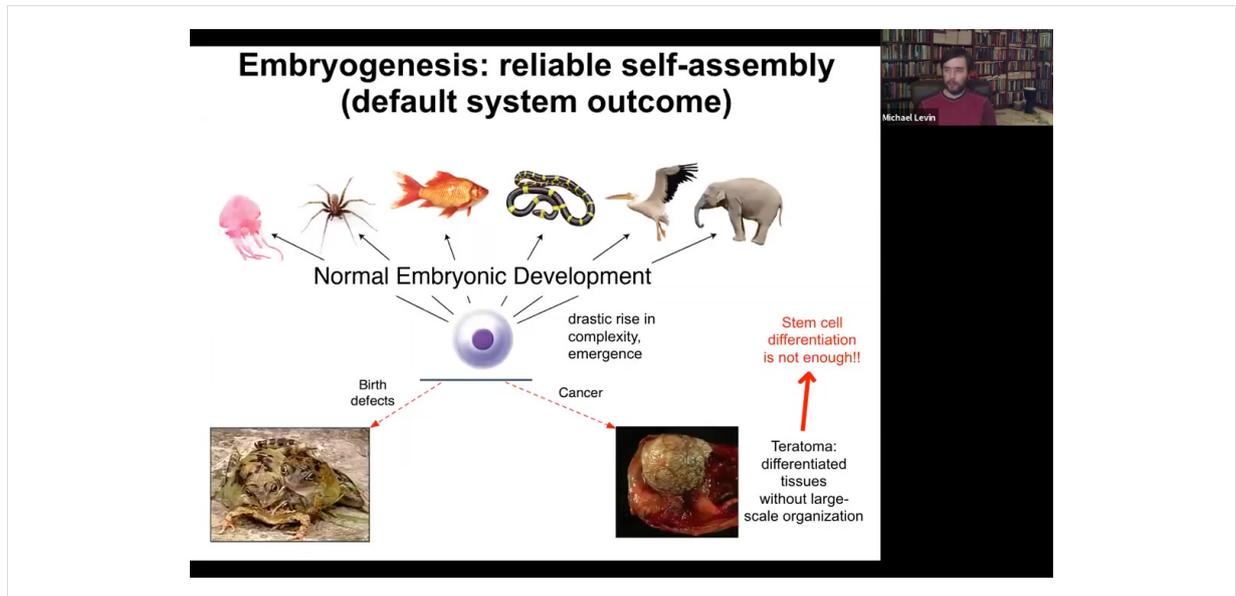
they did not give up their smarts when joining into bodies, but they did have to learn to cooperate to work toward goals on a much larger spatio-temporal scale

Computational boundary expands drastically, when making a metazoan body



Michael Levin

Here you see one cell. This is a single-celled organism. This is called *Lacrymaria*. There is no brain, there's no nervous system, there are no stem cells, there's no cell-to-cell communication, just one cell handling all of its local goals. It's handling its physiological goals, anatomical control, metabolic, behavioral, everything is handled by this one cell in real time. The amazing thing is that these cells, which are extremely competent in their local, very small environment, when they work together to make a metazoan body, they can work on much bigger goals.

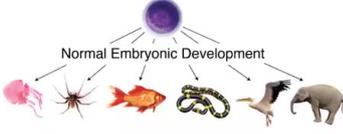


What you see is an inflation, not only of body size, but actually of their ability to pursue states in various kinds of problem spaces, including anatomical morphospace, physiological space.

A single cell can give rise to a collection of cells that self-assembles into some incredibly complex morphology.

We know that simply understanding stem cell biology is not going to be enough because here we have a teratoma and this thing might have hair and teeth and bone and muscle and skin. The work of the stem cells has proceeded fine. You have all your derivatives, you have your various tissues. What you don't have is this three-dimensional structure. We need to understand how this works.

Current Paradigm of Patterning

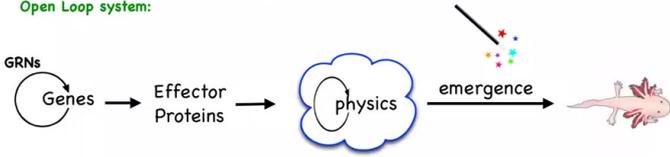


Normal Embryonic Development

- cell differentiation
- cell proliferation
- cell migration
- apoptosis

under progressive unrolling of genome

Open Loop system:



Fundamental difficulty: inverse problem
(what molecules to tweak for desired system-level outcomes?)



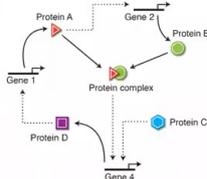
Michael Levin

In standard developmental biology, the story that we're told is a kind of feed-forward open-loop system, which is very much based around emergence. The idea is that there are gene regulatory networks, so genes turn each other on and off. Some of these genes are effector genes; they code for effector proteins, so they are sticky or they diffuse or they exert force or something like this. Then there's this physical process where all of these things interact with each other. Then through things that are studied by the science of complexity, out come these amazingly complex results. Like the salamander here.

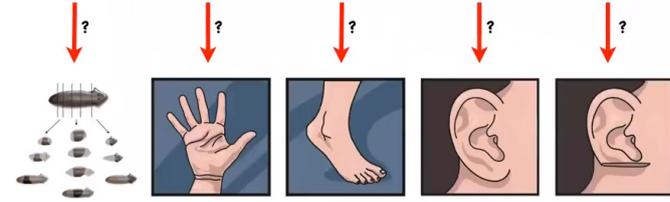
This story is certainly true in the sense that these things all happen. But it's incomplete, and it has a fundamental difficulty, which is this inverse problem. If we're committed to this feed-forward emergence story, then making changes here requires us to exert our interventions, for example, at the genetic level. That means that we have to try to invert this process of emergence, which is fundamentally insolvable. Most of these inverse problems are too difficult to solve. How do you know what genes to tweak to make desired changes at the three-dimensional anatomy level?

Current State of Affairs:

This is what we are good at:



This is what we want to understand:

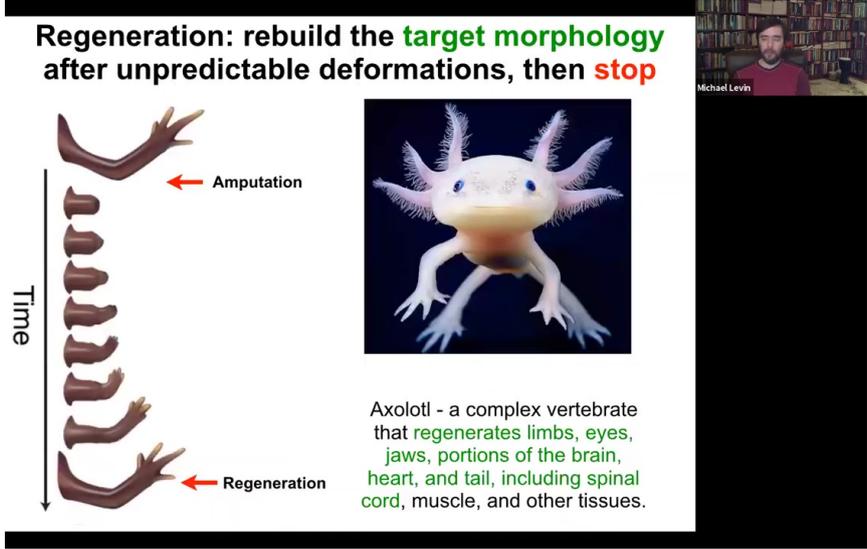


what node to tweak to repair shape?



The current state of affairs is this. We're very good at figuring out the lowest hardware level of which genes and proteins interact with which other genes and proteins. But what we'd really like to understand are things like this: the ability of planaria to regenerate from pieces, the shape of the hand and the shape of the foot and why they're different, and what you would have to do to get these specific shapes back. It's not obvious what you would do at this level.

Regeneration: rebuild the target morphology after unpredictable deformations, then stop



The diagram shows a sequence of stages for limb regeneration over time. It starts with a complete limb, followed by an arrow labeled 'Amputation' pointing to a shorter limb. A vertical arrow labeled 'Time' indicates the progression. The sequence continues with several intermediate stages of regrowing tissue, and finally an arrow labeled 'Regeneration' pointing to a fully restored limb. To the right is a photograph of a white axolotl with blue eyes and gills.

Axolotl - a complex vertebrate that regenerates limbs, eyes, jaws, portions of the brain, heart, and tail, including spinal cord, muscle, and other tissues.

Michael Levin

So the amazing thing is that... Some animals are very good at this, and their bodies show remarkable plasticity in this kind of collective activity of the cells. So for example, here, this animal is a salamander. It regenerates its limbs, its eyes, its jaws, its portions of the brain and the heart. So if they're amputated, they will grow back. And what's cool is that not only is this process incredibly flexible, meaning if you amputate at the shoulder, you grow the whole thing. If you amputate at the wrist, you start here and you just grow the parts you need, but then it stops. And that's the most remarkable part of all of this. And so lots of people work on trying to kickstart regeneration. But actually, how does it know when to stop? Because when it stops is when it has built a correct salamander arm. That's when it stops. How does the system know when it's built a correct salamander arm?

Regeneration is not just for “lower” animals



The human liver is highly regenerative

Every year, deer regenerate meters of bone, innervation, and skin

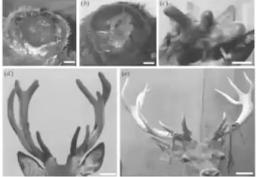




Fig. 2. (A) Amputation of finger tip in 5-year-old girl. (B), (C) Twelve weeks after accident.

Human children below 7-11 years old regenerate fingertips



Michael Levin

It's important to note that this is not just some weird quirk of salamanders. Humans and other mammals can do some of this. The human liver is highly regenerative. That's been known for a long time. Unclear to me how the ancient Greeks knew that, but they clearly did. Deer regenerate huge amounts of bone, up to a centimeter and a half of new bone per day. Bone, vasculature, innervation, and skin can regenerate. Even human children can regrow fingertips. It usually stops at a particular age, but if you keep it clean, the amputation will give rise to a cosmetically perfect finger.

Planarian Regeneration: restoring global order

Cells from same position make radically different structures = non-local decision-making

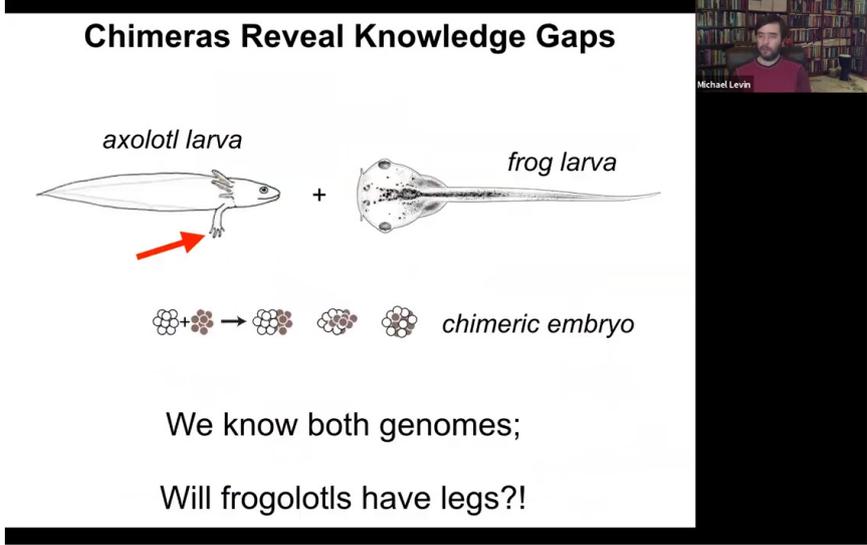
Immortality!

So the champions of this process are these guys. These are planaria. These are flatworms. They have a true brain, central nervous system, the same neurotransmitters that you and I have. And the amazing thing about them is that you can cut them into lots of pieces. The record is 275. And every piece will regrow exactly what's missing, no more, no less, to give you a perfect tiny little worm.

While the new stuff is growing, the remaining tissue is shrinking so that they will all, as quickly as possible, get to proportion, to correct proportionality. And the other thing about them is they're immortal. There's no such thing as an old planarian. So if you're interested in aging or these ideas that things inevitably wind down and accumulate errors and so on, planaria are telling us that is not absolutely required. Here's a life form that's basically immortal.

We're really not very far along in understanding how the cellular collectives make decisions.

Chimeras Reveal Knowledge Gaps



axolotl larva + *frog larva*

chimeric embryo

We know both genomes;

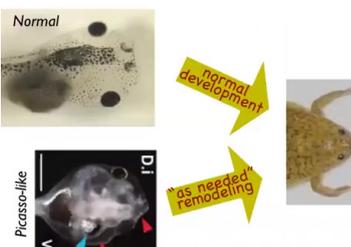
Will frogolotls have legs?!

Michael Levin

One of the easiest ways to see that is to think about chimeric organisms. Here's a simple example, which we're making in my lab right now. Here's an axolotl larva. Axolotl larvae have legs. Here's a frog larva, a tadpole. Tadpoles do not have legs.

One can ask a simple question. If I combine early axolotl tissue with early frog tissue in early embryogenesis, I make a chimeric embryo. They're perfectly healthy. We call them frogolotls. We have the genomes, we have the axolotl genome, we have the frog genome. Now we ask a simple question: do frogolotls have legs? Even though we have all this information, we have no idea how to predict in advance whether frogolotls will have legs. If so, will they be made entirely of axolotl cells or of both types of cells? These are the kinds of things that we would like to understand.

Remodeling until a “correct frog face” is made



Normal

Picasso-like

normal development

as needed remodeling

Genetics does not specify hardwired rearrangements: it specifies a system that executes a highly flexible program that can recognize unexpected states and take corrective action.

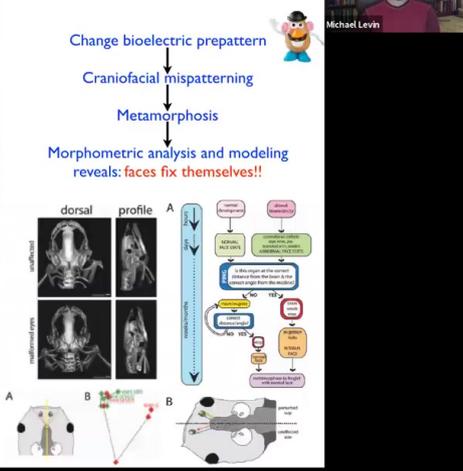
Cannot just follow a rote set of steps. How does it know when it's "right"?

Change bioelectric prepatterning

Craniofacial mispatterning

Metamorphosis

Morphometric analysis and modeling reveals: **faces fix themselves!!**



dorsal profile

metamorphosing face

metamorphosed face

A

B

B

One thing that's very important as part of this process is specifically the algorithm that enables robustness and the handling of novelty. I'll show you a simple example of that. We discovered this a few years ago.

Here is a tadpole. It has eyes here, it has nostrils and a mouth down here. All of these things have to move around in order to get to a frog face. During metamorphosis, the face has to deform. The jaws have to come out, the eyes have to move forward, everything has to move. It was thought that somehow what the genome did was to give each piece of the face a particular direction and amount of movement. That way standard templates become standard frogs.

What we did was we created what we call Picasso tadpoles. Everything's in the wrong position. The eyes are on top of the head, the mouth is off to the side, everything is mixed up. I'll show you in a minute how we do it. But the amazing thing is that these animals become pretty much normal frogs because all of these different organs move around through unnatural paths and sometimes they go too far and actually have to back up.

Remodeling until a “correct frog face” is made

Normal

Picasso-like

normal development

needed remodeling

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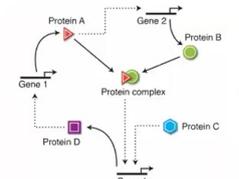
dorsal profile

Michael Levin

But everything moves around until it gets to a correct frog face and then the remodeling stops. So in fact, what the genetics specify is not a bunch of hardwired rearrangements, but a system that executes a really flexible error minimization scheme. It's able to start off at incorrect or abnormal positions and still get to where it needs to be. This parenthetically matches William James's definition of intelligence, which is the ability to reach the same outcome despite perturbations and starting from novel starting configurations. So how does this system know what a correct face is, and how do we get there?

We are good at manipulating molecules and cells;

We are a long way from control of large-scale form and function

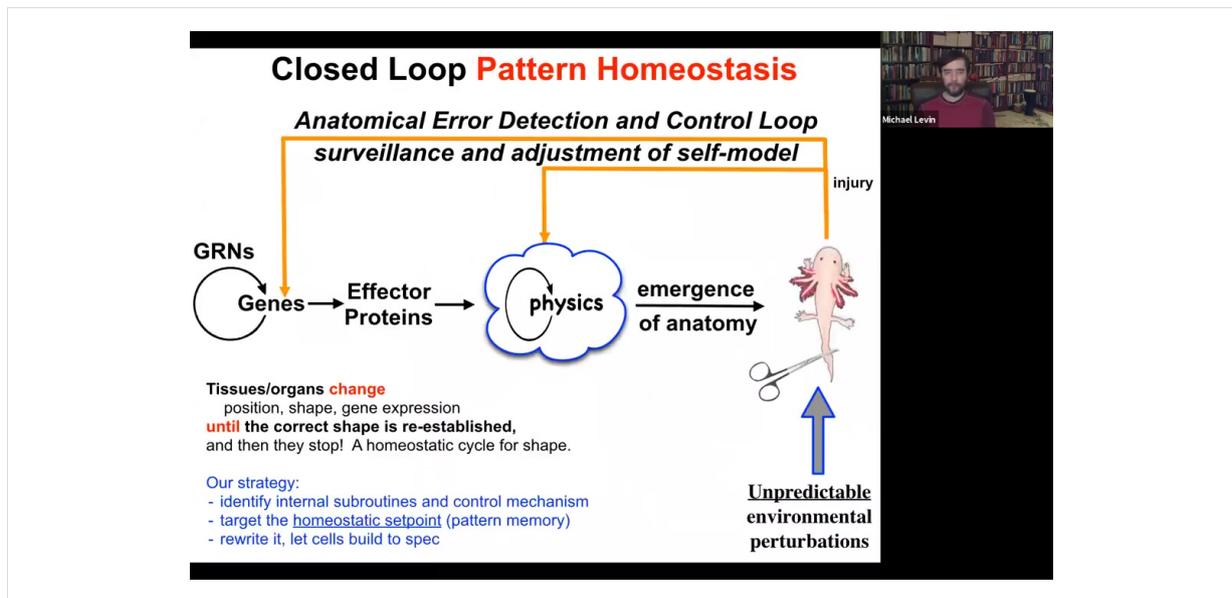


Can we move biology beyond operating at the machine code by reverse-engineering the algorithms of anatomical control?

- what are the native modules and their triggers?
- what does the cellular collective measure?
- how are global patterning goal states specified?
- how is anatomical error detected?
- how reprogrammable is the hardware??



So we've been thinking about this problem. My background is computer science. And so to me, all of this looks like a problem of information processing at different levels. And what I would love to know is: could we go beyond the hardware and operating at this level and ask about the algorithms? How are these decisions being made? What do the cellular collectives measure? What are their modules or subroutines? How are these global patterning goals specified and stored? And in particular, how reprogrammable is any piece of biological hardware?



What we try to do is to come up with a scheme where there's more feedback here. When the system is deviated from its normal target morphology, be that with injury, teratogenic drugs, or pathogens, feedback loops kick in both at the level of genetics and physics, and we're going to talk about this physical one here, that try to minimize the error, the delta between where we are now and where we need to be. This is a classic homeostatic loop. It's what the thermostat in your house does. It measures against a set point, and if error is beyond a tolerable amount, it will undertake corrective action.

Several things to note here. The first is that feedbacks are not new in biology. There's something different here. The first is that the set point of this process: every homeostatic system has to have a set point towards which it tries to reduce error. The set point here is not a single number or a scalar like pH or a metabolic hunger rate. It's a complex set of information that in some rough, coarse-grained way describes what a correct anatomy should be.

The other thing is that this is very much a goal-directed process. When I say goal-directed, I don't mean something magical or mysterious. It's goal-directed in the cybernetic sense. We've had devices that are goal-directed agents since the 40s and 50s. All it means is that it's able to execute this error minimization loop.

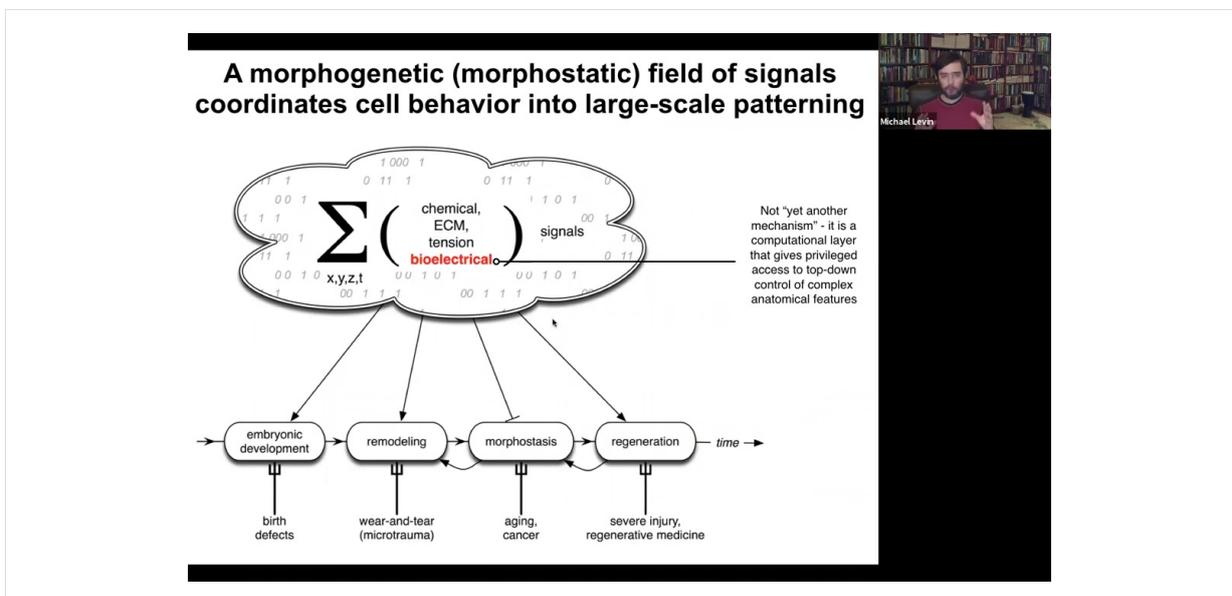
But the cool thing about systems that work like this is the following. When you want to change what they do, you don't have to be rewiring the hardware back here. If you understood how the set point was encoded, you could change the set point and get the exact same cells to build something different. That's the amazing thing about any system that utilizes this kind of architecture, that you can make changes without even necessarily knowing how all the parts of the loop work. All you have to do is rewrite the set point.

So our strategy for some years now was this. We tried to identify some of the mechanisms at work here, in particular, to understand the homeostatic set point. How does the tissue store the pattern towards which it tries to remodel? Then let's rewrite that pattern and let's let the cells build. That's part of trying to decode this aspect of the collective behavior of these cells.

Let's talk about how we've been doing it. We have started looking at something called developmental bioelectricity. What I'm going to show you now are, first of all, the methods. How do we do this? and then show you some proof of principle applications.

Why is this interesting? I want to point out at the beginning that all cells in vivo sit within this complex morphogenetic field of information that tells them what to do as part of a larger unit.

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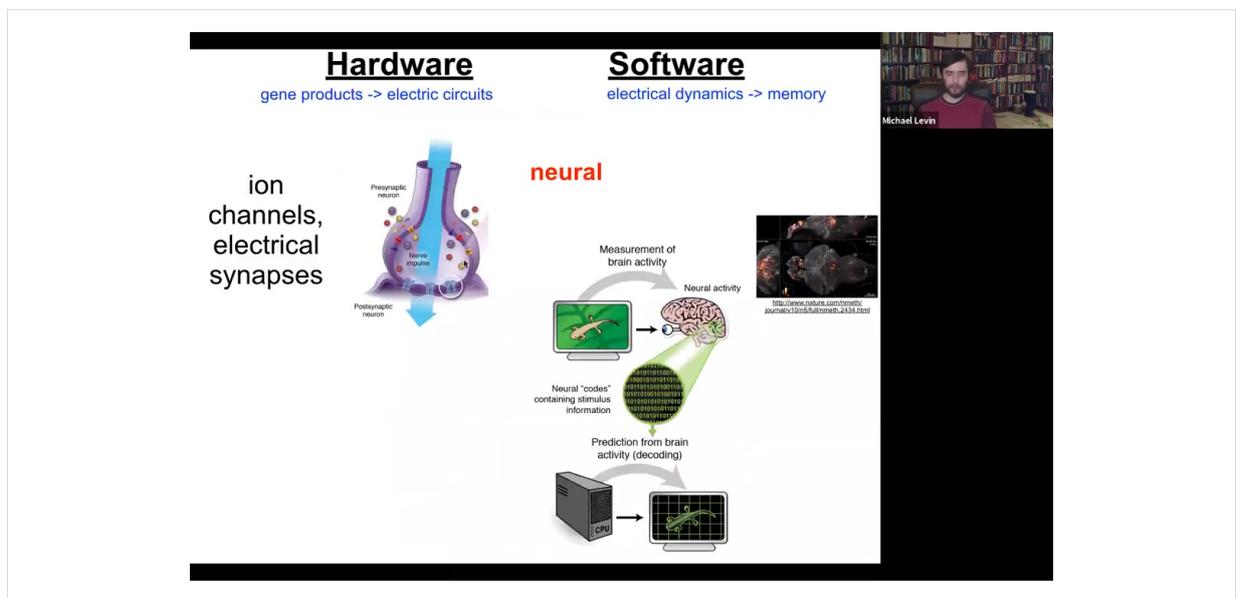


This information comes in many flavors. Chemical, extracellular matrix, tensions and stresses, biomechanics, all kinds of things, and bioelectricity.

Bioelectricity doesn't do this alone. It works with all of this other stuff. I'm going to focus today on bioelectricity because it has a particular aspect to it, which is that it's not just another piece of physics that you need to know to understand anatomies. It's actually a computational layer that gives some privileged access to the control of complex anatomical features. It is the medium in which the computations are being

made to make decisions about the length and the size and the shape of things. Accessing this bioelectricity gives you a remarkable insight into what's going on beyond bottom-up molecular mechanisms.

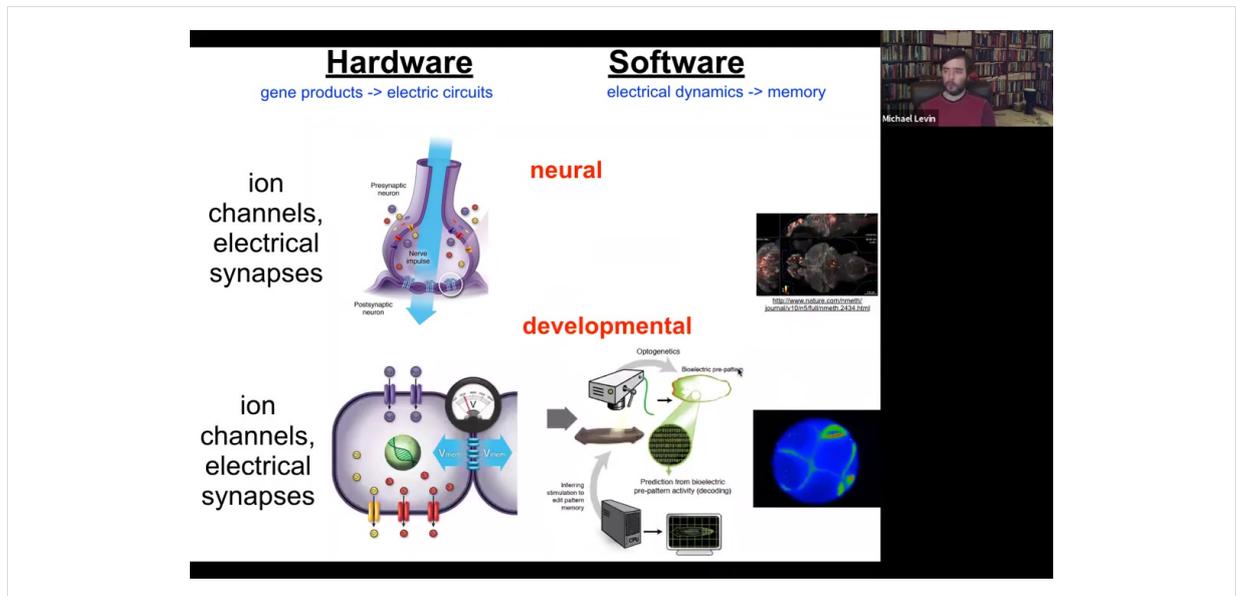
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The kind of inspiration for how to think about this we took directly from neuroscience. In neuroscience, this story will be familiar to everybody. The hardware is simple. You have a collection of cells. Each cell has ion channels in the plasma membrane. They set voltage values across the membrane; that's the resting potential, which can go up and down, and it can propagate to the neighbors via these electrical synapses.

Those kinds of networks underlie this amazing software. Here you can see this physiology in a living zebrafish brain as the fish undergoes cognitive activity. You can see here the electrical activity. The commitment of neuroscience is that if we were able to decode this, if we understood how to read this information, through a computational approach we should be able to figure out what the animal is remembering, what it's thinking about, and what decisions it's making. The semantic and functional cognitive structures are to be read out from the electrical activity of the brain.

That's what we believe.

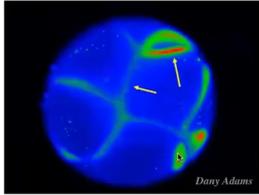


All cells do this. All cells have ion channels. Most cells have electrical synapses known as gap junctions to their neighbors. We have undertaken this project to try to do the same kind of decoding. Here's an embryo, and we would like to read all the electrical conversations that bind the individual cells into a collective that can undergo anatomical homeostasis towards large states, build a limb, build a kidney.

On the one hand, it's strange and surprising to many people to think about somatic cells as having this kind of neuroscience-like aspect to them, as if they're processing information, they're having goal states. On the other hand, if you ask where neurons came from, they didn't just appear out of nowhere. Evolution just sped up, optimized things that cells have been doing for a really long time, since around the time of bacterial biofilms. All of the components of neurons that are really important for this—the channels, the neurotransmitter machinery—existed before multicellularity, and even bacteria were using this to coordinate information across the biofilm.

How we detect and model bioelectric signals:

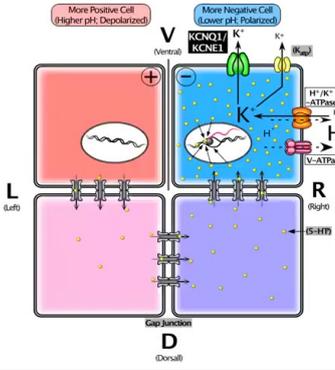
Characterization of endogenous voltage gradients - direct measurement and correlation with morphogenetic events



Dany Adams

Voltage reporting fluorescent dye in time-lapse during *Xenopus* development

Quantitative computer simulation: synthesize biophysical and genetic data into predictive, quantitative, often non-linear models

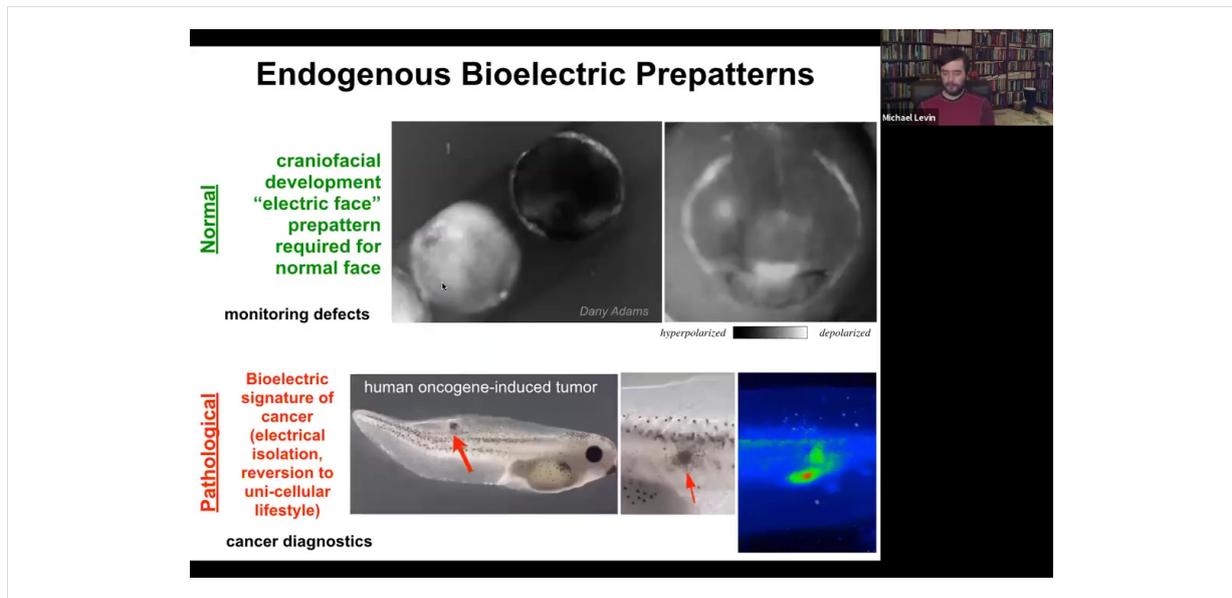




Michael Levin

So we developed some tools to study this. The first is just how to detect and model them. This is a voltage-sensitive fluorescent dye, which reveals in the living state, non-invasively. You don't have to poke the cells with electrophysiological tools. Every cell now reports, around its membrane, all the voltages. This is an early frog embryo in time-lapse, sorting out which cells are going to be left and right, then dorsal, ventral, and so on.

In addition to that, we do a lot of computational modeling. If we know what ion channels and pumps are in the membrane, can we explain why the voltage is the way it is and why it changes as a function of time?



Let me show you a couple of native bioelectrical patterns. This is what we call the electric face. This was discovered by Danny Adams in my group. She used voltage dyes to look at the frog embryo putting its face together. This is, of course, a time lapse. This is one frame taken from that movie. Long before the genes come on to regionalize the face into the eye, the mouth, and everything else, all of the bioelectrical properties set up a pre-pattern, a scaffold that tells you where everything is going to be. Here's where the animal's right eye is going to be. The left eye will come in shortly. Here's the mouth. Here are some placodes.

This bioelectrical pattern is a native instructive pattern for gene expression and for anatomy. The reason we know it's instructive is because if you go in with optogenetics or other tools and change this electrical pattern, not move any cells, but just change the pattern, then you can get, for example, those Picasso tadpoles. You can move organs around at will.

This is a normal pattern that is necessary for correct craniofacial development. Here's a pathological pattern. This embryo was injected with a human oncogene, for example a KRAS mutation. Here it's going to form a tumor, and the tumor is going to start to spread. But before that happens, before it becomes histologically apparent, you can already see with this voltage dye that here are the cells that have depolarized and shut off the electrical connections to their neighbors in a way that's going to basically revert them back to their unicellular ancient lifestyle. Once you're electrically disconnected from this grid, the rest of the animal becomes just external environment, that computational boundary. Whereas before it was large, it was a group working on a liver or a kidney. It shrinks to the level of a single cell and all your goals become single-cell goals. You proliferate, migrate to where life is good. This ends up being metastasis and a conversion of the metastasis. This is a pathological pattern.

Manipulating Bioelectric Networks in vivo

Non-neural cell group

hyperpolarized ← → depolarized

Neurotransmitter (moving via V_{mem})

- Transporter or receptor mutant overexpression
- Drug agonists or antagonists of receptors or transporters
- Photo-uncaging of neurotransmitter

Tools we developed
(no applied fields!)

- Dominant negative Connexin protein
- GJC drug blocker
- Cx mutant with altered gating or permeability

Synaptic plasticity

- Dominant ion channel over-expression (depolarizing or hyperpolarizing, light-gated, drug-gated)
- Drug blocker of native channel
- Drug opener of native channel

Intrinsic plasticity

Michael Levin

What we then developed was a set of tools that were designed to allow us to manipulate these bioelectrical gradients in vivo. If we thought they were functional, we ought to be able to control them to get the collective to do something different. It is important to say that we do not do any external field application. We don't have any electromagnetic components here. There are no electrodes. There are no waves. There's no magnetism. It's all molecular physiology.

The way it works is you have two things you can control in any tissue. You can control which cells electrically couple to which other cells. We target these gap junctions. We can mutate them. We can open them, close them, controlling synaptic plasticity, or we can control the various ion channels, again opening and closing them, to actually set the voltage of the individual cells. This would be the equivalent of some sort of intrinsic plasticity if this was neuroscience. We can use dominant mutations of channels, we can use drugs, we can use optogenetics, light, and so on.

This was back around the year 2000, when I was first setting up these tools and we wanted to control these bioelectric states, it was thought that resting potential is a housekeeping parameter. The standard assumption was that if we perturb it, the cells would die and you would get uninterpretable toxicity. That was the thought. I'm going to show you that, in fact, that's not at all what happens because these bioelectrical states are not just readouts and they're not just yet more molecular machinery, but they're actually the information-bearing medium for these large-scale modifications.

Manipulation of V_{mem} in vivo enables *coherent, modular changes to large-scale anatomy: eye induction*

ion channel mRNA targeted to ventral or posterior regions

can reprogram many regions into complete ectopic eye!

note modularity - not cell level!

Induction of complete, beating hearts

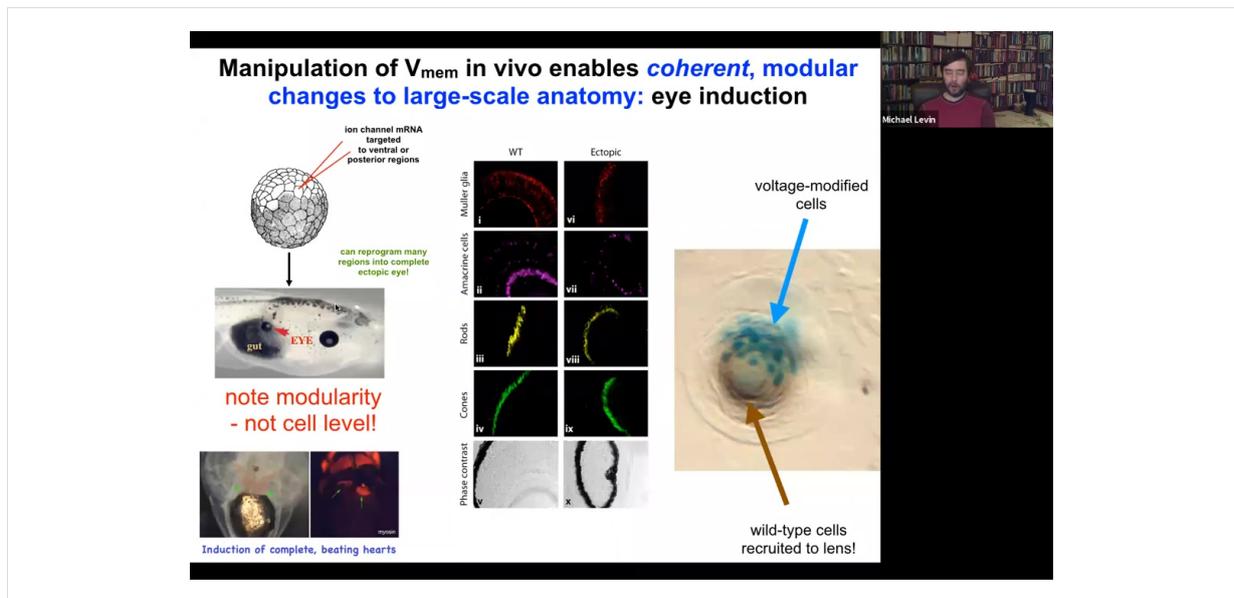
	WT	Ectopic
Muller glia	i	vi
Amacrine cells	ii	vii
Rods	iii	viii
Cones	iv	ix
Phase contrast	v	x

voltage-modified cells

wild-type cells recruited to lens!

Michael Levin

One thing you can do is you can take some cells in the frog that are going to become gut, so they're endodermal cells, and you can inject one of several types of ion channels that in this region that's going to be part of the gut will set up a voltage pattern, a distribution of voltage that's very similar to what happens when the native eyes come in. And sure enough, if you recapitulate that pattern somewhere else, the cells are using that pattern to decide what they're going to build. That pattern tells them to build an eye. They will build an eye out of endodermal cells. Now, in the textbook, it will say that only anterior ectoderm is competent to become eye up here.



And that's true if you use canonical inducers like PAX6, the master regulator transcription factor. But if you go upstream of that and actually re-specify the bioelectric pattern, then you can induce these eyes anywhere, on the tail, in the gut, anywhere you want. And if you make these eyes, they can have all of the same components that normal eyes have. So they'll have lens, and retina, and optic nerve, and so on.

If you label the cells that you've directly injected with the ion channel, let's say we label them with beta-galactosidase, so they're blue. This is a cross-section through a lens sitting out in the flank somewhere. What you'll see is that there are two inductions here. The first induction is by us imposing a particular bioelectrical state that causes these cells to decide to become a lens. That's the first induction. The second induction is that these cells, with their aberrant voltage potential, actually recruit their neighbors, the brown cells here, which are not labeled, which were never directly modified by us, into this project of building this larger scale structure, this nice round lens. So it's a non-cell autonomous effect. And we see this again and again, that there's this ability of these bioelectrical signals to not just specify cell fate, but to actually specify organ type and position and things like that. So we can make eyes, we can make ectopic hearts. Here's a secondary heart.

We can make limbs and brains and some other things, and then many things that we can't yet make.

Manipulation of V_{mem} in vivo enables *coherent, modular* changes to large-scale anatomy: axial patterning

Head and tail amputation

body-wide voltage gradient

Tatsaku Nogi

Hyperpolarized

Depolarized

use drugs and RNAi to change V_{mem}

bipolar 2-head worm

bipolar no head worm

Michael Levin

Let's look at another example. Here's a planarian. One of the most interesting things is that if you chop off the head and the tail, this middle fragment knows exactly how many heads it's supposed to have. It puts one head here, it puts a tail here. The way it does that is because as soon as you amputate, there's this voltage gradient that's set up where the red, the depolarized region tells the worm how many heads it's supposed to have.

Manipulation of V_{mem} in vivo enables *coherent, modular* changes to large-scale anatomy: axial patterning

Head and tail amputation

body-wide voltage gradient

Taisaku Nogi

Hyperpolarized

Depolarized

use drugs and RNAi to change V_{mem}

bipolar 2-head worm

bipolar no head worm

Michael Levin

What we can do is we can go in and target this region using drugs, ion channel modifying drugs to depolarize this region.

When you do that, you get a two-headed animal. These cells are perfectly happy to make a head. The information of how many heads you're supposed to have comes from this electrical gradient. If you change the gradient, they'll happily make two heads. You can make two-headed animals. You can make no-headed animals.

What's important here is that there's no genomic editing here, so this is purely physiological, and I can ask this animal to make a second head of the correct type.

Bioelectric circuit editing over-rides default genome-specified target morphology and switches among species

Tweaking of bioelectric network connectivity causes regeneration of head shapes appropriate to other species! (also includes brain shape and stem cell distribution pattern)

D. dorocephala

cut off head, perturb network topology

like: *P. felina* like: *S. Mediterranea* like: *D. japonica*

quantitative morphometrics

Alexis Petrák

Michael Levin

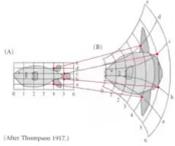
I can actually ask it to make heads belonging to other species. We started off with a triangular-headed worm like this, amputated the head and perturbed for about 48 hours the ability of cells to talk to each other electrically. What happens is the electric circuit is unable to settle to the correct attractor. When they do settle stochastically, sometimes they settle to the correct shape, but sometimes they make round heads or flat heads. In doing so, they recapitulate the state of flat-headed species like this *P. felina* or round-headed species like this *S. mediterranea*. It's not just the shape of the head, it's also the shape of the brain and the distribution of stem cells that is exactly like these other species. No genomic editing here, perfectly wild-type genome. These animals, once the electric circuit settles, there's a good chance that it'll settle into an inappropriate attractor that's actually associated with a completely different head shape that other species are using natively. You can also knock the system into attractors that are not being used by any system.

Drastic body-plan editing: flatworms, with a normal planarian genome, don't have to be flat!

Normal **Bioelectric Circuit Altered After Bisection**



We can reach regions of the morphospace not explored by evolution, purely by changing electric circuits' dynamics *in vivo*



(After Thompson 1917)



And you can really get far from the normal planarian shape. You can make animals that are spiky. You can make animals that are cylindrical, or a combination of a flat animal with a tube growing out into the third dimension. I'm going to show you in a minute that what this is all about is predictive control over large-scale anatomy. This is not about making weird, aberrant teratologies. The idea is that we can show how much of this is under bioelectrical control and then use computational techniques to gain good predictive control over it.

This is a good time to think about the role of the genome in the anatomy of this process. The typical metaphor that we hear is that the DNA or the genome is the software and that the cell is the hardware that interprets this.

That's not a bad analogy at the single cell level when you're thinking about phenotypes of proteins and pathways.

But at the level of anatomy, I would suggest a different metaphor. The different metaphor that works better to understand anatomical control is this.

A Different Code Metaphor

FLIP-FLOP

PARTS LIST = DNA
- 2 TRANSISTORS
- 6 RESISTORS
- 1 BATTERY

Calvin Bradbury-Jost

SOFTWARE → self-organization, memory, feedback loops

Proteomically Identical Cells
Yoshida, Brock, Axtell, Saito, Komatsu, Ohta & Oda, 2017

Alexis Petuk

HARDWARE →

genome determines cellular hardware

Bioelectric decision-making runs on the real-time physics, not necessarily visible to transcriptional or proteomic profiling!

What the genome does is specify all of the hardware that the cell gets to have. All of the specific ion channels that are here are determined by gene expression. But we know from basic neuroscience and also from computer technology that if you make a very simple circuit, this is a flip-flop circuit, if you nail down the hardware, you can have a circuit that can store multiple different pieces of information depending on the current flow through the system and store it stably, which is a memory. A flip-flop is a basic kind of memory. And you don't need to move or change any of the hardware in order to store a zero or a one in this very simple electrical circuit.

So in fact, with our modeling, you can see that the same thing is true if you set up a field of cells where every single cell is expressing exactly the same set of ion channels. What you actually make is an excitable tissue where very rich patterning can take place. It's a little like Turing patterns. There's spontaneous symmetry breaking and self-organization. But all of this happens with a completely constant proteome. You don't need to change these ion channels because they can open and close. And now all of the dynamics are at the electrical circuit level, not with the proteins underneath. The prediction of this way of thinking about it is this: you should be able to edit the software, meaning change the information stored in the system while keeping the hardware constant.

In particular, the piece of information that we want to change is the set point. We want to change the set point towards which cells are working in this error minimization scheme.

An organism's genome sets its long-term anatomy, doesn't it?

Cut, and briefly perturb bioelectric circuit

weeks, cut in plain water

surely a normal worm must result once ectopic heads are removed in plain water (no more reagents), since genome is wild-type...

Michael Levin

Relatively Hyperpolarized (make more negative) Voltage Relatively Depolarized (make more positive)

Anterior: Hyperpolarized, No Head; Depolarized, Head

Posterior: Hyperpolarized, Tail; Depolarized, Head

Amputation Point: 72 hpa

14 dpa

Headless, 2-headed

Energy landscape

Single head (H-I)

Double head (H-I)

Widen the network

Let's think about how to do that. Here's our normal planarian. We've cut off the head and the tail. We got the middle fragment. We've perturbed the electric circuit according to this model that we've developed. Here's your two-headed animal.

We ask a simple question. Here's our two-headed animal. We give it a couple of weeks to get everything settled down. Cut off the primary head. Cut off this crazy ectopic secondary head. Some people think you somehow epigenetically reprogrammed this posterior tissue. Fine, we'll cut it off. We'll throw it away. All that remains is a normal middle fragment here that didn't have any head tissue in it. We do this in plain water, no more manipulations of any kind. The genome is wild type. We haven't edited the genomic sequence at all.

Surely the prediction would be that you should get back to a single-headed worm. Once you cut off this thing, you should be back to a single-headed worm. The interesting thing is that if you model the state space of the electric circuit that's involved here, what you find is that there are multiple stable points. One is here, a very stable point around the single-headed shape, but there's another stable point around the double-headed shape. That suggests an interesting idea: is it possible that when you amputate this thing, the bioelectrical circuit will still remember that it needs to make two heads.

Re-wiring of bioelectric networks causes permanent (stable) changes to target morphology without genomic editing

Cut, and **briefly** perturb bioelectric circuit

or, can force V_{mem} state back to normal

weeks later, cut in **plain water**

Keep trunk

weeks later, cut in **plain water**

Keep trunk

in perpetuity

•••

Basic properties of memory

- Long-term stability
- Lability (rewritable)
- Latency (conditional recall)
- Discrete possible outcomes (1H v. 2H)

Michael Levin

And sure enough, that's exactly what happens. When you amputate these in perpetuity, as far as we can tell, forever, two-headed animals will continue to give rise to two-headed animals, even despite their wild-type genome in plain water, no more manipulation. And we can, in fact, if we want to, we can set the bioelectric circuit back to normal, and it goes back to being a single-headed animal. So a normal planarian body, as you're going to see, is able to store a couple of different, at least, probably more, but we've nailed down two, ideas of what a correct planarian is supposed to look like.

Bioelectrically-Encoded Pattern Memory

normal anatomy

normal molecular histology

middle-third regenerates:

The bioelectric pattern doesn't indicate what the anatomy is now, it encodes the pattern that will guide anatomy if it is cut at a future time

Biophysical Journal

Michael Levin

The Same Body can Store different Electrical Pattern Memories

Here's a single-headed planarian. If we look at the molecular markers, the anterior marker is here in the head, not in the tail. If you amputate, you get a perfectly normal single-headed animal. Here's another anatomically normal animal.

Bioelectrically-Encoded Pattern Memory

normal anatomy

normal molecular histology

middle-third regenerates:

The bioelectric pattern doesn't indicate what the anatomy is now, it encodes the pattern that will guide anatomy if it is cut at a future time

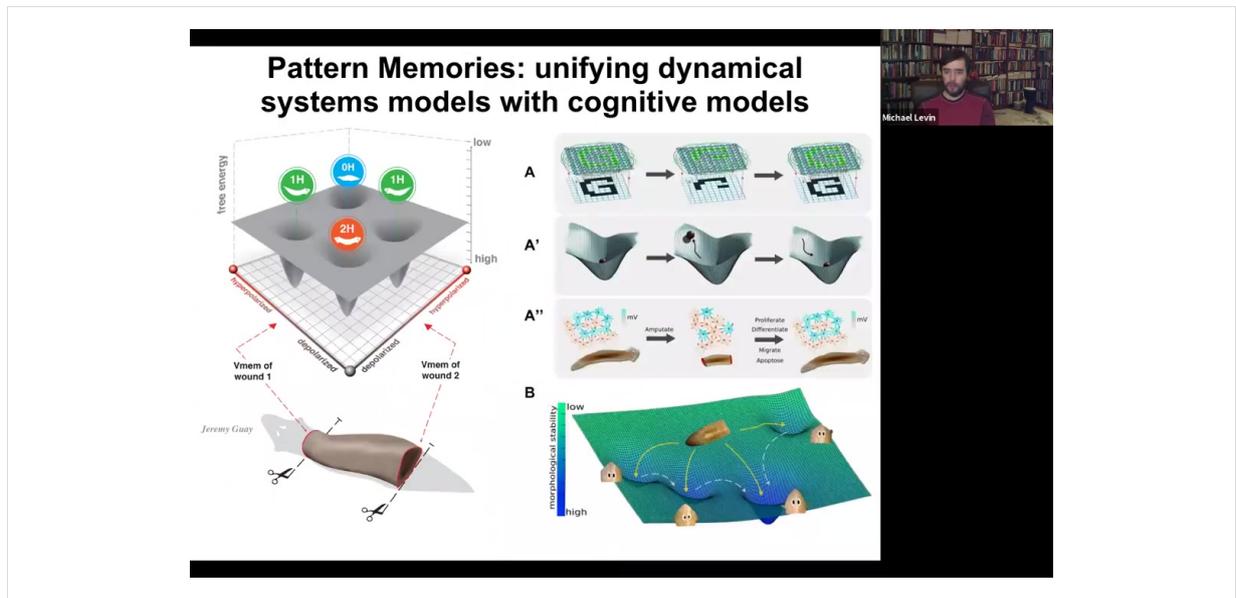
Michael Levin

Biophysical Journal

The Same Body can Store different Electrical Pattern Memories

Again, molecular markers are all normal, anterior in the head, no anterior marker back here. But if you amputate this one, suddenly you get a two-headed animal. Why would you get a two-headed animal? Because in the meantime, what we've done is we've bioelectrically changed the stable pattern to say, no, now you need to have two heads. Now, here's the critical part of this: the electrical map here is not a map of this two-headed animal. This electrical map is a map of this one-headed animal, meaning that the bioelectricity distribution is not simply a map of whatever the anatomy is doing. The same single-headed anatomy can store at least one of two different stable representations of what it's going to do if it gets injured in the future. If you're interested in neuroscience and how counterfactuals are remembered in the brain, this is the evolutionary precursor to being able to remember things that are not happening now, either things that happened before or might happen in the future. It's a counterfactual memory. It's the ability to store a representation of a worm towards which the fragment is going to build if it gets injured. So it sits perfectly comfortably as a single-headed animal with this pattern until you cut. At that point, that memory is no longer latent and it actually becomes functional.

And that's how we get this propagation of these two-headed worms.



So we are building very quantitative molecular biophysical models to understand the state space of the circuit, to understand how the circuit minimizes free energy and eventually lands in one of these attractors, hopefully the right one, but not necessarily. And tying it into exactly the same formalism for how people in machine learning think about networks, electrical networks that can store patterns and can repair those patterns when pieces of information are missing. This is all very well-trodden ground in machine learning. And we think that some of these same strategies that we use now were discovered by evolution long ago.

And beyond worms, this is the beginning of our roadmap for regenerative medicine.

Brief bioelectric signals kickstart long-term, self-limiting modules (low info-content trigger, high complexity output)



Hind-leg amputation
*
designed ionophore
cocktail regimen



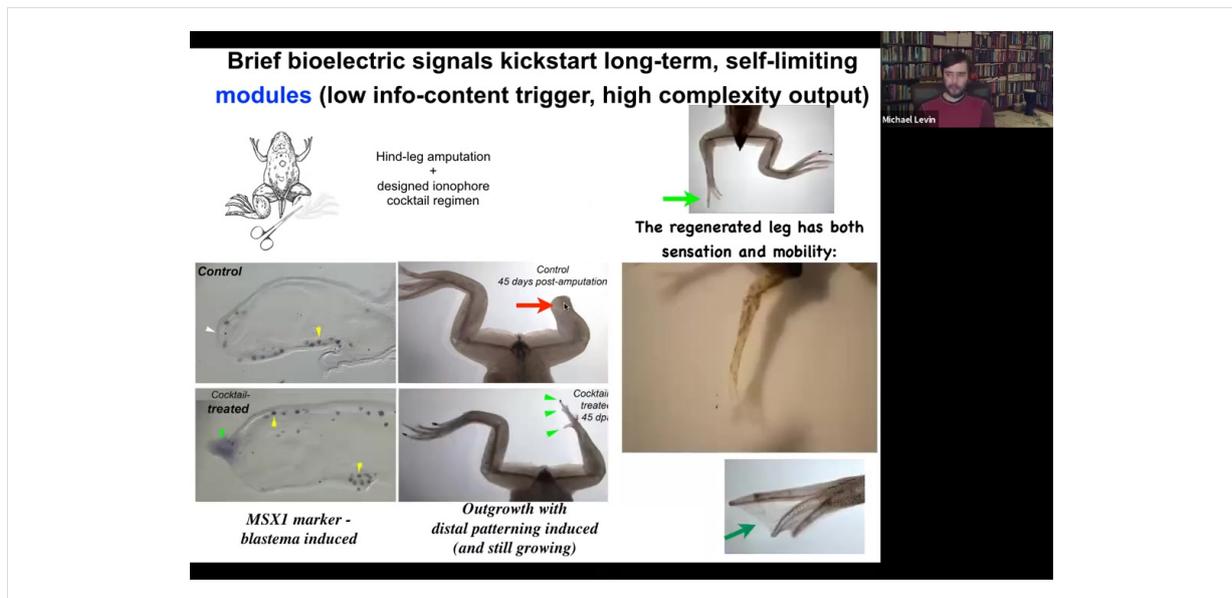
Control

Control
45 days post-amputation



Michael Levin

Because what we found is that, for example, if you have a frog and the frogs do not regenerate their legs the way that salamanders do, you amputate the leg, 45 days later, there's basically nothing. There's no blastema and there's no regenerate. What we can do is we can apply a cocktail of ion channel drugs that serve as a stimulus to kickstart a very complex cascade.



What you see here is that immediately the MSX1 blastema marker becomes turned on, and the leg starts growing. Already you have the toes and here's a toenail. And eventually you get a very respectable leg here and it's touch sensitive and motile. The animal can use it.

There are a couple of interesting things about this. One is that much like when we created an eye or induced a second head on a planarian, inducing this leg, we don't provide all the information to micromanage the process. We have no idea how to build a planarian head from scratch or how to make an eye or how to make a leg. What we have found is a trigger for a subroutine that this tissue already knows how to do. And the decision for what it's going to do, scar or produce various types of organs, is part of an electric circuit that we can guide.

The second thing is that, in keeping with this idea from the beginning that one of the hallmarks of the collective intelligence of these cells in responding to novel situations is that they can get to the final outcome through novel paths. If you actually look at this intermediate stage of this leg, this is nothing like what frog legs look like when they're developing in the first place. It eventually gets to a very good frog leg shape here, basically indistinguishable from the control. But the intermediate path through morphospace, the ability of the system to navigate that anatomical morphospace is amazing because it does not follow the same path that frog limbs normally follow where they make a paddle and then there's apoptosis that kills off the space between the digits. It's not what they do. They grow; it's almost like a plant meristem where you get the central stalk with this nail and then off to the side you get these toes and eventually the whole thing remodels into a leg. The remarkable ability to get its job done in a different way.

How much of this applies beyond frog and worm?

- **Human MSCs, cardiomyocytes in vitro:**

Depolarization Alters Phenotype, Maintains Plasticity of Preadifferentiated Mesenchymal Stem Cells
Sarah Sundtshaus, PhD¹, Michael Levin, PhD² and David L. Kaplan, PhD²

SCIENTIFIC REPORTS
OPEN **Comparison of the depolarization response of human mesenchymal stem cells from different donors**
Sarah Sundtshaus, Michael Levin¹, David L. Kaplan²

Biometric modulation of wound healing in a 3D in vitro model of tissue-engineered skin
Sarah Sundtshaus^{1,2}, Chuanwei Li^{1,2}, Young Jun Choi^{1,2}, Michael Levin^{1,2}, David L. Kaplan^{1,2}

Depolarization of Cellular Resting Membrane Potential Promotes Neonatal Cardiomyocyte Proliferation *In Vitro*
Kun-Yu Lee¹, Grah Williams¹, Michael Levin^{1,2} and Lauren Black Black III^{1,2}

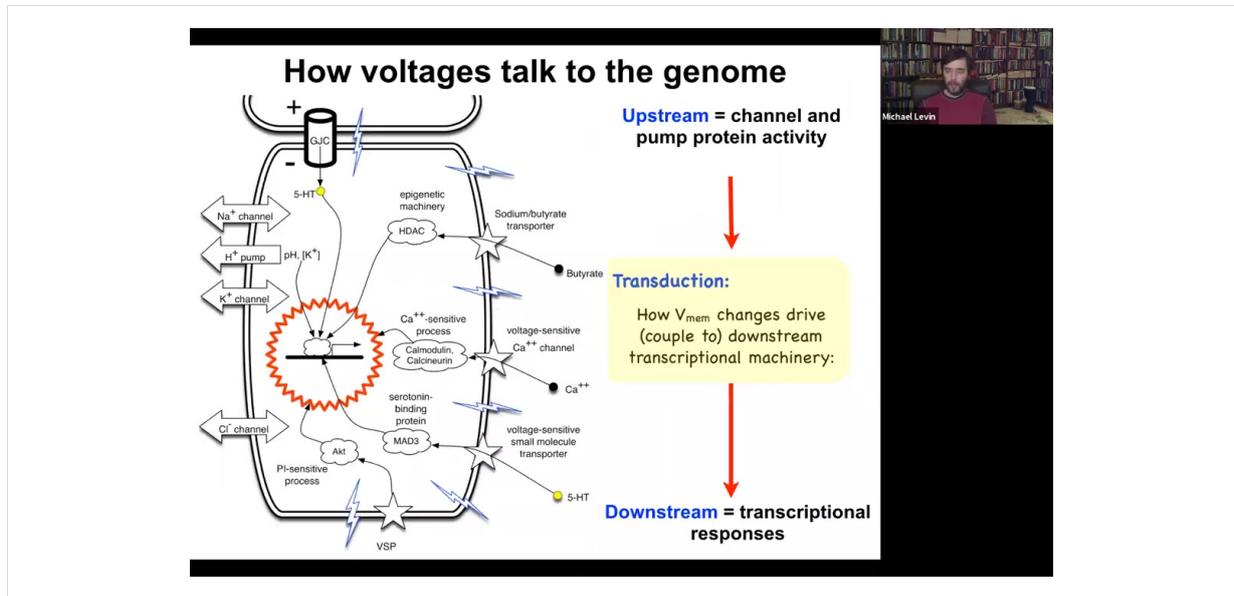
The H⁺ Vacuolar ATPase Maintains Neural Stem Cells in the Developing Mouse Cortex
Christine Lumb^{1,2}, Ross Pennington¹, Peter Knappke¹, Tashin Taylor^{1,2}, Michael Levin^{1,2} and Roberto Gallego¹

Membrane Potential Controls Adipogenic and Osteogenic Differentiation of Mesenchymal Stem Cells
Sarah Sundtshaus¹, Michael Levin¹, David L. Kaplan^{1,2}

Michael Levin

This is not just things that apply to frog and worm. We've done some work with David Kaplan and Lauren Black and others on human mesenchymal stem cells and cardiomyocytes. You can control the kinds of things that you can see in vitro, differentiation, proliferation, wound healing. But I think that's not what the bioelectricity is for. What the bioelectricity is really for in terms of why evolution uses it and why we should be interested in it is because it allows us to exploit the modular nature of the anatomical decision-making, because we can control very large-scale outcomes, not micromanage the details.

Here I have to do a disclosure. David Kaplan and I are both co-founders of this company called Morphoseuticals Inc. where we're trying to take the things that we learned about frog leg regeneration and move them towards mammals. Our goal is we're now in rodents trying for limb regeneration using the same strategies and these wearable bioreactors. The idea is to kickstart regeneration, not micromanage it, not try to babysit all of the different cell types and growth factors, but kickstart a trigger very early on and convince the cells with an aqueous protective environment that regeneration can proceed in the way that it would in a salamander or in an embryonic mammal during this adult phase. That's where one of the research programs is going.



I should point out that it is already known how voltage change impacts the transcriptional machinery. People often say that it would be great if we understood how voltage controls gene expression. We do. We already know about six ways that voltage change transduces down into second messenger pathways and controls gene expression. These include typical mechanisms like calcium, which neuroscience is very familiar with, other things like voltage-sensitive phosphatases, and neurotransmitter transporters. We already know how this works at a single cell level. This transduction machinery has been dissected.

Cracking and Exploiting the Bioelectric Code

- Cell level pathway details are straightforward
- Understanding decision-making in bioelectric networks is the real challenge
- Analogous to multi-scale problem in neuroscience

Common differentially expressed genes as a response to depolarization

Downstream Process Networks	Cell Signaling Network Categories	Disease Network Categories
Apoptosis Cytoskeletal Cell Cycle Cell Metabolism Cell Death Cell Fate Cell Interactions Cell Stress	Developmental Processes SMAD, TGF-β/BMP, FGF, PDGF, EGF, NGF, IGF1, FGF, Conabotrogan Wound Healing MAPK1, 3, 14, MMP/DK1 Regeneration YF92, Notch, HIF-1α, NF-κB, PI3K, Akt1	Neoplasms Metabolic Disorders Wound healing/Regeneration defects Neural Disorders Immune Disorders Cardiac Disorders Pulmonary Disorders Developmental Disorders/Birth Defects

Pai et al. Figure 7

Michael Levin

Also some of the downstream targets have been identified. The genes that are controlled by this — all of your favorite BMPs, Sonic Hedgehog, FGFs — are in fact the redistribution of morphogens in the examples that I showed you. All of this is known both from specific candidates and through unbiased RNA-seq and microarray experiments.

We know this, but what's interesting is that these answers about how it works at a single-cell level have been fairly unhelpful in understanding the large-scale picture that we're really interested in. It's analogous to this multi-scale problem in neuroscience. You can track the pathway, and in every paper you have to go from the channel to the transduction machinery, which genes are downstream. But that actually leaves open some much deeper questions about how the collective makes decisions.

Machine Learning for Model Discovery

<https://gitlab.com/betse/plimbo>
<https://gitlab.com/betse/betse>

Exploring Instructive Physiological Signaling with the Bioelectric Tissue Simulation Engine

Inferring Regulatory Networks from Experimental Morphological Phenotypes: A Computational Method Reverse-Engineers Planarian Regeneration

Research Article
Developmental Biology
Serotonergic regulation of melanocyte conversion: A bioelectrically regulated network for stochastic all-or-none hyperpigmentation

Michael Levin

And the other nice thing about these models is that they are the perfect fodder for machine learning approaches to do two things: to infer better models from data. And we've done some of that. And then interrogate those models for interventions. And so here's some software that anyone can play with if you want to download and simulate all this. And we've shown that you can use machine learning to find models that are really good at explaining functional data sets. In other words, you did something to the system and then something else happened. What is a model that explains that? And then find these needle-in-the-haystack interventions that are ways in which you could perturb it to get it to do what you want. So that is the future of using this for regenerative medicine.

So let's talk specifically about a couple of applications in the last few minutes.

Depolarization (more broadly, a bioelectric signature) reveals tumors (caused by *dom-neg-p53*, *Rel3*, *Gli1*, *RAS*, etc.) before they become morphologically evident

many leukemia basal cell carcinoma rhabdomyosarcoma



Fluorescent dyes reporting V_{mem}

Goal:
Non-invasive detection of pre-cancer and tumor margins *in vivo*

We are now optimizing physiological signature metrics (profile) to improve predictive power

Michael Levin

The cancer issue. I've shown you that you can track the shrinking of these kinds of computational boundaries from the organ or whole-body scale to single cells. You can use this as a diagnostic modality, screening for cancer.

Depolarization of rare instructor cells causes transformation of melanocyte behavior:

Depolarization of selected cells induces a melanoma-like EMT phenotype:

- Shape change (arborization)
- MMP-dependent invasion of many organs (metastasis)
- Over-proliferation
- Vasculature changes

Hyperpigmented tadpoles (%)

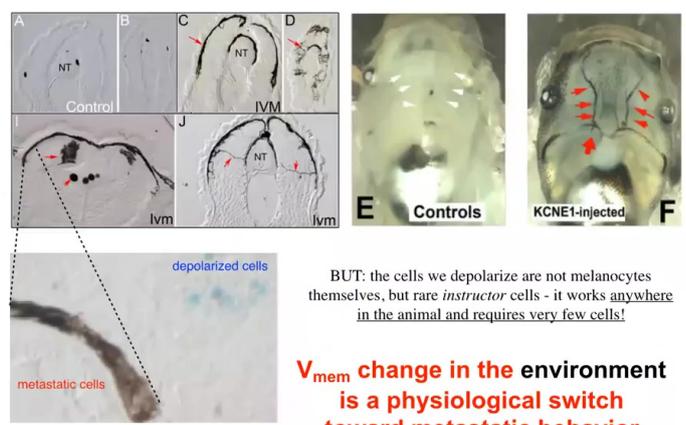
Group	Hyperpigmented tadpoles (%)
Control	~5
Depolarized	~100

Depolarized + MMP blocker

Michael Levin

Functionally, what's really important is that you can induce a conversion of normal melanocytes. So here they are. These little black dots are pigment cells; they're melanocytes. If you depolarize a very specific cell population in the animal, not the melanocytes themselves, but a different cell population, we call them instructor cells. Those cells tell the melanocytes what to do, in particular to stay nice, cooperative melanocytes under control. If you block their ability to signal, what happens is metastatic melanoma.

Depolarization of instructor cells causes melanocytes to become more arborized and invade numerous tissues



E Controls **F KCNE1-injected**

G depolarized cells
metastatic cells

BUT: the cells we depolarize are not melanocytes themselves, but rare *instructor* cells - it works anywhere in the animal and requires very few cells!

V_{mem} change in the environment is a physiological switch toward metastatic behavior

Michael Levin

These normal melanocytes convert to these crazy, long stringy things that start to crawl away. They dig into the brain, they dig into the neural tube, they start to invade. Here they are all through the blood vessels. This is like a melanoma-type behavior. You can see here, these blue cells, these are the ones that we actually depolarized. They are the ones that are now failing to keep the normal melanocytes behaving correctly. The melanocytes are going on their own.

So it's a voltage change in the environment. There's nothing genetically wrong with these animals. There are no oncogenes, no carcinogens. But this voltage change is a physiological switch away from the cooperation of cells toward embryogenesis and toward single-cell behavior.

Bioelectric state overrides genetic mutation (e.g., KRAS) in tumorigenesis - suppression and reprogramming

Tumor formed

Tumor suppressed

Morphology

Oncogene

Oncotarget
Volume 7, Number 15
May 15, 2016

Use of genetically encoded, light-gated ion translocators to control tumorigenesis

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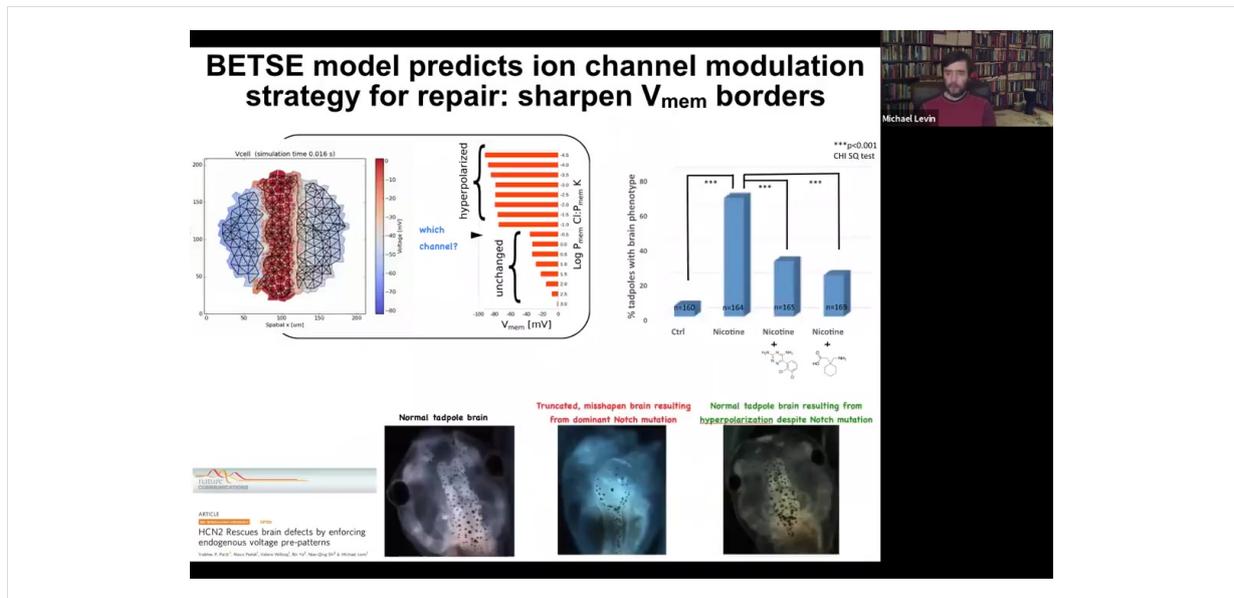
Keywords: Bioelectricity, voltage, KAS, optogenetics

Received: November 22, 2015 **Accepted:** February 11, 2016 **Published:** March 16, 2016

You can override this. If we inject an oncogene and they make these tumors, they're labeled with the red fluorescent protein. You can co-inject a channel and force these cells to stay electrically coupled to their neighbors despite what the oncogene is trying to get them to do.

Dominant negative KRAS, p53, while blazingly expressed, there's no tumor. It's the same animal. There's no tumor because these cells are coupled to this electrical network that forces them towards a proper tissue fate.

That's where our cancer applications are going: we've done this with light, with optogenetics, and now we're in human cells to try to use this to target carcinogenic behavior.



The other thing that is important is to think about the level of control that's possible with this. Here is a normal tadpole brain. You see forebrain, midbrain, and hindbrain. If you, for example, introduce a mutated Notch protein. Notch is a very important neurogenesis gene. You can mutate it and introduce a dominant mutation. You can see forebrain is basically gone, midbrain and hindbrain are just a bubble. These animals have no behavior to speak of. They lie there, they do nothing. You can do the same thing with alcohol, nicotine, various teratogen exposures.

You can build a computational model. This is the work of Alexis Pytak, who's a staff scientist in my group, and a few collaborators. You can build a computational model that asks a simple question: what determines the shape and size of the brain? There's a particular bioelectrical pattern that's required for the brain. On these backgrounds of teratogens or even Notch mutation, you can ask the model a question. You can say, if this pattern is disturbed, such as it is here, what channels would I have to open or close to get back to the correct pattern? The model told us there's a particular channel, HCN2, which can help us sharpen these boundaries. Even though there are massive defects in the Notch signaling and with these various other pathways, you can still reinforce these boundaries by opening HCN2. Sure enough, if you do it with drugs or just misexpress HCN2, you can get back to a normal brain shape, a normal brain gene expression, and in fact, normal IQ. These animals get their behaviors back and their learning rates back.

So here's what this is: using a very specific computational model to rationally manipulate the electrical signaling to get back to a very complex organ morphogenesis.

Electroceuticals beyond electrodes + CNS

Simulator -> exploiting specificity of channel expression with human-approved ion channel drugs for targeted V_{mem} state induction

ion channel expression data

what V_{mem} pattern/state is desired?

Cell Physiology, Tissue Physiology, Final Preparation

BETSE simulator

design cocktail of channel openers/blockers

global or meso-local application

SIGMA-ALDRICH

Michael Levin

<https://science.sciencemag.org/content/347/6200/1300419.full>

The whole roadmap looks like this. We have expression data, profiling data on which tissues in the body express which channels. That's known.

Electroceuticals beyond electrodes + CNS

Simulator -> exploiting specificity of channel expression with human-approved ion channel drugs for targeted V_{mem} state induction

ion channel expression data

what V_{mem} pattern/state is desired?

Cell Physiology

Tissue Physiology

Final Depattern

BETSE simulator

design cocktail of channel openers/blockers

global or meso-local application

SIGMA-ALDRICH

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This needs a lot more work: if you knew what the voltage pattern was supposed to be in various healthy tissues under various conditions. Physiomic profiling is needed. Then we have this computational simulation engine, which is able to say, if you want to go from the incorrect pattern to the correct pattern, which of these channels you would have to open and close. That tells you immediately what cocktail of ion channel drugs you would need to use.

Software Tools for Electroceutical Design

What channels to open and close, to get desired system-level bioelectrical state?

<http://34.215.62.180/db/>

What ion flows would repair the physiological state?

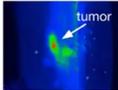
Which channels/pumps can be targeted to achieve desired ion flows?

What drugs are available to implement the intervention?

w/ Jack Tuszynski and Philipp Winter

EDeN - Electroceutical Design Environment

A tissue specific ion channel database



tumor

1. Select one or more tissues:

Normal tissues:

- bronchus
- temporal lobe
- testis
- thalamus
- thymus
- thyroid gland
- tongue
- testis
- trachea
- urinary bladder
- uterine cervix
- uterine endometrium
- uterus
- zadax nerve
- adipose tissue

Cancer tissues:

- colorectal adenocarcinoma cell
- breast cancer cell
- ovarian carcinoma cell
- thyroid cancer cell
- testicular cancer cell
- gastric cancer cell
- glioma cell
- lung cancer cell
- pancreatic cancer cell
- liver cancer cell
- uterine adenocarcinoma cell
- K562 cell
- kidney cancer cell
- carcinoid cell
- HL-60 cell

EDeN platform

Ion channel expression profiling



Database of ion channel drugs

2. Show ion channels with mRNA expression level greater than 10

3. Select comprehensive to show all channels expressed in the selected tissues (ordered by most Select Unique to show only channels that are specific to the selected tissues.)

4. Select one or more ion channels:

- Sulfonylurea receptor; K-ATP channels (Q15842|KCNJ8)
- Transient receptor potential cation channel subfamily M member 7 (Q96Q74|TRPM7)
- Transmembrane protein 102 (Q99010|TMEM102)
- Vesicle-associated membrane protein 2 (P63027|VAMP2)
- Voltage-dependent anion-selective channel protein 1 (P21796|VDAC1)
- Voltage-dependent calcium channel gamma-like subunit (Q94X54|TMEM37)
- Voltage-gated L-type calcium channel (Q05801|CACNA1F)
- Voltage-gated L-type calcium channel (Q13701|CACNA1S)
- Voltage-gated L-type calcium channel alpha-1C subunit (Q13936|CACNA1C)
- Voltage-gated calcium channel (Q00355|CACNA1A)
- Voltage-gated calcium channel (Q13978|CACNA1E)
- Voltage-gated potassium channel (P48547|KCNK3)

And so we've developed this pipeline. You can play with it here and choose your tissue and so on. The idea is that something like 20% of all drugs are ion channel drugs. These form an incredibly convenient toolkit of what we call electroceuticals because if you have the right computational model, you can repurpose these; the human safety data is already available. People already take them for all kinds of uses, and they have massive applicability in tweaking bioelectrical signals.

Another nice thing about this approach is that once the bioelectric circuit has made its decision, the downstream steps — second messengers, gene expression, everything else — can run as long as you want. For example, in the case of the frog, a one-day, 24-hour application of a particular cocktail gives 13 months of leg growth. You don't have to micromanage that whole process. It's just figuring out what electrical state is going to shift the tissue towards a particular goal state that all of the cells are going to work towards.

Conclusions

- Physiological software layer between the genotype and the anatomy is a tractable target for biomedicine and synthetic bioengineering
- Evolution discovered very early that bioelectric signaling is a convenient medium for computation and global decision-making.
- Cracking the bioelectric code will reveal how cell networks make group decisions for anatomical homeostasis. We can now re-write patten memories in vivo to reprogram large-scale shape (heads, eyes, limbs).
- New AI tools are coming on-line for design of strategies for regenerative medicine of birth defects, cancer, and traumatic injury repair, and synthetic living machines: electroceuticals!



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I'm going to close and summarize as follows. There's this important layer of physiological decision-making that sits between the genotype and the anatomy. It's becoming a tractable target for biomedicine and synthetic bioengineering because we can now see how the electrical dynamics operating in that tissue encode particular anatomical layouts towards which the cells continue to build.

Evolution apparently discovered very early on that this electrical signaling is a convenient medium for computation and global decision-making. It's not an accident that all nervous systems use it and all of our computer technology uses it. Ion channels, especially the voltage-gated ion channels, are basically voltage-gated current conductances. They're transistors. That's a powerful architecture that evolution found long before we did for forming feedback loops and memory circuits.

We think that cracking this bioelectric code can help reveal how cell networks make decisions in large-scale anatomy, not just how individual cells decide what type of cell they're going to be, but the real question of growth and form. Where do these complex patterns reside? We can now rewrite some of these patterns in the planarian or in the frog. New machine learning tools are coming online to help us design strategies for all kinds of applications.

Thank you to:

Post-docs: **Al-Sun Tseng** - Celia H-Rincon, Nirosha Murugan - **limb regeneration**
Douglas Blackiston - brain-body interface plasticity, synthetic living machines
Yalbhav Fai - **voltage gradients in eye/brain induction and repair**
Patrick McMillen - bioelectrics and calcium signaling in development

Students: **Sherry Aw** - bioelectric basis of eye development
Maya Emmons-Bell - planarian trans-species physiological reprogramming
Fallon Durant - V_{mem} and pattern memory in planarian regeneration
Brook Chernet, Maria Lobkin - V_{mem} and control of cancer

Technical support:
Rakela Colon, Jayati Mandal - lab management
Erin Switzer - vertebrate animal husbandry
Junji Morokuma - planarian molecular biology
Joan Lemire - molecular biology

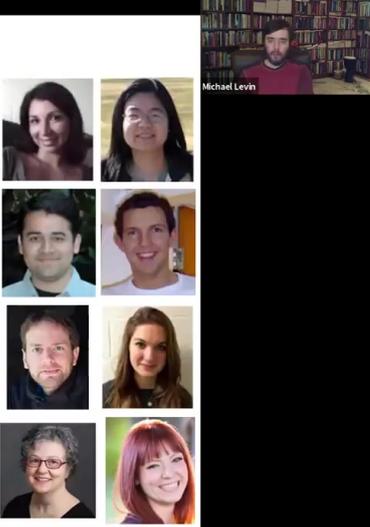
Collaborators: **Allen Center members +**
Joshua Bongard, Samuel Kriegman - **computer modeling of synthetic organisms**
Alexis Pietak, Salvador Mafe, Javier Cervera - **computer modeling of bioelectrics**
Dany Adams - bioelectrics of craniofacial patterning
David Kaplan - V_{mem} and human MSC differentiation, regenerative bioreactors
Giovanni Pizzullo, Francisco Vico - cognitive science models of pattern regulation
Vitaly Volpert, Chris Fields - mathematical models of pattern regulation
Paul C. W. Davies, S. I. Walker, Karl Friston - top-down causation models
Don Ingber, Richard Novak, J. H. Dungan - mammalian bioengineering
Jack Tuszynski - biophysics/chemistry modeling

Model systems: tadpoles, planaria, zebrafish, slime molds, ants, chick embryos, Xenobots

Funding support:
Paul G. Allen Frontiers Group, TWCF, Mathers Foundation, NIH, NSF, DARPA

Illustrations: Jeremy Guay @ Peregrine Creative, Calvin Bradbury-Jost, Alexis Pietak

Disclosure:
Morphochemicals Inc.



I would like to thank the people in my group who did the work. These are all the postdocs and students and others who did the work that I showed you today. Here are some of our collaborators who are working with us.

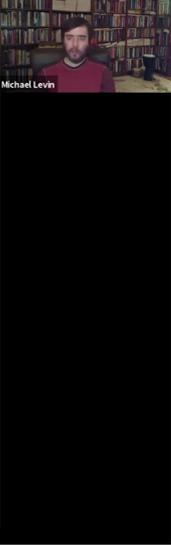
The model systems that we work with are all kinds of animals. And our funders — very grateful to them. Disclosure: Morphochemicals Inc. is the company for our frog limb regeneration work.

Impossible Biological Objects:



**2-head planaria continue to result from cuts in plain water:
software-level, modular control with no genomic editing**

<http://www.dr-michaellevin.org>
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In the end, I always like to show a video of these two-headed animals. The first time I reported these data at a meeting, someone stood up and said, "That's impossible, those animals can't exist." Now I make sure that I bring a video.

Thank you for reading.

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