REGENERATION

Cross-body communication is electric in the froglet

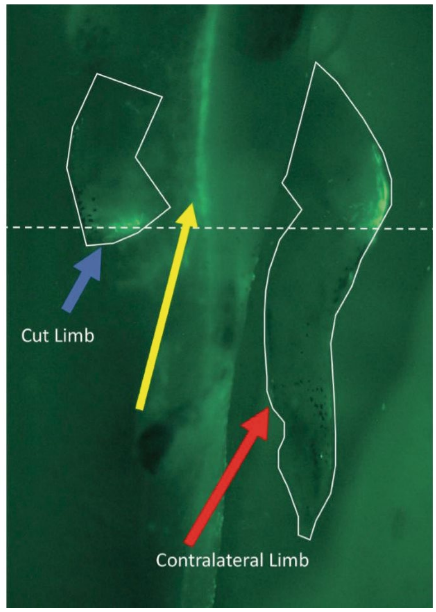
Busse, S.M., McMillen, P.T., and Levin, M. *Development* **145**, dev164210 (2018)

Michael Levin and his lab at Tufts University in Boston, Massachusetts study computation in living media. "The idea is to understand how cells, tissues, organs, and entire organisms make decisions," Levin says. Consider regeneration as one example. In the animals that are capable of regenerating lost limbs or organs, their cells and control networks need to know a few things: first, that there's been some kind of damage; second, how to go about rebuilding the missing structures to a precise anatomical specification; and finally, when to stop. "All of this requires cells to make decisions about things much larger than themselves," he says.

One of the models that Levin's lab uses to study regeneration is the African clawed frog, *Xenopus laevis*. The animals are capable of regenerating their hind limbs, but only for a short time while they are very young froglets—tadpoles with legs. As they complete their metamorphosis into adult frogs, they lose the ability, unlike other popular amphibious regeneration models like the axolotl. "The frog is actually more similar to humans in that respect," says Levin.

Recently, Levin tasked an undergraduate student working in his lab, Sera Busse, with recording electrical signals after experimental amputations in regenerative froglets and non-regenerative older animals. One means of intercellular communication is electrical, so Busse's work was intended to identify the electrical changes that occur during hind limb regeneration and whether and how those signals might be manipulated to influence the process. She soaked froglets in a solution of $DiBAC_4$, a voltage-sensitive fluorescent dye that lights up when the charge of a cell changes, amputated part of one hind limb, and visualized the results in the cut leg; the intact contralateral hind limb was intended to serve as a control for the experiments.

Busse saw light indicating depolarization, but in both limbs. She could have just subtracted out the signal observed in the intact leg and continued on, Levin says, but instead she brought up the peculiarity for a closer look. The fluorescent signal was indeed observable in the contralateral leg within 30 seconds of the amputation and closely mirrored the pattern observed in what remained of the cut leg.



Similar patterns of depolarization in the cut (left) and intact (right) hind limb of a *Xenopus laevis* froglet. Credit: Sara Busse, Patrick McMillen, Michael Levin – Tufts University

Their original research plans were put on hold.

"We wanted to make sure that this was actually real," says Levin. He, Busse, and postdoctoral fellow Patrick McMillen recorded numerous measurements to confirm and quantify the pattern and timing of fluorescence in the amputated and contralateral limb, establish that amputation, not just injury, is needed to spark the effect, and check whether the cross-body signaling phenomenon they were observing was specific to the animals' limbs or if it could also occur in other paired organs, like the kidney or eye.

The speed with which the signal was transmitted from amputated side to intact was intriguing: it was too fast for diffusion of molecules but too slow for neurons, Levin says. They experimentally removed a section of spinal cord to confirm that the central nervous system wasn't playing a role. One likely explanation is electrical signaling across cells, such as those in the frogs' epidermis. The animals aren't transparent, limiting the reporting ability of the dye to just the top few layers of skin. "The fact that we can see this at all is remarkable," say Levin. "If this was happening in the middle of the limb, we would never have seen it."

In most froglets, the intercellular electrical signal was traveling long distances across the body without losing the spatial content that it carried. "What made it really quite incredible was how specific the information was," says Levin, "By looking at the contralateral leg you could tell where the damage occurred because the positioning of the signal was directly related to where the cut occurred."

The phenomenon, which they dub "bioelectric injury mirroring," has two larger implications, suggests Levin. If it turns out to have a functional role in the animals, it could be tapped for long-range interventions, since electrically manipulating cells in one part of the body could influence outcomes in other areas as well. "We're going to dig more into the mechanism," says Levin. "We're going to learn everything we can in the frog model and then once we get to the point where we know enough to be able to design functional interventions, at that point we'll go into rodents."

It also has the potential to be used as surrogate site diagnostic tool to "understand what's happening at one part of the body by taking measurements in another part," he says. "We are in the process of starting another project specifically to test this out much more broadly."

The possibility of bioelectric injury mirroring also merits a word of caution about the use of contralateral controls. "I do think people have to be very careful about using the contralateral side and drawing conclusions as if it were un-manipulated," says Levin. "It hasn't been cut, but that doesn't mean it's the equivalent of a completely baseline, wild-type situation."

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