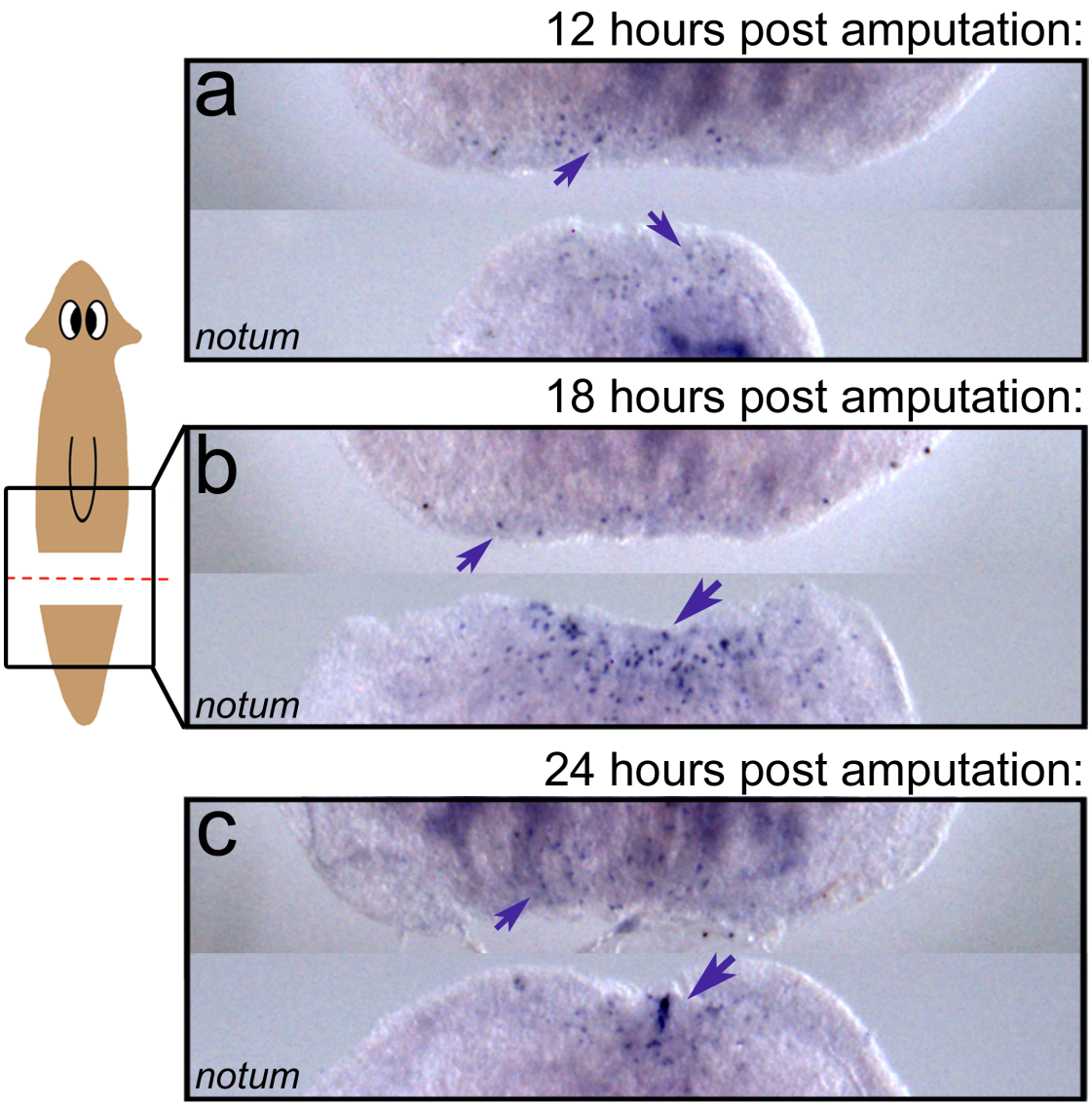
Biophysical Journal, Volume *116*

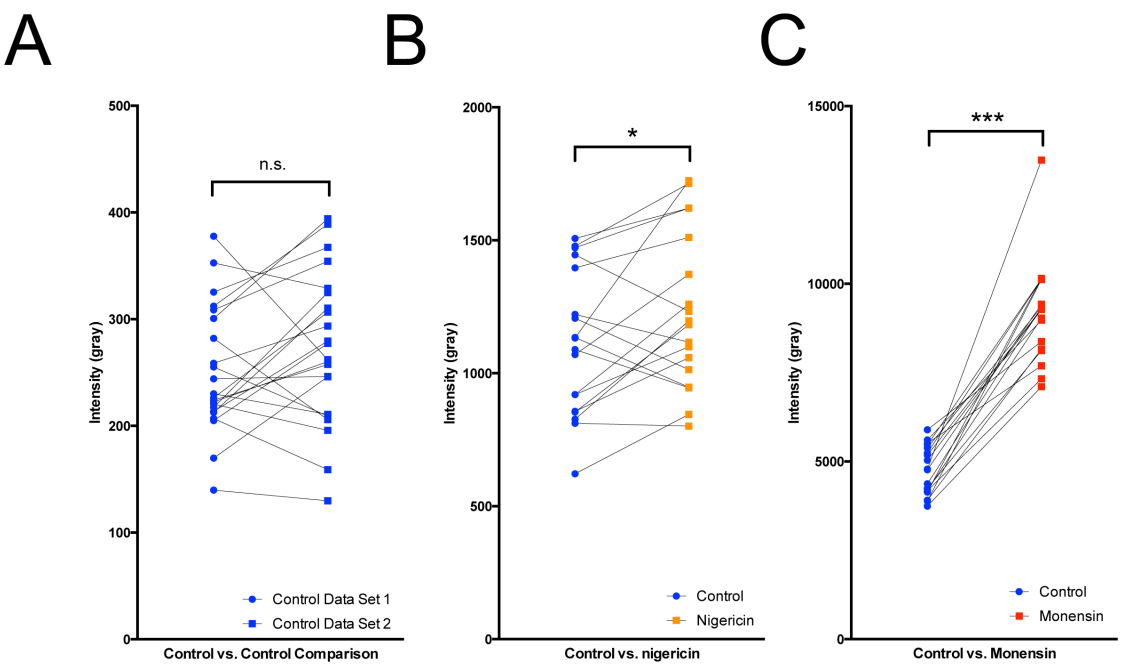
Supplemental Information

The Role of Early Bioelectric Signals in the Regeneration of Planarian Anterior/Posterior Polarity

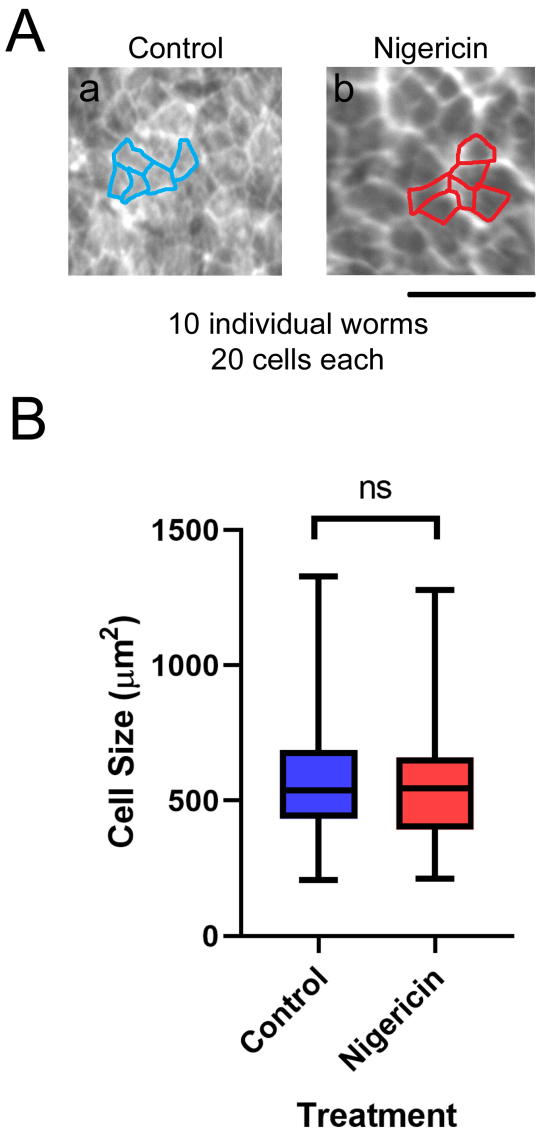
Fallon Durant, Johanna Bischof, Chris Fields, Junji Morokuma, Joshua LaPalme, Alison Hoi, and Michael Levin



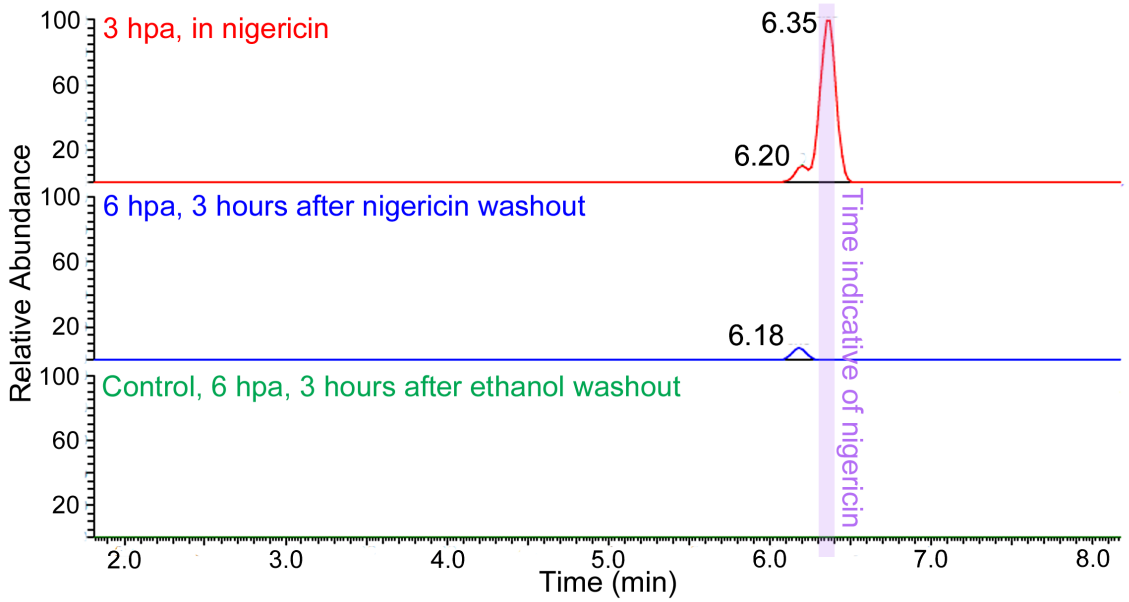
Supplemental Figure 1. *Notum* expression at later timepoints of regeneration. Timeline indicating *notum* expression in WT regenerating DJ at (a) 12 hours, (b) 18 hours and (c) 24 hours post-amputation, as determined by *in situ* hybridization. Amputation plane indicated in red on the sketch. Each panel representative of a timepoint includes the posterior wound site of the anterior portion of an amputated worm (top) and the anterior wound site of the posterior portion of an amputated worm (bottom). Purple arrows indicate punctate expression pattern. Scale bars represent 1 mm throughout.



Supplemental Figure 2. Alternate quantification of Figure 2Bg and 2Cg. Re-quantification of the data used in Figures 2Bg and 2Cg, now directly comparing pairs of worm fragments imaged on the same platform. Data indicates overall average $DiBAC_4(3)$ fluorescence intensity of pairs of (A) two control fragments (n=22 pairs, blue, $p>0.05$, paired t-test), (B) pairs of one nigericin-treated fragment (orange) with a side-by-side control (n=18 pairs), and (C) pairs of one monensin-treated fragment (red) with a side-by-side control (n=18 pairs) all 3 hours post amputation. Paired samples are indicated with a line. n.s.: not significant, * $p<0.05$, *** $p<0.001$.



Supplemental Figure 3. Control of cell size after nigericin treatment. (A) Cell membranes stained with SS44 dye and epidermal cells imaged on the dorsal side of the animal in fragments treated with (a) control solution and (b) nigericin solution, showing a few example outlines traced (20 cells per animal in 10 animals were measured for the quantification). (B) Quantification of the cell sizes measured in the control and nigericin-treated fragments, showing no significant difference in cell size ($p>0.05$, N=200, unpaired t-test). Scale bar represents 50 μM.



Supplemental Figure 4. Washout of the ionophore. (A) Liquid chromatography mass spectrometry indicating the presence of nigericin. Peak at 6.35 min indicates presence nigericin (lavender region). Peak is clearly defined in animals soaking in nigericin solution at 3 hours post-amputation (red). By 6 hours post-amputation (3 hours post washout) nigericin is no longer present (blue) similar to corresponding 3 hour (brown) and 6 hour (green) corresponding ethanol controls. Peaks seen at 6.20 and 6.18 minutes are other compounds having ions in a similar m/z range. These signals are low and can be considered noise.

As ΔV depends sensitively on the extent to which the fragment polarization profile is amplified by the wound Ca^{2+} response toward the V_{mem} profile of the intact animal as discussed above, the predicted Head - Tail branching ratios also depend on this parameter, as illustrated by the example results shown below (all calculations were performed using the model implementation available at https://chrisfieldsresearch.com/bcar-model.htm). A full-trunk fragment has an anterior cut much closer to the head that the PT fragments used in the experiments reported here; hence it requires less amplification to return to the intact-animal V_{mem} profile.

Supplementary Table 1: Example model results showing nonlinear dependence of Head - Tail branching ratio as a function of amputation-fragment polarization amplification for a full trunk fragment (anterior cut at 20% of animal length, posterior cut at 80% of animal length) with all other model parameters at default values.

Polarization Amplification [%]	Anterior Wound		Posterior Wound	
	Heads [%]	Tails [%]	Heads [%]	Tails [%]
10	20	80	0	100
20	39	61	0	100
30	66	34	0	100
40	90	10	0	100
50	100	0	0	100
…				
100	100	0	1	99

Supplementary Table 2: Example model results showing nonlinear dependence of Head - Tail branching ratio as a function of amputation-fragment polarization amplification for a PT trunk fragment as employed in the experiments reported here (anterior cut at 60% of animal length, posterior cut at 80% of animal length) with all model parameters at default values.

Polarization Amplification [%]	Anterior Wound		Posterior Wound	
	Heads [%]	Tails [%]	Heads [%]	Tails [%]
10	0	100	0	100
20	0	100	0	100
30	0	100	0	100
40	1	99	0	100
50	2	98	0	100
60	6	94	0	100
70	35	65	0	100
80	98	2	0	100
90	100	0	1	99
100	100	0	1	99