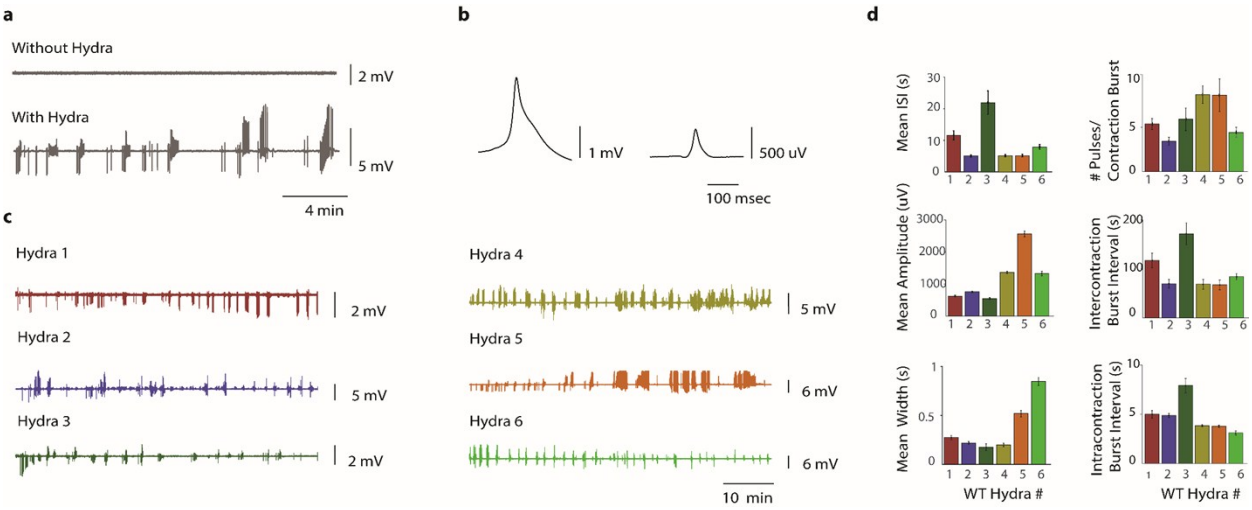
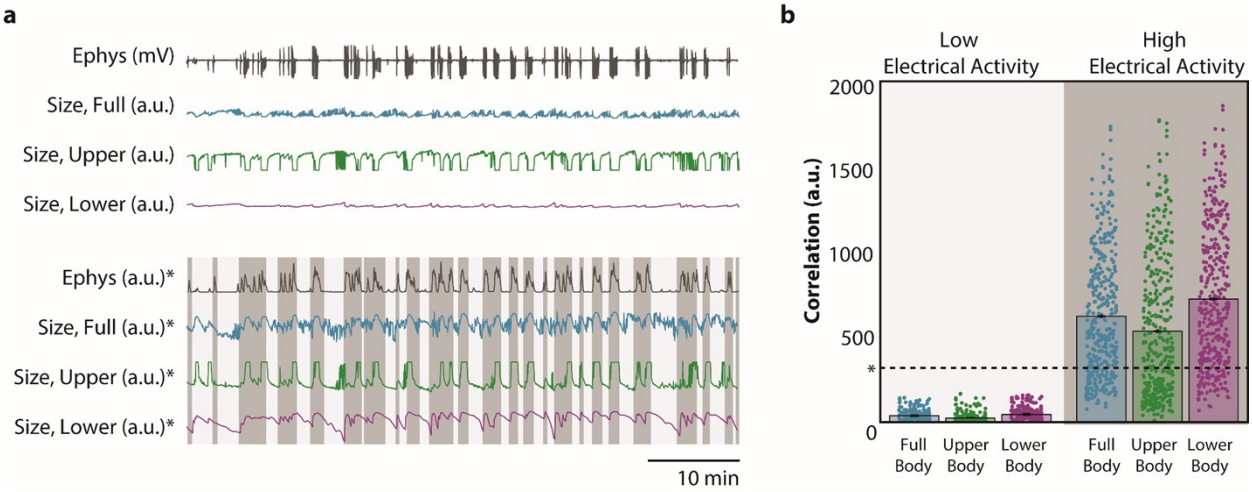
Electronic Supplementary Material (ESI) for Lab on a Chip.

SUPPLEMENTARY INFORMATION

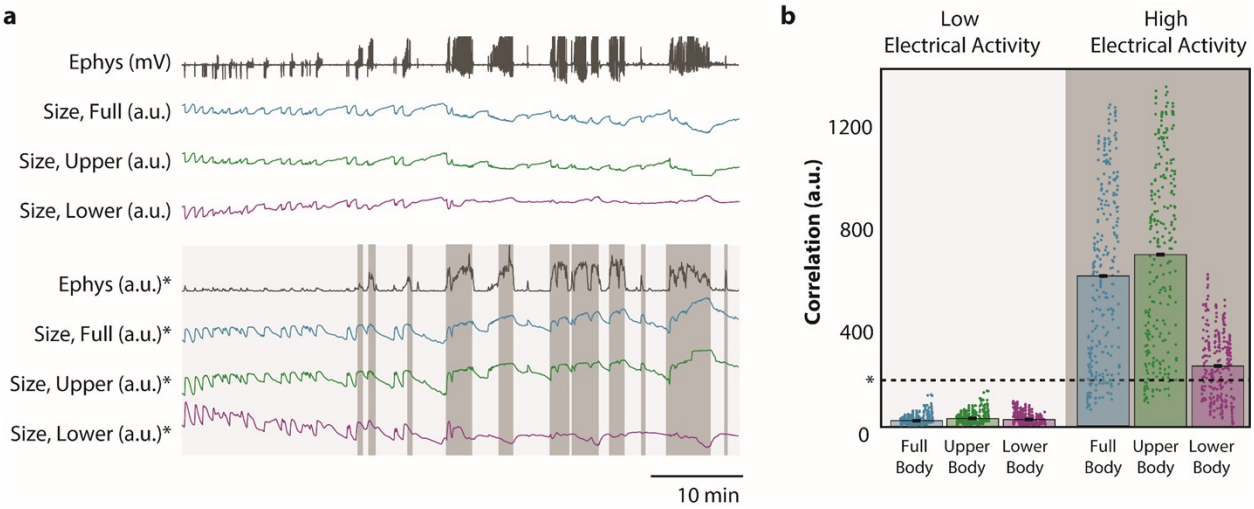
Supplementary Figures



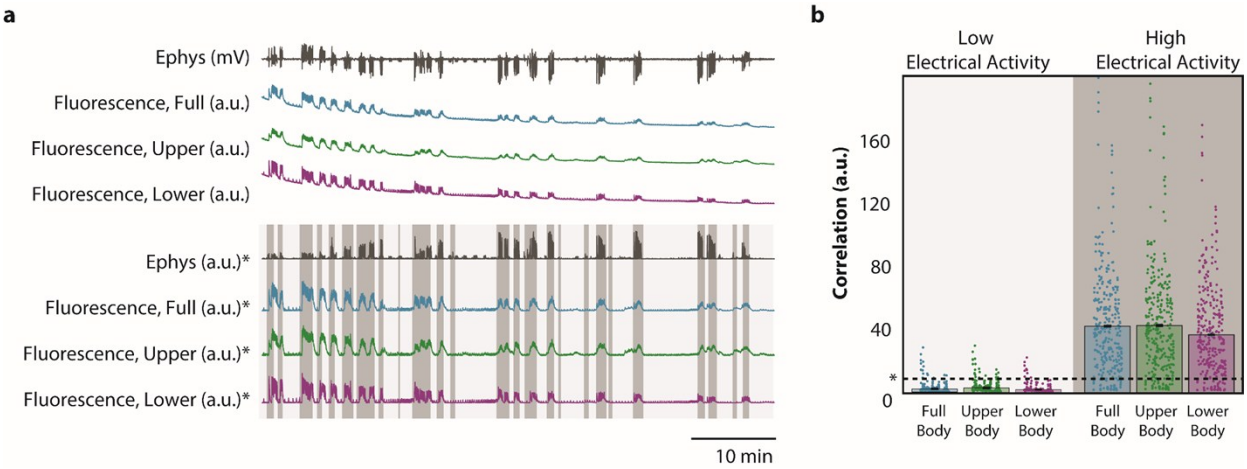
Supplementary Figure 1: Analysis of electrophysiology of *Hydra*. (a) Shows twenty minutes of electrical measurements from nano-SPEAR electrode with and without *Hydra* immobilized. **(b)** Peak-aligned average small and large amplitude waveforms determined by PCA clustering. **(c)** 1 hour long electrical recording from six individual WT *Hydra vulgaris* AEP. **(d)** Shows contraction burst activity across six individual *Hydra* with analysis of large amplitude waveforms. Mean ISI is the time between large amplitude spikes. Mean amplitude is the absolute value of peak amplitude for large large amplitude spikes. Mean width is the average spike duration. #pulses/contraction bursts is number of individual large amplitude spikes from a single contraction burst event. Intercontraction Burst Interval is the time between contraction events. Intracontraction Burst Interval is the time between individual spike occurances in a single contraction burst event.



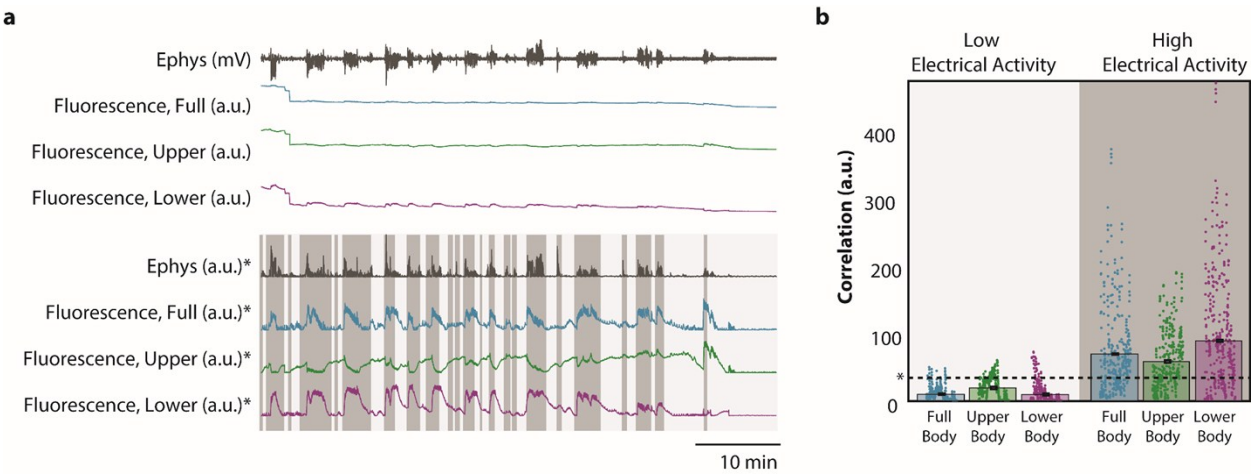
Supplementary Figure 2: Correlation analysis of electrophysiology and movement of *Hydra*. (a) Shows sixty minutes of electrical measurements from nano-SPEAR electrode and the animal body size (number of pixels comprising the whole body or body segments). Change in entire body size or upper and lower portion of the body represent muscle movements (size decreases during contractions). The original data (shown on top) is normalized (shown on bottom) then separated into 30 s time intervals. Each interval is classified as high activity (dark gray) or low activity (light gray) periods based on the mean electrical activity during the interval. **(b)** The maximum value of the cross-correlation between the electrical measurements and movement for each interval yields correlation values for the time-aligned (unshuffled) data shown as dots. The mean cross-correlation value for each body segment is represented by bar. Error bars represent S.E.M. To test the statistical significance of the correlation values, the time intervals for all data sets are randomly shuffled to calculate correlation values from uncorrelated data. The correlation values that fell outside the 99% confidence interval calculated for randomized (shuffled) time intervals (represented by dashed line; * = 315 a.u.) are considered significant. Mean correlation values for high activity periods above the significance threshold are considered statistically significant.



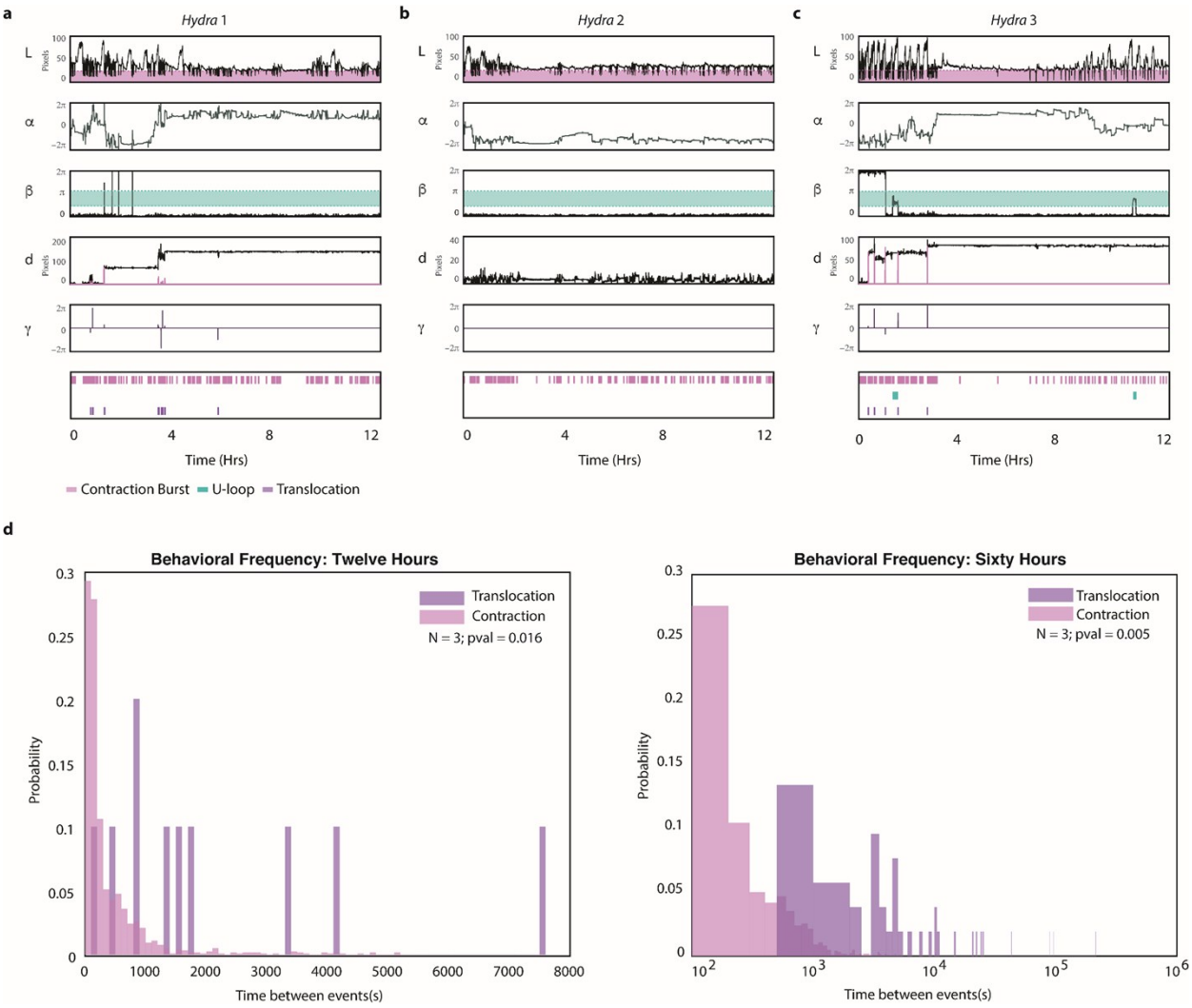
Supplementary Figure 3: Correlation analysis of electrophysiology and movement of Hydra. (a) Shows sixty minutes of electrical measurements from nano-SPEAR electrode and the animal body size (number of pixels comprising the whole body or body segments). Change in entire body size or upper and lower portion of the body represent muscle movements (size decreases during contractions). The original data (shown on top) is normalized (shown on bottom) then separated into 30 s time intervals. Each interval is classified as high activity (dark gray) or low activity (light gray) periods based on the mean electrical activity during the interval. (Note during animal elongations in the last 20 min of the recording, the entire body of the animal slowly shifted down and was partially outside of the imaging field of view.) (**b)** The maximum value of the cross-correlation between the electrical measurements and movement for each interval yields correlation values for the time-aligned (unshuffled) data shown as dots. The mean cross-correlation value for each body segment is represented by bar. Error bars represent S.E.M. To test the statistical significance of the correlation values, the time intervals for all data sets are randomly shuffled to calculate correlation values from uncorrelated data. The correlation values that fell outside the 99% confidence interval calculated for randomized (shuffled) time intervals (represented by dashed line; * = 175 a.u.) are considered significant. Mean correlation values for high activity periods above the significance threshold are considered statistically significant.



Supplementary Figure 4: Correlation analysis of electrophysiology and neural calcium imaging of *Hydra*. (a) Shows 60 minutes electrical measurements from nano-SPEAR electrode and neural calcium activity (average fluorescence) entire body size or upper and lower portion of the (fluorescence increases during contractions). The original data (shown on top) is normalized (shown on bottom) then separated into 30 s time intervals. Each interval is classified as high activity (dark gray) or low activity (light gray) periods based on the mean electrical activity during the interval. (**b)** The maximum value of the cross-correlation between the electrical measurements and movement for each interval yields correlation values for the time-aligned (unshuffled) data shown as dots. The mean cross-correlation value for each body segment is represented by bar. Error bars represent S.E.M. To test the statistical significance of the correlation values, the time intervals for all data sets are randomly shuffled to calculate correlation values from uncorrelated data. The correlation values that fell outside the 99% confidence interval calculated for randomized (shuffled) time intervals (represented by dashed line; * = 10 a.u.) are considered significant. Mean correlation values for high activity periods above the significance threshold are considered statistically significant.



Supplementary Figure 5: Correlation analysis of electrophysiology and neural calcium imaging of Hydra. (a) Shows 60 minutes electrical measurements from nano-SPEAR electrode and neural calcium activity (average fluorescence) entire body size or upper and lower portion of the (fluorescence increases during contractions). The original data (shown on top) is normalized (shown on bottom) then separated into 30 s time intervals. Each interval is classified as high activity (dark gray) or low activity (light gray) periods based on the mean electrical activity during the interval. (Note the large decrease in fluorescence in raw data was due to a change in excitation light.) **(b)** The maximum value of the cross-correlation between the electrical measurements and movement for each interval yields correlation values for the time-aligned (unshuffled) data shown as dots. The mean cross-correlation value for each body segment is represented by bar. Error bars represent S.E.M. To test the statistical significance of the correlation values, the time intervals for all data sets are randomly shuffled to calculate correlation values from uncorrelated data. The correlation values that fell outside the 99% confidence interval calculated for randomized (shuffled) time intervals (represented by dashed line; * = 35 a.u.) are considered significant. Mean correlation values for high activity periods above the significance threshold are considered statistically significant.



Supplementary Figure 6: Behavioral analysis of *Hydra.* (a-c) Behaviors and locomotion patterns of three separate *Hydra* imaged simultaneously. Plots show change in body length (L), body orientation (α), difference orientation of top half and bottom half of the body (β), displacement of foot (d) and direction of foot displacement (γ). Shaded regions show threshold values used to generate raster plot. Contraction bursts are when body length falls within the shaded region. U-loop body bending is when β is within shaded region. Translocation event is when the displacement from start position (d, black) has sudden change. d) Histograms show distribution of time elapsed between successive translocation and contraction events for the first twelve hours (left) and the entire sixty hours (right) of imaging from N=3 animals. Translocation events are considered less frequent (longer time between events) than contraction events when P value is less than 0.05 using one-sided student's t-test with unequal variances.

Supplementary Movies

Supplementary Movie 1: Microfluidic Immobilization of *Hydra*

Shows *Hydra* inserted through entry port and immobilized at the pinch point in hour-glass microfluidic device.

Supplementary Movie 2: Simultaneous electrophysiology and brightfield imaging

Shows simultaneous electrophysiology and brightfield imaging in *Hydra*. Trace at the bottom shows the electrical activity coinciding with movements.

Supplementary Movie 3: Simultaneous electrophysiology and fluorescence imaging, neurons

Shows simultaneous electrophysiology and calcium imaging of neurons in transgenic *Hydra*. Trace at the bottom shows the electrical activity coinciding with observed calcium activity.

Supplementary Movie 4: Brightfield imaging of mouth opening during chemical stimulation

Shows chemical stimulation of *Hydra* in wheel-and-spoke device. Labels at the top indicate flow conditions: i) no flow, ii) media flow, iii) GSH flow, and iv) media flow. Tentacles start to contract half way into GSH flow and mouth cavity later forms. *Hydra* loses rigidity after mouth opening and is easier to push aside with minimal flow conditions. Mouth begins closing and contractions slowly return half way into media flow following stimulation.

Supplementary Movie 5: Calcium imaging of mouth opening during chemical stimulation

Shows fluorescence imagine of calcium indicator in neurons during chemical stimulation of transgenic *Hydra* (GCaMP6s, neurons) in wheel-and-spoke device. Labels at the top indicate flow conditions: i) media flow, ii) GSH flow, and iii) media flow.

Supplementary Movie 6: Time-lapse imaging of *Hydra* behavior in 2D

Time-lapse movie shows three individual *Hydra* behaving in separate chambers in evenly illuminated microfluidic arena. Simplified movement track is overlaid to show detected translocation events.