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**Non-linear elasticity and short-range mechanical coupling  
govern the rate and symmetry of mouth opening in Hydra**

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*Statement (if applicable):*

This research uses the freshwater invertebrate Hydra and therefore does not require IACUC approval.

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This paper involves new data generated in laboratory experiments and a computational model. Both, the data and the code associated with the manuscript have been deposited to Zenodo at: <https://doi.org/doi:10.5281/zenodo.10498855>

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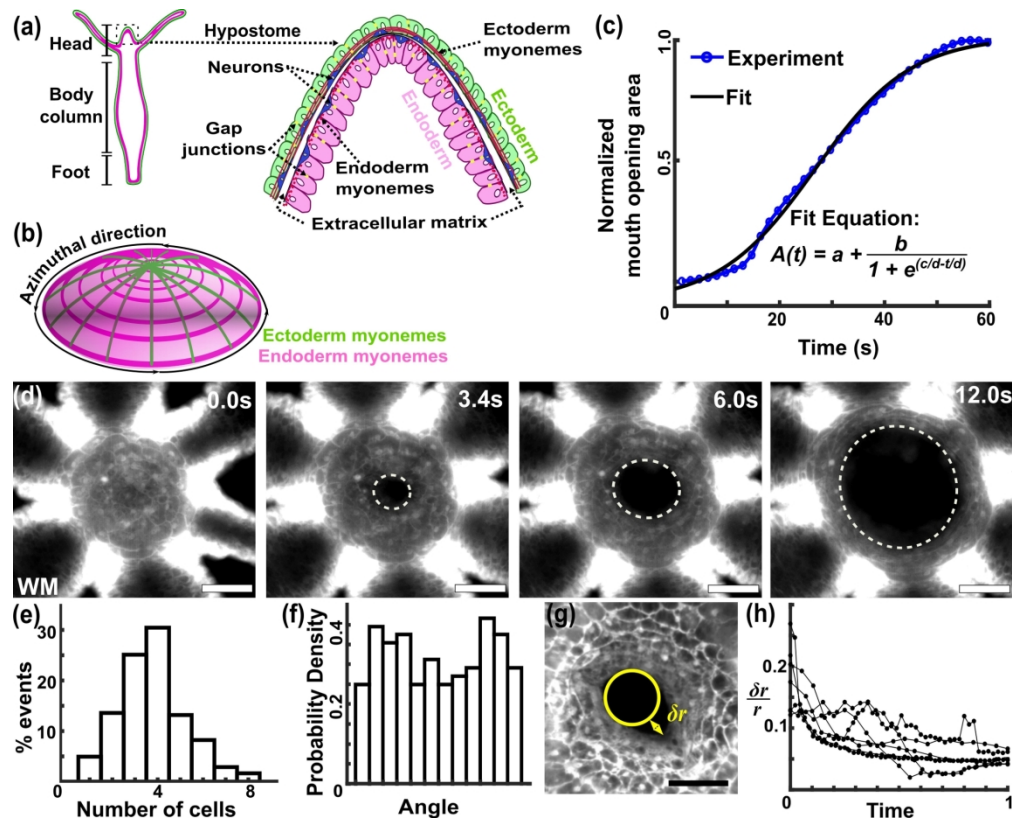


Figure 1: Hydra head anatomy and mouth opening dynamics. (a) Schematic showing basic Hydra anatomy and cell types including a zoomed-in cross-section of the hypostome. (b) Schematic top-down view of the myoneme organization in the ectoderm (green) and endoderm (magenta) in the Hydra head. (c) Area of the mouth (normalized to the maximum area) as a function of time. The blue curve shows representative experimental data. The black curve corresponds to a logistic fit as described by the equation on the right. 'a', 'b', 'c' and 'd' correspond to the minimum normalized area, maximum normalized area, time to reach half maximum and timescale of mouth opening respectively. (d) Still images from Movie S1 showing quinine hydrochloride (0.5 mM) induced opening of the ectodermal epithelial layer in a Watermelon (WM) Hydra (scale bar: 100  $\mu\text{m}$ ). (e) Histogram of number of cells involved in each tugging event ( $n = 364$  tugging events from  $N = 8$  independent events). (f) Probability density function for the relative angle between successive tugging events ( $n = 184$  tugging event pairs from  $N = 5$  independent mouth opening events). (g) Representative image of a radial tug during mouth opening induced by 0.2mM reduced glutathione (scale bar: 200  $\mu\text{m}$ ). (h) Time evolution of the relative fluctuation of the mouth radius,  $\delta r/r$ , averaged over the azimuth ( $N = 6$  mouth opening events). Time is normalized from the beginning of mouth opening to when the mouth reaches its maximum area.

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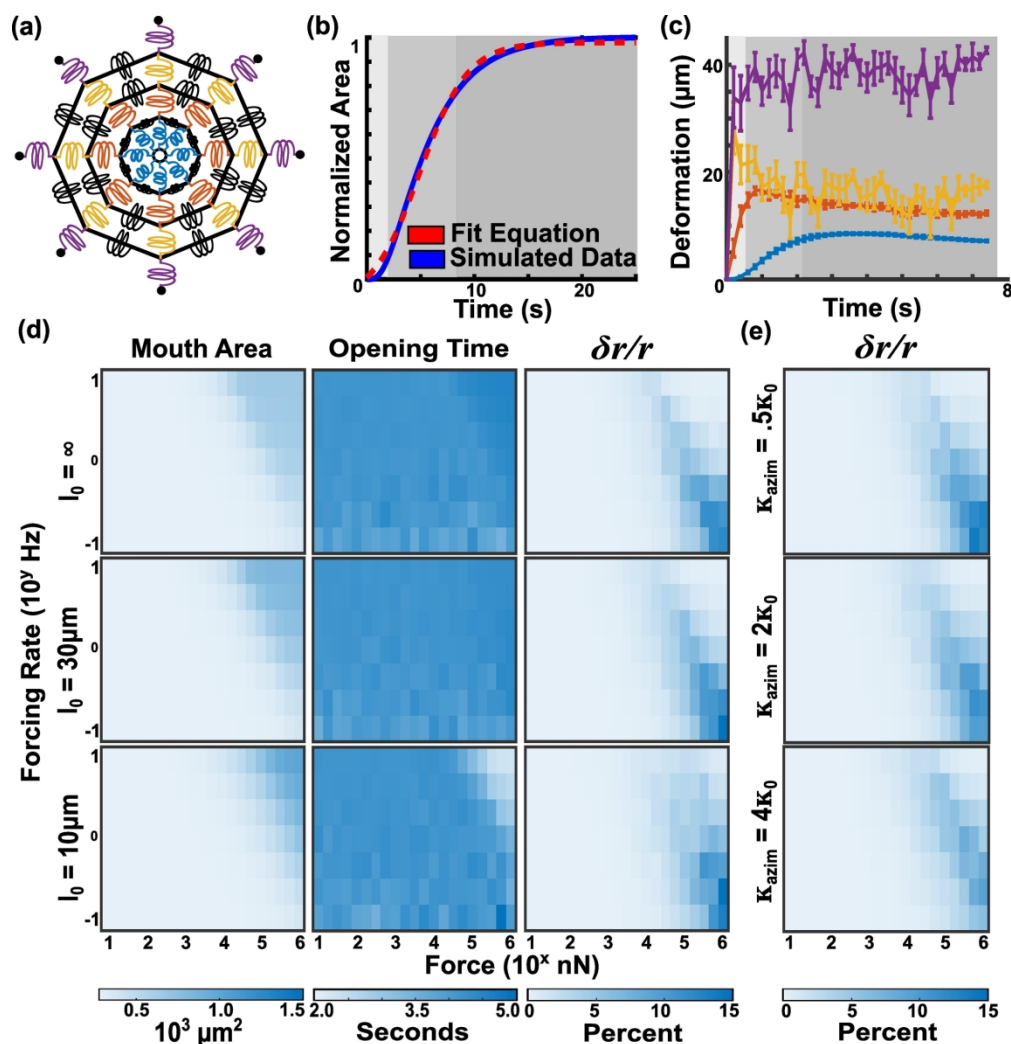


Figure 2: Non-linear spring network model captures macroscopic features of mouth opening. (a) Schematic of the spring network. Color-coding indicates distance from center. (b) Comparison between mouth opening time series obtained from model simulations (blue) and the logistic curve fit (red). Intensity of grey background denotes phases of mouth opening (from light to dark): slow increase, fast increase, and saturation. (c) Azimuthally averaged radial deformation of vertices in each N-gon. Line colors match those of the radial springs in (a). Grey background matches that of (b). (d) Variation of final mouth area (left column) fitted mouth opening time (middle column), and relative fluctuations in mouth radius,  $\delta r/r$  (right column), as functions of the active force strength (x-axis) and the average forcing rate (y-axis) for different non-linear regimes: without non-linearity (top), weak non-linearity (middle) and strong non-linearity (bottom). Note the axes have logarithmic scales. A darker blue color indicates larger mouth areas (left), longer opening times (middle) and larger relative fluctuations in mouth radius (right). (e) Changes in relative fluctuations in mouth radius with changes in the stiffness of the azimuthal springs relative to stiffness of radial springs ( $\kappa_0$ ).  $l_0 = 30 \mu\text{m}$  for all three heat maps. Parameters for all simulations along with descriptions and relevant sources can be found in Table S1.

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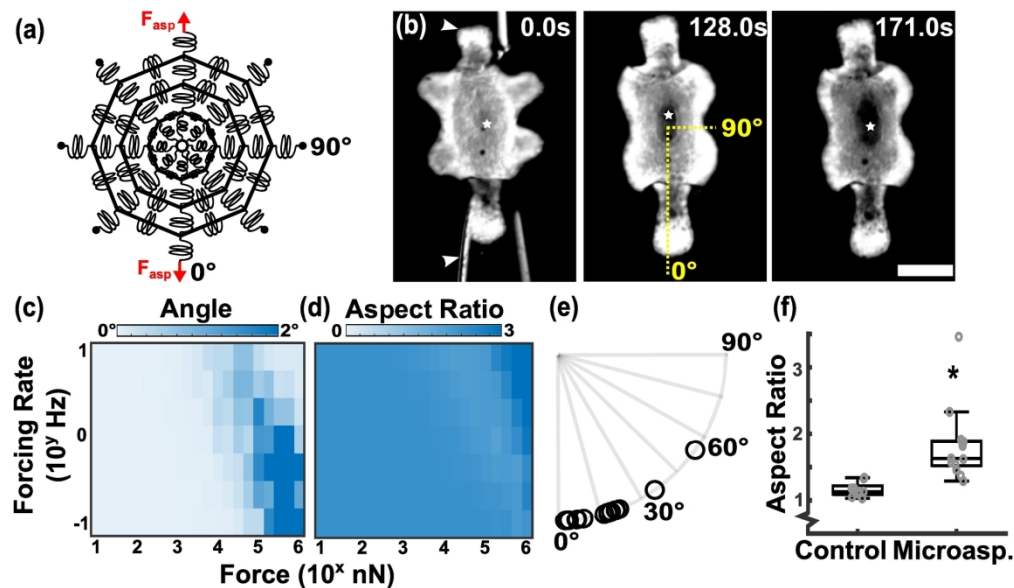


Figure 3: Application of external uniaxial tension affects symmetry of mouth opening. (a) Schematic of the hypostome under uniaxial tension.  $F_{asp}$  is the strength of the microaspiration force. (b) Still image sequence of mouth opening when the head is placed under uniaxial tension by applying negative pressure. Arrowheads indicate the location of needles used to apply negative pressure on the head. Star indicates the mouth opening (Scale bar:  $100 \mu\text{m}$ ). (c) Orientation angle of major axis (relative to the direction of tensile force) and (d) aspect ratio of best fit ellipse to the mouth, in simulations, as functions of the strength and rate of the active force ( $l_0=20\mu\text{m}$ ). (e) Orientation angle of major axis (relative to the direction of tensile force) ( $N = 13$ ) and (f) aspect ratio of best fit ellipse to the mouth, from experiments ( $N = 9$  (Control),  $N = 13$  (Microaspirated)). (\*) indicates  $p\text{-value} = 0.0006$  for a one-tailed two-sample Student's t-test.

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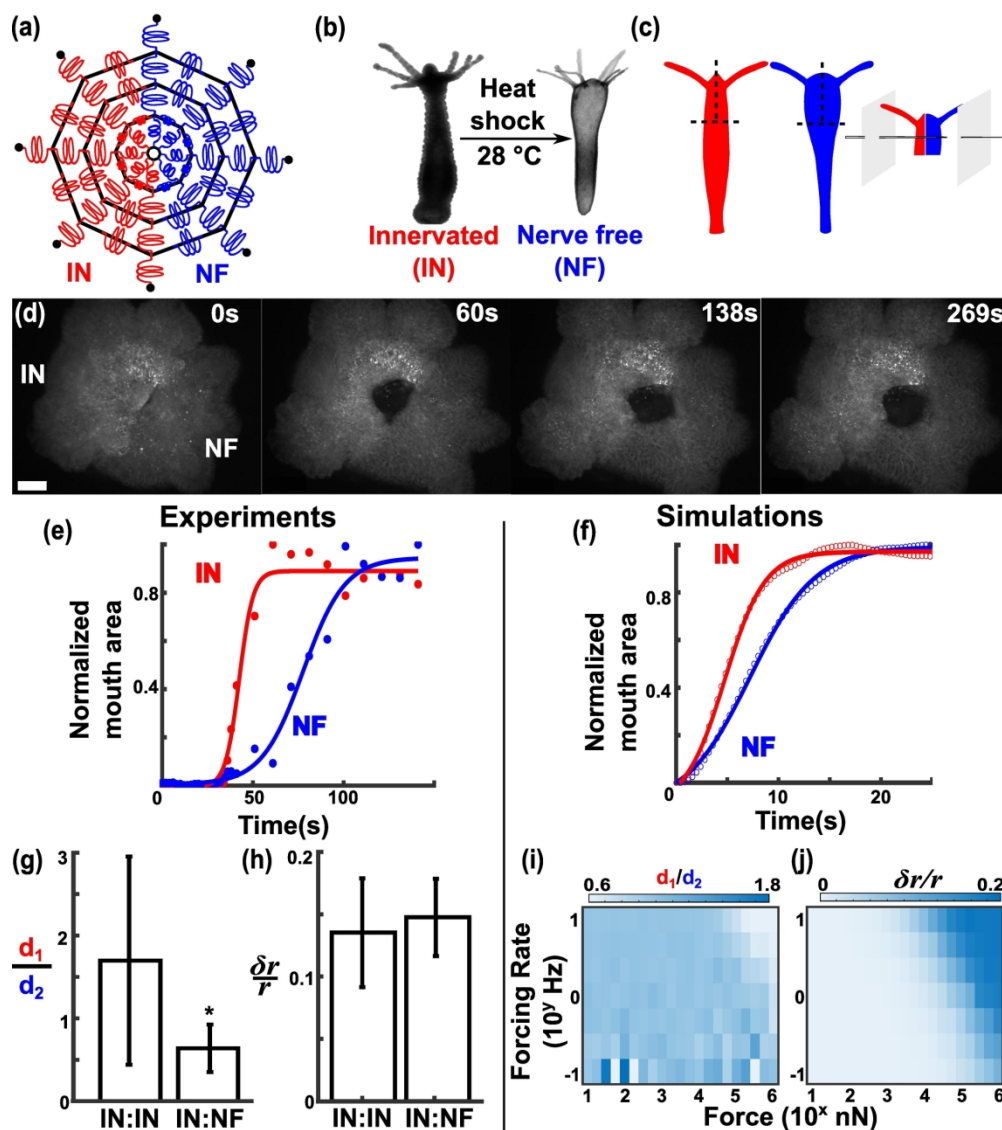


Figure 4: The nerve-net is required to initiate but not to execute mouth opening. (a) Schematic of the simulated chimera. (b) Greyscale images of innervated and nerve-free Hydra generated by heat shock treatment of the A10 strain. Note the nerve-free animal is more bloated at the oral end due to buildup of fluid. (c) Schematic showing the process of creating chimeras. (d) Still image sequence of mouth opening in an innervated:nerve-free chimera (scale bar: 100  $\mu$ m). Representative mouth area-time curves (e) in vivo and (f) in silico. Comparison of the (g) ratio of mouth opening times and (h) relative fluctuations in mouth radius between control chimeras (N = 6) and innervated:nerve-free chimeras (N = 9). (\*) denotes p-value < 0.05 (i) Ratio of mouth opening times and (j) relative fluctuations in mouth radius for innervated:nerve-free chimeras, in simulations, as functions of the strength and rate of the active force in the strongly non-linear regime ( $l_0=10\mu$ m). Simulation parameters provided in Table S1.

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1 **Non-linear elasticity and short-range mechanical coupling govern the rate and**  
2 **symmetry of mouth opening in *Hydra***

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13 **Keywords:** epithelium; viscoelastic; chimera; strain hardening; nearest-neighbor interaction

14 **Abstract**

15 Hydra has a tubular bilayered epithelial body column with a dome-shaped head on one end and a foot on the  
16 other. Hydra lacks a permanent mouth: its head epithelium is sealed. Upon neuronal activation, a mouth  
17 opens at the apex of the head which can exceed the body column diameter in seconds, allowing Hydra to  
18 ingest prey larger than itself. While the kinematics of mouth opening are well characterized, the underlying  
19 mechanism is unknown. We show that Hydra mouth opening is generated by independent local contractions  
20 that require tissue-level coordination. We model the head epithelium as an active viscoelastic non-linear  
21 spring network. The model reproduces the size, timescale, and symmetry of mouth opening. It shows that  
22 radial contractions, travelling inwards from the outer boundary of the head, pull the mouth open. Non-linear  
23 elasticity makes mouth opening larger and faster, contrary to expectations. The model correctly predicts  
24 changes in mouth shape in response to external forces. By generating innervated:nerve-free chimera in  
25 experiments and simulations, we show that nearest-neighbor mechanical signaling suffices to coordinate  
26 mouth opening. Hydra mouth opening shows that in the absence of long-range chemical or neuronal signals,  
27 short-range mechanical coupling is sufficient to produce long-range order in tissue deformations.

28

29 **Introduction**

30 Epithelial tissues experience extreme deformations, such as bending, stretching, and compression.  
31 Withstanding these deformations is essential for maintaining tissue integrity and physiological function.  
32 Mouth opening in the freshwater cnidarian *Hydra* is a physiologically relevant process to examine the role  
33 of extreme deformations for epithelial tissue function. *Hydra* lacks a permanent mouth and creates a new  
34 mouth opening in the apex of its head epithelium every time it needs to feed, egest, or vent fluid from its  
35 body cavity. A previous study (1) revealed the kinematics of *Hydra* mouth opening (**Figure 1**): a) The mouth  
36 area grows as a logistic function of time (S-shaped curve) over seconds to tens of seconds, b) the initial mouth  
37 opening is asymmetric but as opening progresses the circularity of mouth opening increases, and c) the mouth  
38 opening is due to extreme cell deformations, with azimuthal cell strains of up to 200% and no cell  
39 rearrangements (1) (**Figure S1** and **Movie S1**). This behavior is in contrast to the large-scale tissue

1

40 deformations observed during development which rely on cell migration on minute to hour long timescales  
41 (2). Thus, as reviewed in (3), *Hydra* mouth opening is an excellent *in vivo* system to study how cell-level  
42 deformations give rise to organismal level behaviors.

43 *Hydra* consists of a cylindrical body column, about 1 cm long and a few hundred microns in diameter, with  
44 a foot at one end and a dome-shaped head surrounded by a ring of tentacles at the other end (**Figure 1a**). As  
45 a cnidarian, *Hydra* lacks a mesoderm: it is composed of two epitheliomuscular cell layers, an outer ectoderm  
46 and inner endoderm, separated by an extracellular matrix (ECM) (**Figure 1a**). The epithelial cells have  
47 epitheliomuscular processes (myonemes) that originate from the basal side of the epithelial cells and extend  
48 into the ECM (4). Individual myonemes only span over 1-2 cells (4-6). However, when visualized using  
49 phalloidin staining, myonemes appear as long fibers on a tissue scale (1). In the endoderm, myonemes are  
50 organized in concentric circles in both body column and the head (**Figure 1b**). Ectodermal myonemes are  
51 organized perpendicular to endodermal ones: They run parallel to the oral-aboral axis in the body column  
52 and appear as radial spokes in the head (**Figure 1b**) (1,4-6). Treatment with 2.5% (w/v) magnesium chloride  
53 ( $\text{MgCl}_2$ ), a muscle relaxant used for jelly fish (7), blocked both longitudinal body column contractions and  
54 mouth opening and it was shown that the endoderm starts opening only after the ectoderm has opened to a  
55 critical area (1). Based on these observations, it was proposed that the active contraction of ectodermal  
56 myonemes generate the forces necessary for mouth opening (1). However, how cell-level myoneme  
57 contraction is coordinated to cause the S-shaped opening kinematics (**Figure 1c**) and symmetric mouth  
58 opening (**Figure 1d**) is unknown.

59 This study integrates previous and new experimental observations for a mechanistic understanding of mouth  
60 opening. Using fluorescence imaging of different transgenic *Hydra* lines, we deduce the length- and  
61 timescales of the “individual” (cell-level) deformations produced by local myoneme contractions and the  
62 spatiotemporal pattern of myoneme activation in the head. With this information, we develop a non-linear,  
63 viscoelastic spring network model of the *Hydra* head. Despite its simplicity, the model captures the main  
64 macroscopic features of mouth opening in *Hydra*. It can also predict changes to these macroscopic features  
65 in response to perturbations, which we have experimentally tested. Because the model uses only a few  
66 parameters that can be experimentally constrained, we can develop a physical intuition for the mouth opening  
67 process and gain new biological insights.

## 68 **Results and Discussion**

69 It has been shown that the mouth opening is less circular initially and becomes more circular over time (1).  
70 This initial asymmetry likely arises from local myoneme activity. Ectoderm cells are about 20  $\mu\text{m}$  in diameter  
71 and individual ectodermal myonemes range between 5-20  $\mu\text{m}$  in length (4). Thus, they generate short-range  
72 contractile forces. These short-range forces act across neighboring cells to generate ‘tugs’ – radial tissue  
73 deformations at the mouth perimeter involving 3-4 adjacent cells (**Figure 1e**), on the order of  $\sim 70\text{-}90 \mu\text{m}$   
74 (compared to a radius of  $\sim 200 \mu\text{m}$  of the head). The angles between successive tugs, binned into 100 ms  
75 intervals, are distributed uniformly (**Figure 1f**, p-value = 0.87, two-sample Kolmogorov Smirnov test against  
76 a uniform distribution), showing that the tugs are azimuthally uncorrelated. These uncorrelated tugs lead to  
77 the initial non-circularity of the mouth that has previously been reported (1). Despite this lack of azimuthal  
78 coordination at short timescales, mouth opening is radially symmetric over long timescales of tens of seconds:  
79 relative fluctuations in the (azimuthally) averaged mouth radius decrease over the course of mouth opening  
80 and settle to about 5% (**Figure 1g,h**). This implies that local deformations are spatially synchronized to  
81 achieve the observed symmetric mouth opening. Note that very large openings that lead to endoderm  
82 evagination, as sometimes observed with chemical induction or during feeding (1,8), are not considered in  
83 our analysis.

84 While transgenic *Hydra* lines exist for imaging myonemes *in vivo* (9), the large-scale tissue deformation of  
85 mouth opening prevents the resolution of individual myonemes (**Figure S2**). However, because *Hydra*'s  
86 epitheliomuscular cells require intracellular calcium ( $\text{Ca}^{2+}$ ) for contraction, we can visualize the spatio-  
87 temporal pattern of calcium in the epithelial tissue using genetically encoded  $\text{Ca}^{2+}$  indicators (GCaMP6s) and  
88 thus obtain the spatio-temporal pattern of active contractile forces (10-12). This allowed us to determine  
89

90 whether the initial active forces are localized within a specific region of the head. Previous work using low  
 91 magnification lateral imaging of transgenic *Hydra* that express the GCaMP6s in the ectodermal epithelial  
 92 layer suggested that epithelial cell activity begins at the center of the mouth and propagates outward as mouth  
 93 opening continues (10,13). We performed both low-resolution lateral and high-resolution top-down imaging  
 94 of heads of *Hydra* expressing GCaMP6s in the ectoderm. Mouth opening was induced via exposure to quinine  
 95 or reduced glutathione (1,14), which bind to receptors in sensory neurons (15,16). The neurons are thought  
 96 to activate epithelial cell contraction using neuropeptides (12,17,18). Upon stimulation, variations in  $\text{Ca}^{2+}$   
 97 signaling across the head were observed (**Figure S3**). However, consistent  $\text{Ca}^{2+}$  activity was seen along the  
 98 base of the *Hydra* head near the tentacle ring at the onset of mouth opening in all 16 top-down and in 7/9 of  
 99 all sideview movies (**Figure S3**, SI Section 1.1 and **Movie S2**). Thus, myoneme contractions at the head  
 100 boundary appear to induce opening.

101 The active contraction of ectodermal myonemes generate the forces necessary for mouth opening, while the  
 102 endoderm follows with the same logistic kinematics for the opening area with a time delay (1). In this study,  
 103 we focus on the dynamics after both epithelia have opened. Because active forces are only being produced  
 104 in the ectoderm, the passive mechanical response of the tissue can be effectively captured by modifying the  
 105 elastic modulus and viscosity of a single layer. Further, because out-of-plane deformations are limited  
 106 (Methods), we can model the head epithelial tissue as a 2D network of coupled non-linear springs (**Figure**  
 107 **2a, S1b**), representing both epithelial layers and the ECM. Since neuronal signals are required to trigger  
 108 mouth opening (19,20), they are incorporated as sources that generate active forces.

109 The network of non-linear springs is arranged in  $R+1$  concentric regular  $N$ -gons (polygons with  $N$  vertices)  
 110 (**Figure 2a**). For simplicity, we present results for a model using 5 concentric octagons (see **Figure S5**  
 111 for other cases of  $R$  and  $N$ ). Each vertex is connected to its four nearest neighbors by non-linear springs, except  
 112 the vertices on the innermost and outermost octagon. All vertices are movable, except for those in the  $(R+1)^{\text{th}}$   
 113 -octagon, which represents a fixed boundary – the tentacle ring at the base of the head. The spring network  
 114 represents the elastic behavior of the coupled epithelial tissue layers and not myonemes or individual cells.  
 115 Since mouth opening is a low Reynolds number process ( $Re = \frac{\rho l v}{\eta} \sim 10^{-10}$ ), we ignore the inertial term in the  
 116 momentum balance equations describing the motion of the vertices. The forces produced by the ectodermal  
 117 myonemes are represented by short-lived “external” radial forces acting on the vertices in the network.  
 118

119 Guided by the experimental data, we make two more assumptions: 1) At timescales  $> 100$  ms, the active  
 120 forces generated by the radial myonemes can be treated as uncorrelated, and 2) the active forces, act only on  
 121 the head boundary, because  $\text{Ca}^{2+}$  activity was always observed at the boundary in experiments (**Figure S1a**,  
 122 **Figure S3**, Supplementary Appendix Section 1.1 and **Movie S2**). Therefore, we model the active forces as  
 123 independent Poisson processes and we constrain them to only act on the vertices of the  $R^{\text{th}}$  polygon, in the  
 124 radially outward direction (See Figure S4 and Supplementary Appendix Section 1.1 for results corresponding  
 125 to activation in the center). The dynamical equations for the vertices of the non-linear spring network under  
 126 these assumptions (see Methods), primarily depend only on two adjustable parameters: the ratio of the  
 127 strength of the active force to the strength of the characteristic elastic force of the non-linear springs ( $\frac{\tilde{f}_0}{\kappa l_0}$ ) and  
 128 the ratio of the rate of active forcing to the viscoelastic relaxation rate ( $\frac{\lambda \kappa}{\mu}$ ). Using published data, we estimated  
 129  $\kappa$  and  $\mu$  (**Table S1**). Because the forces exerted by individual myonemes are unknown, we varied the active  
 130 forcing rate  $\lambda$  and the active force strength  $\tilde{f}_0$ , over several orders of magnitude to investigate how the  
 131 macroscopic behavior of the system would change across a wide range of parameters. We also varied the  
 132 strength of non-linear elasticity in the model by changing  $l_0$  to understand how changes in the mechanical  
 133 properties of the epithelial tissue might affect the macroscopic features of mouth opening.

134 *Mechanical cascade generates the logistic (S-shape) mouth area-time curve*

135 The model reproduces the experimentally observed logistic time curve (defined in **Figure 1c**) for the mouth  
 136 area (**Figure 2b**). The slow initial increase of the mouth area is caused by a cascade of tissue deformations  
 137 (**Figure 2c**): The active forces at the head boundary act at random timepoints on the outermost ( $R$ )<sup>th</sup> ring of

138 vertices causing the outermost set of radial springs to contract. This transmits force to the springs connecting  
139 the (R-1)<sup>th</sup> and R<sup>th</sup> polygons, causing them to pull on the vertices in the (R-1)<sup>th</sup> polygon. This transmits forces  
140 to the springs connecting the (R-2)<sup>th</sup> and (R-1)<sup>th</sup> polygons, causing them to pull on the vertices in the (R-2)<sup>th</sup>  
141 polygon, and so on. Thus, force is transmitted from the outermost to the innermost ring with a time delay as  
142 the springs contract sequentially. This is seen in the time shift between the peak deformation of the springs  
143 in the outermost and those in the innermost ring (**Figure 2c**) and creates the observed slow initial increase of  
144 the mouth area curve. Once the outer springs reach their steady state lengths – effectively functioning as rigid  
145 rods transmitting force to the inner vertices – the mouth area increases quickly. The mouth area saturates  
146 once the elastic restoring forces of the springs equal the strength of the stochastic active forces. When this  
147 point is reached, the active forces only produce transient local deformations that undergo very fast  
148 viscoelastic relaxation, and the mouth area remains constant (plateau in **Figure 2b,c**). This mechanism of a  
149 deformation cascade differs from the hypothesis proposed in (1) that the initial dynamics are a result of  
150 increasing active myoneme forces (See Supplementary Appendix Section 1.2).

#### 151 *The non-linearity facilitates wider and faster mouth opening*

152 The model shows that the mouth area increases with increasing strength and increasing rate of the active  
153 force (**Figure 2d (Left)**). As the total amount of work done by the active forces on the tissue increases, either  
154 through increasing the magnitude of the force or its frequency, an increased amount of elastic energy is stored  
155 in the spring network resulting in a larger mouth opening. The non-linear elastic term  $\left(\frac{1}{l_0}\right)$  makes the springs  
156 stiffer as they deform. Strain stiffening has been reported for a variety of biological materials in response to  
157 mechanical perturbation (21–24). With increased stiffness, one would expect tissue deformations to be  
158 smaller because stiffer tissue requires a greater force to be deformed to the same extent as less stiff tissue.  
159 However, for a given active force strength and forcing rate, as the strength of non-linearity increases, the  
160 mouth area increases (**Figure 2d (Left)**): as the non-linearity increases, more force is transferred from the  
161 outer springs to the inner springs for the same deformation of the outer springs, resulting in larger mouth  
162 opening (see Supplementary Appendix Section 1.3). In addition, the timescale of mouth opening showed a  
163 functional change in its response to the strength and rate of the active force, depending on the strength of the  
164 non-linearity (**Figure 2d (Middle)**). Without non-linearity ( $l_0 = \infty$ ) the spring stiffness is constant, and the  
165 opening time increases with the strength and the rate of the active forcing. For intermediate values of the  
166 non-linear coefficient ( $l_0 = 30 \mu\text{m}$ ), the timescale of mouth opening is insensitive to the active force. In the  
167 strongly non-linear regime ( $l_0 = 10 \mu\text{m}$ ), the timescale of opening decreases as the strength and rate of the  
168 active force increases. Thus, for the same strength and rate of active forcing, the timescale of mouth opening  
169 reduces as the non-linearity increases (for a detailed explanation, see Supplementary Appendix Section 1.3).

#### 170 *Nearest-neighbor coupling controls the symmetry of mouth opening*

171 The model reproduces the radial symmetry of mouth opening that is experimentally observed. The measure  
172 of asymmetry of the mouth radius, the relative fluctuations in mouth radius ( $\delta r/r$ ), is on the order of 5% for  
173 most of the parameter space explored, in agreement with experiments (**Figure 1h, 2d (Right)**). To understand  
174 how this symmetry arises despite the uncorrelated stochastic forces, we modified the model by increasing the  
175 stiffness of the azimuthal springs (black springs in **Figure 2a**), keeping everything else constant. The  
176 increased stiffness caused a reduction in the relative fluctuations in radius ( $\delta r/r$ ), i.e., an increase in symmetry  
177 (**Figure 2e**). This symmetry increase can be attributed to the nearest neighbor coupling of the vertices by  
178 springs. When an individual vertex moves radially due to a short-lived active force, the azimuthal springs  
179 connecting it to its neighbors pull it back to its original position and distribute some of that force to the  
180 neighbors. This decreases the asymmetry caused by the active force (for a detailed explanation, see  
181 Supplementary Appendix Section 1.4).

182 Because the tentacle ring acts as a circular boundary of the head epithelium, we had assumed that the vertices  
183 on the outer boundary of the spring network are stationary – imposing a fixed circularly symmetric boundary  
184 condition on the spring network. To investigate the role of this boundary condition regarding mouth opening  
185 symmetry, we simulated the response of the spring network to a constant uniaxial force. The uniaxial force  
186 caused the head boundary to distort from a circular to an elliptical shape during the mouth opening process.

187 In simulations, we accomplished this by adding a constant radial force to a pair of diametrically opposite  
188 vertices that were originally constrained to be stationary (**Figure 3a**) The shape of the mouth opening was  
189 quantified by measuring the aspect ratio (the ratio of the major axis length to the minor axis length) and major  
190 axis orientation of the best fit ellipse to the mouth. The model predicted that the major axis of the ellipse  
191 should align with the direction of the uniaxial tension (**Figure 3c**) and that the aspect ratio of the ellipse  
192 should exceed 1 across a range of active forces and forcing rates (**Figure 3d**).

193  
194 We tested these predictions experimentally by recording mouth opening in *Hydra* heads subjected to constant  
195 uniaxial tension generated by microaspiration (**Figure 3b**, **Figure S6**, **Movie S3**) and found qualitative  
196 agreement. The measured aspect ratio of the microaspirated mouth opening ( $1.8 \pm 0.6$ , mean  $\pm$  standard  
197 deviation,  $N = 13$  microaspirated heads) was significantly larger ((p-value = 0.0006, one-tailed two-sample  
198 Student's *t*-test) than that of the control ( $1.2 \pm 0.1$ ,  $N = 9$  control heads) (**Figure 3f**). The major axis of the  
199 ellipse was closely aligned to the direction of the uniaxial force (angle between uniaxial force and major axis  
200 of best fit ellipse to the mouth =  $16.5 \pm 14.9^\circ$ , mean  $\pm$  standard deviation,  $N = 13$  microaspirated heads)  
201 (**Figure 3e**). Thus, the model correctly predicted how the shape of the head boundary affects the shape of the  
202 mouth opening.

203  
204 To verify that mechanical nearest neighbor interactions were sufficient for mouth opening in the real system,  
205 we tested mouth opening behavior in nerve-free chimera and in *Hydra* in which we pharmacologically  
206 blocked gap junction communication to prevent the exchange of ions and small molecules between cells.

207 Nerve-free *Hydra* (19,25) cannot open their mouths (20,26). Sensory neurons initiate mouth opening by  
208 activating the ectodermal epitheliomuscular cells that pull the mouth open. In our model we assume that once  
209 activated by a neuronal signal, signal propagation is mechanical and thus does not require a nervous system.  
210 Supporting this assumption, we found that nerve-free *Hydra* open their mouths upon electrical stimulation  
211 (**Movie S4**). This suggests that the nerve net is only necessary to trigger mouth opening.

212 We could not use electrical stimulation to quantify the kinematics of mouth opening in nerve-free *Hydra* due  
213 to the size of the electrode being comparable to the size of the *Hydra* head. Therefore, to investigate whether  
214 the nerve net affected opening kinematics, we took advantage of *Hydra*'s unique regenerative properties and  
215 created chimeric animals with a nerve net in only half of the head, i.e., innervated:nerve-free *Hydra* (**Figure**  
216 **4a-c**, **S6a**). To account for possible effects from the grafting, we compared the behavior to  
217 innervated:innervated *Hydra* chimera. We also simulated the chimeric innervated:nerve-free *Hydra* using our  
218 model: we set the active forces to zero on half of the vertices in the outermost polygon, creating a "semi-  
219 circular" passive sector of the network (**Figure 4a**). In both simulations and experiments, we found that upon  
220 activation (chemical stimulation with quinine in experiments) (**Movie S5**) only the innervated half of the  
221 chimeric head deformed initially, and the mouth started opening initially only in the innervated half (**Figure**  
222 **4d-f**), whereas in innervated:innervated *Hydra* mouth opening occurred in both halves. After a short delay,  
223 the nerve-free part of the innervated:nerve-free *Hydra* head also deformed, albeit slower than the innervated  
224 half (**Figure 4d-f**), creating a more symmetric final mouth opening (**Figure 4h,j**). The mouth areas associated  
225 with innervated and nerve-free halves both showed the logistic time dependence observed in control  
226 (innervated:innervated) *Hydra*. The ratio of the timescale of mouth opening ( $\frac{d_{innervated}}{d_{nerve-free}}$ ) in experiments was  
227 significantly lower (p-value = 0.03 Student's *t*-test) for the innervated:nerve-free chimeras (ratio =  $0.64 \pm$   
228  $0.29$  (mean  $\pm$  standard deviation),  $n = 9$  movies,  $N = 9$  chimeras) compared to control (innervated: innervated)  
229 chimeras (ratio =  $1.70 \pm 1.26$ ,  $n = 8$  movies,  $N = 6$  chimeras) (**Figure 4g**). The ratio of the timescales obtained  
230 from the simulations in the strongly non-linear regime was the same order of magnitude as seen in the  
231 experiments (**Figure 4g, i**). Thus, both simulations and experiments with innervated:nerve-free chimeras  
232 suggest that non-neuronal cellular communication suffices for the propagation of mouth opening and  
233 produces the logistic opening kinematics.

234 *Hydra* ectodermal cells are connected via gap junctions (27) and gap junctions have been found to control  
235 other large-scale shape changes that are due to ectodermal myoneme contraction, such as body column  
236 shortening (28,29). Therefore, we tested whether ectodermal gap junction communication was necessary for

237 mouth opening. When gap junctions were blocked with 0.04% 1-heptanol, which blocks body column  
238 shortening (29), no difference in the time history or timescale of mouth opening was observed compared to  
239 untreated *Hydra* (**Figure S7b**). These results imply that neither neuronal signaling nor gap junction  
240 communication are necessary to coordinate mouth opening once initiated. Instead, mechanical nearest  
241 neighbor interactions are sufficient to coordinate and execute mouth opening.

242 In summary, our model shows how elastic coupling of nearest neighbors can produce a symmetric  
243 macroscopic deformation despite stochastic forces acting on a short length scale. By considering different  
244 strengths of non-linearity, we demonstrate the role of strain stiffening in tissue mechanics. Strain stiffening  
245 is a common phenomenon reported in a variety of tissues (21–24). Our model illustrates how strain stiffening  
246 can lead to larger and faster macroscopic deformations. This allows *Hydra* to ingest prey larger than its own  
247 diameter and react more quickly to external stimuli.

248  
249 The model also shows how mechanics alone can be sufficient to coordinate complex, physiologically  
250 important behaviors in the absence of neuronal control. It complements recent work in *Trichoplax adhaerens*,  
251 a primitive “epithelial” organism that lacks muscles and neurons. In *T. adhaerens*, mechanical forces between  
252 epithelial cells have been shown to suffice for maintenance of epithelial integrity in response to external  
253 forces. It has been proposed that *T. adhaerens* maintains its epithelial integrity by “active cohesion” -  
254 balancing individual cell contractions with local stress softening that prevents detachment of cell-cell  
255 junctions (30). In contrast, mouth opening in *Hydra* involves strain stiffening and it begins with loss of  
256 epithelial integrity and disruption of cell-cell junctions. Thus, different forms of mechanical coupling could  
257 be a fundamental mechanism of generating complex behaviors that evolutionarily precede the neuronal  
258 control of behavior. Our study also shows that the rich network of neurons in the *Hydra* hypostome (31,32)  
259 may primarily occupy a sensory role as neuronal signaling is only required to trigger mouth opening.

260

#### 261 **Concluding Remarks**

262 The cigar comb jelly *Beroë* also lacks a permanent mouth and its mechanisms of mouth opening share key  
263 similarities with the process we have described in *Hydra*. In both cases, neuronal activity triggers stochastic  
264 local contractions which eventually lead to mouth opening (33). Thus, both processes depend on the  
265 coordination of local forces to achieve rapid large tissue deformations. How this coordination is achieved  
266 depends on the cellular mechanisms of opening: While *Hydra* mouth opening requires the formation of a  
267 circular hole in the epithelium originating from the center of the hypostome, *Beroë* mouth opening requires  
268 the peeling off of an adhesive strip that holds the two ‘lips’ of the *Beroë* sealed (33). Both processes are  
269 effective solutions for achieving the rapid extreme epithelial deformations that are required for feeding.  
270 Studying how these simpler organisms use mechanical nearest neighbor interactions to generate complex  
271 behaviors allows us to extract fundamental design principles for tissue bioengineering applications that  
272 require extreme deformations of tissue while maintaining structure (as opposed to fluidization of cells as in  
273 morphogenesis).

274

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287 **References**

- 288 1. Carter JA, Hyland C, Steele RE, Collins EMS. Dynamics of Mouth Opening in Hydra. *Biophysical*  
289 *Journal*. 2016 Mar 8;110(5):1191–201.
- 290 2. Trepap X, Chen Z, Jacobson K. Cell Migration. *Compr Physiol*. 2012 Oct;2(4):2369–92.
- 291 3. Gooshvar S, Madhu G, Ruszczyk M, Prakash VN. Non-Bilaterians as Model Systems for Tissue  
292 *Mechanics*. *Integrative and Comparative Biology*. 2023 Jun 24;icad074.
- 293 4. West DL. The epitheliomuscular cell of hydra: Its fine structure, three-dimensional architecture and  
294 *relation to morphogenesis*. *Tissue and Cell*. 1978 Jan 1;10(4):629–46.
- 295 5. Mueller JF. Some Observations on the Structure of Hydra, with Particular Reference to the Muscular  
296 *System*. *Transactions of the American Microscopical Society*. 1950;69(2):133–47.
- 297 6. Haynes JF, Burnett AL, Davis LE. Histological and ultrastructural study of the muscular and nervous  
298 *systems in Hydra*. I. The muscular system and the mesoglea. *Journal of Experimental Zoology*.  
299 1968;167(3):283–93.
- 300 7. Abrams MJ, Basinger T, Yuan W, Guo CL, Goentoro L. Self-repairing symmetry in jellyfish through  
301 *mechanically driven reorganization*. *Proc Natl Acad Sci USA [Internet]*. 2015 Jun 30 [cited 2023 Jun  
302 28];112(26). Available from: <https://pnas.org/doi/full/10.1073/pnas.1502497112>
- 303 8. Loomis WF. Glutathione Control of the Specific Feeding Reactions of Hydra. *Annals of the New York*  
304 *Academy of Sciences*. 1955;62(9):211–27.
- 305 9. Aufschnaiter R, Wedlich-Söldner R, Zhang X, Hobmayer B. Apical and basal epitheliomuscular F-  
306 *actin dynamics during Hydra bud evagination*. *Biology Open*. 2017 Jan 1;bio.022723.
- 307 10. Szymanski JR, Yuste R. Mapping the Whole-Body Muscle Activity of *Hydra vulgaris*. *Curr Biol*. 2019  
308 Jun 3;29(11):1807-1817.e3.
- 309 11. Kuo IY, Ehrlich BE. Signaling in Muscle Contraction. *Cold Spring Harb Perspect Biol*. 2015 Feb  
310 1;7(2):a006023.
- 311 12. Wang H, Swore J, Sharma S, Szymanski JR, Yuste R, Daniel TL, et al. A complete biomechanical  
312 *model of Hydra contractile behaviors, from neural drive to muscle to movement*. *Proceedings of the*  
313 *National Academy of Sciences*. 2023 Mar 14;120(11):e2210439120.
- 314 13. Dupre C, Yuste R. Non-overlapping Neural Networks in *Hydra vulgaris*. *Curr Biol*. 2017 Apr  
315 24;27(8):1085–97.
- 316 14. Forrest H. Lack of dependence of the feeding reaction in hydra on reduced glutathione. *The Biological*  
317 *Bulletin*. 1962 Jun 1;122(3):343–61.
- 318 15. Venturini G. The hydra GSH receptor. Pharmacological and radioligand binding studies. *Comparative*  
319 *Biochemistry and Physiology Part C: Comparative Pharmacology*. 1987 Jan 1;87(2):321–4.
- 320 16. Pierobon P. Regional modulation of the response to glutathione in *Hydra vulgaris*. *Journal of*  
321 *Experimental Biology*. 2015 Jul 1;218(14):2226–32.
- 322 17. Takahashi T, Hayakawa E, Koizumi O, Fujisawa T. Neuropeptides and their functions in Hydra. *Acta*  
323 *Biol Hung*. 2008;59 Suppl:227–35.
- 324 18. Yum S, Takahashi T, Koizumi O, Ariura Y, Kobayakawa Y, Mohri S, et al. A Novel Neuropeptide,  
325 *Hym-176, Induces Contraction of the Ectodermal Muscle in Hydra*. *Biochemical and Biophysical*  
326 *Research Communications*. 1998 Jul 30;248(3):584–90.
- 327 19. Campbell RD. Elimination by Hydra interstitial and nerve cells by means of colchicine. *J Cell Sci*.  
328 1976 Jun;21(1):1–13.
- 329 20. Tran CM, Fu S, Rowe T, Collins EMS. Generation and Long-term Maintenance of Nerve-free Hydra.  
330 *JoVE (Journal of Visualized Experiments)*. 2017 Jul 7;(125):e56115.
- 331 21. Trepap X, Grabulosa M, Puig F, Maksym GN, Navajas D, Farré R. Viscoelasticity of human alveolar  
332 *epithelial cells subjected to stretch*. *American Journal of Physiology-Lung Cellular and Molecular*  
333 *Physiology*. 2004 Nov;287(5):L1025–34.
- 334 22. Trepap X, Deng L, An SS, Navajas D, Tschumperlin DJ, Gerthoffer WT, et al. Universal physical  
335 *responses to stretch in the living cell*. *Nature*. 2007 May;447(7144):592–5.
- 336 23. Wang Y, Du Y, Xu F. Strain stiffening retards growth instability in residually stressed biological  
337 *tissues*. *Journal of the Mechanics and Physics of Solids*. 2023 Sep 1;178:105360.
- 338 24. Fernández P, Pullarkat PA, Ott A. A Master Relation Defines the Nonlinear Viscoelasticity of Single  
339 *Fibroblasts*. *Biophysical Journal*. 2006 May 15;90(10):3796–805.

- 340 25. Marcum BA, Campbell RD. Development of Hydra lacking nerve and interstitial cells. *Journal of Cell*  
 341 *Science*. 1978 Feb 1;29(1):17–33.
- 342 26. Marcum BA. Culturing Epithelial Hydra. In: Lenhoff HM, editor. *Hydra: Research Methods* [Internet].  
 343 Boston, MA: Springer US; 1983 [cited 2022 Oct 1]. p. 287–90. Available from:  
 344 [https://doi.org/10.1007/978-1-4757-0596-6\\_38](https://doi.org/10.1007/978-1-4757-0596-6_38)
- 345 27. Wood RL. Intercellular Attachment in the Epithelium of Hydra As Revealed by Electron Microscopy.  
 346 *The Journal of Cell Biology*. 1959 Dec;6(3):343–52.
- 347 28. Fraser SE, Green CR, Bode HR, Gilula NB. Selective disruption of gap junctional communication  
 348 interferes with a patterning process in hydra. *Science*. 1987;237(4810):49–55.
- 349 29. Takaku Y, Hwang JS, Wolf A, Böttger A, Shimizu H, David CN, et al. Innexin gap junctions in nerve  
 350 cells coordinate spontaneous contractile behavior in Hydra polyps. *Sci Rep*. 2014 Jan 7;4:3573.
- 351 30. Armon S, Bull MS, Aranda-Diaz A, Prakash M. Ultrafast epithelial contractions provide insights into  
 352 contraction speed limits and tissue integrity. *Proceedings of the National Academy of Sciences of the*  
 353 *United States of America*. 2018 Oct;115(44):E10333–41.
- 354 31. Kinnamon JC, Westfall JA. A three dimensional serial reconstruction of neuronal distributions in the  
 355 hypostome of a Hydra. *Journal of Morphology*. 1981;168(3):321–9.
- 356 32. Grimmelikhuijzen CJP. Antisera to the sequence Arg-Phe-amide visualize neuronal centralization in  
 357 hydroid polyps. *Cell Tissue Res*. 1985 Jul;241(1):171–82.
- 358 33. Tamm SL, Tamm S. Reversible Epithelial Adhesion Closes the Mouth of Beroe, a Carnivorous Marine  
 359 Jelly. *The Biological Bulletin*. 1991 Dec;181(3):463–73.
- 360 34. Glauber KM, Dana CE, Park SS, Colby DA, Noro Y, Fujisawa T, et al. A small molecule screen  
 361 identifies a novel compound that induces a homeotic transformation in Hydra. *Development*. 2013 Dec  
 362 1;140(23):4788–96.
- 363 35. Shimizu H, Koizumi O, Fujisawa T. Three digestive movements in Hydra regulated by the diffuse  
 364 nerve net in the body column. *J Comp Physiol A*. 2004 Aug 1;190(8):623–30.
- 365 36. Lenhoff HM, Brown RD. Mass culture of hydra: an improved method and its application to other  
 366 aquatic invertebrates. *Lab Anim*. 1970 Apr 1;4(1):139–54.
- 367 37. Goel T, Wang R, Martin S, Lanphear E, Collins EMS. Linalool acts as a fast and reversible anesthetic  
 368 in Hydra. *PLOS ONE*. 2019 Oct 24;14(10):e0224221.

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### 389 **Figures and Tables**

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**Figure 1: Hydra head anatomy and mouth opening dynamics.** (a) Schematic showing basic *Hydra* anatomy and cell types including a zoomed-in cross-section of the hypostome. (b) Schematic top-down view of the myoneme organization in the ectoderm (green) and endoderm (magenta) in the *Hydra* head. (c) Area of the mouth (normalized to the maximum

394 area) as a function of time. The blue curve shows representative experimental data. The black curve corresponds to a  
 395 logistic fit as described by the equation on the right. 'a', 'b', 'c' and 'd' correspond to the minimum normalized area,  
 396 maximum normalized area, time to reach half maximum and timescale of mouth opening respectively. (d) Still images  
 397 from **Movie S1** showing quinine hydrochloride (0.5 mM) induced opening of the ectodermal epithelial layer in a  
 398 Watermelon (WM) *Hydra* (scale bar: 100  $\mu\text{m}$ ). (e) Histogram of number of cells involved in each tugging event ( $n = 364$   
 399 tugging events from  $N = 8$  independent events). (f) Probability density function for the relative angle between successive  
 400 tugging events ( $n = 184$  tugging event pairs from  $N = 5$  independent mouth opening events). (g) Representative image of  
 401 a radial tug during mouth opening induced by 0.2mM reduced glutathione (scale bar: 200  $\mu\text{m}$ ). (h) Time evolution of the  
 402 relative fluctuation of the mouth radius,  $\delta r/r$ , averaged over the azimuth ( $N = 6$  mouth opening events). Time is  
 403 normalized from the beginning of mouth opening to when the mouth reaches its maximum area.

404 **Figure 2: Non-linear spring network model captures macroscopic features of mouth opening.** (a) Schematic of the  
 405 spring network. Color-coding indicates distance from center. (b) Comparison between mouth opening time series  
 406 obtained from model simulations (blue) and the logistic curve fit (red). Intensity of grey background denotes phases of  
 407 mouth opening (from light to dark): slow increase, fast increase, and saturation. (c) Azimuthally averaged radial  
 408 deformation of vertices in each N-gon. Line colors match those of the radial springs in (a). Grey background matches  
 409 that of (b). (d) Variation of final mouth area (left column) fitted mouth opening time (middle column), and relative  
 410 fluctuations in mouth radius,  $\delta r/r$  (right column), as functions of the active force strength ( $x$ -axis) and the average forcing  
 411 rate ( $y$ -axis) for different non-linear regimes: without non-linearity (top), weak non-linearity (middle) and strong non-  
 412 linearity (bottom). Note the axes have logarithmic scales. A darker blue color indicates larger mouth areas (left), longer  
 413 opening times (middle) and larger relative fluctuations in mouth radius (right). (e) Changes in relative fluctuations in  
 414 mouth radius with changes in the stiffness of the azimuthal springs relative to stiffness of radial springs ( $\kappa_0$ ).  $l_0 = 30 \mu\text{m}$   
 415 for all three heat maps. Parameters for all simulations along with descriptions and relevant sources can be found in **Table**  
 416 **S1**.

417 **Figure 3: Application of external uniaxial tension affects symmetry of mouth opening.** (a) Schematic of the  
 418 hypostome under uniaxial tension.  $F_{\text{asp}}$  is the strength of the microaspiration force. (b) Still image sequence of mouth  
 419 opening when the head is placed under uniaxial tension by applying negative pressure. Arrowheads indicate the  
 420 location of needles used to apply negative pressure on the head. Star indicates the mouth opening (Scale bar: 100  $\mu\text{m}$ ).  
 421 (c) Orientation angle of major axis (relative to the direction of tensile force) and (d) aspect ratio of best fit ellipse to the  
 422 mouth, in simulations, as functions of the strength and rate of the active force ( $l_0 = 20 \mu\text{m}$ ). (e) Orientation angle of  
 423 major axis (relative to the direction of tensile force) ( $N = 13$ ) and (f) aspect ratio of best fit ellipse to the mouth, from  
 424 experiments ( $N = 9$  (Control),  $N = 13$  (Microaspirated)). (\*) indicates  $p$ -value = 0.0006 for a one-tailed two-sample  
 425 Student's  $t$ -test.  
 426

427 **Figure 4: The nerve-net is required to initiate but not to execute mouth opening.** (a) Schematic of the simulated  
 428 chimera. (b) Greyscale images of innervated and nerve-free *Hydra* generated by heat shock treatment of the A10 strain.  
 429 Note the nerve-free animal is more bloated at the oral end due to buildup of fluid. (c) Schematic showing the process of  
 430 creating chimeras. (d) Still image sequence of mouth opening in an innervated:nerve-free chimera (scale bar: 100  $\mu\text{m}$ ).  
 431 Representative mouth area-time curves (e) *in vivo* and (f) *in silico*. Comparison of the (g) ratio of mouth opening times  
 432 and (h) relative fluctuations in mouth radius between control chimeras ( $N = 6$ ) and innervated:nerve-free chimeras ( $N =$   
 433  $9$ ). (\*) denotes  $p$ -value  $< 0.05$  (i) Ratio of mouth opening times and (j) relative fluctuations in mouth radius for  
 434 innervated:nerve-free chimeras, in simulations, as functions of the strength and rate of the active force in the strongly  
 435 non-linear regime ( $l_0 = 10 \mu\text{m}$ ). Simulation parameters provided in **Table S1**.

436

437

## 438 Materials and Methods

439

### 440 Model Description

441 The epithelial tissue of the hypostome is modeled as a system of (R+1) concentric regular N-gons (polygons  
 442 with N vertices), all lying in the same plane. Each vertex is connected to its topological nearest neighbors (so  
 443 each vertex is connected to 4 others, except the ones on the innermost and outermost polygon) by a non-  
 444 linear spring (**Figure 2a**). All vertices, except those in the (R+1)<sup>th</sup> polygon are movable. The (R+1)<sup>th</sup> polygon  
 445 represents a fixed boundary, mimicking the role of the tentacle ring in the hypostome. For each movable  
 446 vertex, we can write the momentum balance equation:

447

$$\begin{aligned}
 448 \quad \frac{d\vec{r}_{i,j}}{\mu dt} = & - \left\{ \kappa (\|\vec{r}_{i,j} - \vec{r}_{i-1,j}\| - a) + \frac{\kappa}{l_0^2} (\|\vec{r}_{i,j} - \vec{r}_{i-1,j}\| - a)^3 \right\} \frac{\vec{r}_{i,j} - \vec{r}_{i-1,j}}{\|\vec{r}_{i,j} - \vec{r}_{i-1,j}\|} \\
 449 \quad & - \left\{ \kappa (\|\vec{r}_{i,j} - \vec{r}_{i+1,j}\| - a) + \frac{\kappa}{l_0^2} (\|\vec{r}_{i,j} - \vec{r}_{i+1,j}\| - a)^3 \right\} \frac{\vec{r}_{i,j} - \vec{r}_{i+1,j}}{\|\vec{r}_{i,j} - \vec{r}_{i+1,j}\|} \\
 450 \quad & - \left\{ \kappa (\|\vec{r}_{i,j} - \vec{r}_{i,j-1}\| - b_i) + \frac{\kappa}{l_0^2} (\|\vec{r}_{i,j} - \vec{r}_{i,j-1}\| - b_i)^3 \right\} \frac{\vec{r}_{i,j} - \vec{r}_{i,j-1}}{\|\vec{r}_{i,j} - \vec{r}_{i,j-1}\|} \\
 451 \quad & - \left\{ \kappa (\|\vec{r}_{i,j} - \vec{r}_{i,j+1}\| - b_i) + \frac{\kappa}{l_0^2} (\|\vec{r}_{i,j} - \vec{r}_{i,j+1}\| - b_i)^3 \right\} \frac{\vec{r}_{i,j} - \vec{r}_{i,j+1}}{\|\vec{r}_{i,j} - \vec{r}_{i,j+1}\|}
 \end{aligned}$$

$$452 \quad + \delta_{i,R} \tilde{f}_0 \sum_n \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(t-t_n)^2}{2\sigma^2}} \frac{\vec{r}_{i+1,j} - \vec{r}_{i,j}}{\|\vec{r}_{i+1,j} - \vec{r}_{i,j}\|}$$

453

$$t_n \sim \text{Pois}(\lambda)$$

$$454 \quad \frac{d\vec{r}_{R+1,j}}{\mu dt} = 0$$

455 where,  $\vec{r}_{i,j}$  is the position of the vertex in the  $i$ -th N-gon at the  $j$ -th azimuthal position at time  $t$ .  $a$  is the rest  
 456 length of the radial springs.  $b_i$  is the rest length of the azimuthal springs in the  $i$ -th N-gon.  $\kappa$  is the spring  
 457 constant,  $\mu$  is the coefficient of mobility and  $l_0$  is the length at which the linear and non-linear forces are of  
 458 equal magnitude.  $\delta_{i,R}$  is the Kronecker delta function,  $\tilde{f}_0$  is the strength of the active force,  $\sigma$  is the duration  
 459 over which the force acts and  $t_n$  is the time at which the force acts.  $t_n$  are drawn randomly from a Poisson  
 460 distribution with a mean rate  $\lambda$ .

461 The initial positions of the vertices are:

$$462 \quad \vec{r}_{i,j} = (a_0 + (i-1)a) \left( \cos\left(\frac{2\pi j}{N}\right) \hat{x} + \sin\left(\frac{2\pi j}{N}\right) \hat{y} \right)$$

463  $a_0$  is the initial radius of the mouth, equal to one cell radius. Also,

$$464 \quad b_i = 2(a_0 + (i-1)a) \sin\left(\frac{\pi}{N}\right)$$

465

466 The values of all these parameters are provided in **Table S1**. To model the microaspiration experiment, we  
467 added an additional constant radial force (**Table S1**) and to model the chimera we set the active force to  
468 zero at specific vertices (see Supplemental Appendix Section 1.5).

469

## 470 **Numerical methods**

471 The model equations were solved using custom MATLAB 2021a (MathWorks) scripts. For each set of free  
472 parameters, 5 realizations of the stochastic external force were simulated. The system of coupled ODEs  
473 described in the model description section were then solved for each realization using a 4th order Runge-  
474 Kutta scheme. For each realization, the final area of the mouth, and the azimuthal average and standard  
475 deviation of mouth radius were obtained. The timescale of mouth opening was obtained by fitting the mouth  
476 area from the simulations to the experimentally obtained logistic curve using a linear least squares approach.  
477 Realizations which generated unphysical results (leading either to mouth areas orders of magnitude larger  
478 than the rest of the realizations or mouth opening timescales less than 50ms) were discarded. The outputs  
479 from the remaining realizations were then averaged for each set of parameters. The relevant code is available  
480 on Zenodo (see **Software S1** for the link).

481

## 482 **Hydra strains and culture**

483 The *Hydra vulgaris* AEP strain and various transgenic lines derived from this strain: Epithelial GCaMP,  
484 expressing GCaMP6s in the ectoderm (10); HyBra, expressing GFP under control of the HyBra2  
485 promoter:GFP transgenic animals (34); “Watermelon” (WM) animals (34) expressing GFP in the ectoderm  
486 and DsRed2 in the endoderm with both genes under control of an actin gene promoter; Lifeact-GFP strains  
487 (9), expressing Lifeact-GFP under the control of the *Hydra actinI* promoter, in the ectoderm or in the  
488 endoderm and, *Hydra vulgaris* strain A10 (chimera consisting of *Hydra vulgaris* (formerly *Hydra*  
489 *magnipapillata* strain 105) epithelial cells and sf-1 interstitial cells, which are temperature sensitive  
490 interstitial cells (35) were used for experiments. *Hydra* strains were maintained in mass cultures in *Hydra*  
491 medium (HM) composed of 1 mM CaCl<sub>2</sub> (Spectrum Chemical, Cat#C1096-500GM), 0.1 mM MgCl<sub>2</sub>  
492 (Sigma-Aldrich, Cat#M0250-1KG), 0.03 mM KNO<sub>3</sub> (Fisher Scientific, Cat#P263-500), 0.5 mM NaHCO<sub>3</sub>  
493 (Fisher Scientific, Cat#S233-500), and 0.08 mM MgSO<sub>4</sub> (Fisher Scientific, Cat#BP213-1) prepared with  
494 MilliQ water, with a pH between 7 and 7.3. Cultures were maintained at 18°C in the dark in a Panasonic  
495 incubator (Panasonic MIR-554, Tokyo, Japan). The cultures were fed 2-3x/week with *Artemia* nauplii  
496 (brine shrimp) from the San Francisco Bay or from the Great Salt Lake (Brine Shrimp Direct). Animals  
497 were cleaned daily using standard cleaning procedures (36). Asexual, non-budding polyps starved for at  
498 least 24 hours were used for experiments unless stated otherwise.

499

## 500 **Calcium signaling in mouth opening**

501 The large-scale tissue deformation of mouth opening prevents the resolution of individual myonemes (**Figure**  
502 **S2**). Therefore, we visualized myoneme activation indirectly by imaging transgenic strains with calcium  
503 reporters in the ectoderm (10), using calcium signaling as a proxy for cell activity. Additionally, we quantified  
504 the consequences of myoneme activation by measuring changes in cell shapes. Combining these data, we  
505 were able to infer the length scale associated with individual myoneme contractions and the timescale over  
506 which local myoneme contractions are independent of each other.

507

508 Epithelial GCaMP polyps, expressing GCaMP6s in the ectoderm, were starved for 24-72 hours before  
509 imaging. Movies were recorded on the Olympus IX81 inverted microscope (Olympus Corporation) equipped  
510 with an Zyla sCMOS camera (Andor Technology-Oxford Instruments), an X-cite Xylis (Excelitas,  
511 Model#XT720L) fluorescence light source, and Slidebook 2022 software (Intelligent Imaging Innovations).  
512 For top-down imaging, *Hydra* were decapitated just below the tentacle ring in 1mM linalool (37) (Sigma-  
513 Aldrich, Cat#L2602-100G), rinsed in HM, and allowed to heal for 1-2 hours before imaging. *Hydra* polyps  
514 (for side-view imaging) or heads (for top-down imaging) were mounted on 75 x 25 mm glass slides (Corning,  
515 Cat#2949-75X25) in a few drops of 1mM linalool (Sigma-Aldrich) for side-view imaging or HM for top-  
516 down imaging. Double-sided tape was used as a spacer. Due to the different thicknesses of different brands,  
517 as well as different sizes of *Hydra* polyp heads and body columns, the amount of tape used varied. For side-  
518 view imaging, two pieces of Amazon Basic tape usually sufficed, while 3 pieces of Scotch tape worked  
519 equally well. For top-down imaging of heads, one piece of Scotch double-stick tape spaced with an additional  
520 small rectangle cut from a Kimwipe (Fisher Scientific, Cat#06-666A) was used. A 22x22 mm glass coverslip  
521 (Fisher Scientific, Cat#12-542-B) was overlaid such that there was an opening on one side of the glass slide.  
522 Mouth opening was induced by adding 80  $\mu$ L of 0.5-2.0 mM (-)-quinine hydrochloride dihydrate (Sigma-  
523 Aldrich, Cat#22630-10G-F) or 0.1-0.5 mM reduced L-glutathione (Sigma-Aldrich, Cat#G4251-10G)  
524 through one open end of the tunnel slide while a Kimwipe (Fisher Scientific) was held at the other end to  
525 absorb any extra fluid. Stock quinine solution was protected from light using aluminum foil, and both reduced  
526 glutathione and quinine stock solutions were made fresh weekly and stored at 4°C. Side-view images were  
527 recorded using a 4 $\times$ /0.13 UPlanFL N objective (Olympus Corporation), while top-down images were  
528 recorded using a 20 $\times$ /0.45 LUCPlanFL N objective (Olympus Corporation). Both sideview and top-down  
529 images were recorded with frame rates of 10-20 fps, 2x2 binning and 100% fluorescence intensity.

530

### 531 **Microaspiration experiment**

532 Microaspiration needles were prepared as described in the Supplemental Text.

533

534 Large, 2-3 day starved, WM *Hydra* were decapitated at least 1hr before imaging as follows: polyps were first  
535 incubated in 1mM linalool (Sigma-Aldrich) for 10 minutes and decapitated and the tentacles removed at their  
536 base. Samples were then washed once in HM and incubated at room temperature for 1-2 hrs to allow for  
537 wound healing and for the linalool to wear off.

538

539 A 6 mm glass concavity slide was propped up and positioned under the Leica MZ16FA microscope and filled  
540 with HM. The left and right micromanipulators were positioned such that the needle was immersed in the  
541 HM in the glass well. Aspiration was achieved by suctioning water through the tube by gently pulling the  
542 syringe plunger until approximately 1 mL of HM was inside the syringe, then removing the plunger entirely,  
543 and positioning the syringe so that the water level in the syringe was lower than that of the concavity slide.  
544 Once both the left and right microaspirators were experiencing negative pressure and needles were aligned  
545 approximately 180° to each other and within camera view, fluorescence was turned on using the GFP2 filter,  
546 the brightfield decreased, and the needles were pulled out of camera view. A *Hydra* head was then placed in  
547 the center of the glass well, between the needles. The head was then positioned hypostome-up. Final  
548 positioning of the hypostome was achieved with the aid of a hair loop. The left and right microaspirators  
549 were then positioned to suction the head, approximately 180° apart. Once so positioned, the microaspirators  
550 were adjusted such that the needles pulled the head to distort it from circular symmetry. The height of the  
551 water in the syringe relative to the concavity well was recorded. Enough water was removed from the dish  
552 to minimize image distortions, while maintaining negative pressure. Bright field was then turned off entirely  
553 and a NIGHTSEA SFA light head BNC (Stellar Scientific) was positioned to shine light directly on the *Hydra*

12

554 head. Images were recorded on the Leica MZ16FA microscope using a Leica 10445930 1.0x stereo  
555 microscope C-mount camera adapter connected to a Point Grey Grasshopper3 camera at a rate of 2 fps using  
556 the Flycapture2 Software (Point Grey). Mouth opening was induced by sequentially adding 80  $\mu$ L of 0.5 mM,  
557 1.0 mM, 1.5 mM, and 2.0 mM (-)-quinine hydrochloride dihydrate (Sigma-Aldrich) to the concavity well  
558 until mouth opening was observed.

559

560 Data sets were discarded for samples in which the hypostome was permanently distorted or damaged after  
561 the microaspiration. As a control, we tested whether the symmetry of the mouth opening was affected by  
562 physical contact of the needle with the tissue in the microaspiration setup. This was achieved by fixing only  
563 one side of the *Hydra* head by one microaspirator needle at low pressure and inducing opening. For the  
564 control analysis two types of heads were used: 5 heads post-microaspiration experiment and 4 new heads.  
565 The means of the aspect ratio of the mouth openings for the two types of control heads (post-microaspiration  
566 v. new) were not found to statistically differ ( $p = 0.56$  two-sample Student's *t*-test MATLAB).

567

### 568 **Generation and imaging of innervated:nerve-free chimera**

569 Nerve-free (NF) A10 and innervated HyBra were used to generate chimeras with half of the hypostome  
570 innervated and the other half nerve-free. Only NF A10 polyps that had the bloated body column phenotype  
571 (20), were unable to open their mouths to feed and were similar in size to the HyBra polyps were selected.  
572 The chosen NF A10 and HyBra polyps were put in a dish containing 1.25% methyl cellulose (Acros Organics,  
573 Cat#258111000) and 1 mM linalool (Sigma-Aldrich) in HM for 10 min. Each polyp was then decapitated  
574 one-third of the way down the body column with a scalpel. The resulting head pieces were each prompted to  
575 open their mouth with a tap of the forceps, and a lateral cut across the hypostome and tentacles was made  
576 with the scalpel to bisect the mouth. The half hypostome pieces were matched with the opposite *Hydra* strain  
577 piece and grafted together: Glass needles were pulled from 5  $\mu$ L microcapillaries (Corning Inc.) over a  
578 Bunsen burner to a thickness of around 0.03 mm. The hypostome pieces were then strung onto the needle  
579 and a small square of Kimwipe (Fisher Scientific) was placed on either side of the grafted pieces to ensure  
580 they stayed in contact during healing. These chimeras were allowed to heal on the needle for 1.5 h in the  
581 methyl cellulose solution and were then removed from the needle with forceps. The chimeras were transferred  
582 to a new dish with HM and allowed to heal overnight (between 14 and 24 h) in the 18°C incubator. Any  
583 leftover body column tissue was cut off from the chimeras using a scalpel. The chimeras were incubated in  
584 a solution of 1:1000 (wt:vol) 1-aminoanthracene (Sigma Aldrich, Cat#A38606) for 7 min in the dark to  
585 provide a temporary green fluorescence, then washed three times with HM. Once stained, chimeras were kept  
586 in the dark and imaged on the EVOS FL Auto 2 microscope equipped with a 10 $\times$ /0.3 Plan Fluor objective.  
587 Mouth opening events were either spontaneous (not induced by external stimuli) or induced by adding 0.5-  
588 1.5 mM (-)-quinine hydrochloride dihydrate (Sigma-Aldrich) or 0.1-0.5 mM reduced L-glutathione (Sigma-  
589 Aldrich). Images were recorded at framerates between 10-22 fps.

590 To account for possible effects on behavior due to strain differences, we performed control experiments with  
591 chimeras containing innervated A10 and HyBra using the same protocol, except for the fact that innervated  
592 A10 animals were used in place of the NF A10.

593 RFamide antibody staining was used to confirm the absence of neurons in the nerve-free tissue of the  
594 chimeras after imaging (**Figure S7a**). The chimeras were fixed with 4% paraformaldehyde (Ted Pella, Inc,  
595 Cat#50-00-0) made in HM. Chimeras were first relaxed in 1 mM linalool (Sigma-Aldrich) for 5 min, then  
596 transferred to the paraformaldehyde solution and fixed for 1 hr at room temperature or overnight at 4°C in  
597 the dark. Samples were then washed once quickly with 0.3% PBSTx (0.3% Triton-X (Sigma-Aldrich,  
598 Cat#T9284) in 1x PBS (MP Biomedicals Inc, Cat#092810306), followed by two 5 min washes. The fixed  
599 chimera samples were placed on glass slides for a wet mount antibody stain. Humid chambers for staining

600 were constructed by lining covered 100 mm Petri dishes (Spectrum Scientific, Cat#961-62084) with wet  
601 paper towels and placing the slides inside the dishes. A well was created in the center of each glass slide by  
602 layering two pieces of double-sided tape across both short sides of the slide with one piece of tape running  
603 on both long edges of the slide. The samples were placed in a drop of HM on the slide. All steps were  
604 performed at room temperature unless noted otherwise. The samples were washed three times with 20  $\mu$ L 1x  
605 PBS (MP Biomedicals), followed by a 15 min permeabilization with 20  $\mu$ L 0.5% PBSTx. They were then  
606 incubated for 3.5 h in 20  $\mu$ L blocking solution (10% FBS (Sigma-Aldrich, Cat#F6178), 1% DMSO in 1x  
607 PBS) and placed overnight (16 h) at 4°C in 30  $\mu$ L anti-RFamide primary antibody (gift from Dr. Kathleen  
608 Siwicki) diluted 1:200 in the blocking solution. On the second day, samples were washed quickly 3x with 40  
609  $\mu$ L 1x PBS, followed by four 30-minute washes of 20  $\mu$ L 0.3% PBSTx. The samples were then incubated in  
610 a 1:1000 or 1:500 dilution of Alexa 488 Goat anti-rabbit IgG secondary antibody (Thermo-Fisher Scientific,  
611 Cat#A-11008) for 5 h, followed by three quick and two 10 min washes of 0.3% PBSTx. The samples were  
612 then washed three times with 1x PBS. The 1x PBS was replaced with a 1:1 solution of glycerol (Omnifur,  
613 Cat#4750) and HM. Finally, a coverslip was placed over the samples and nail polish was used to seal the  
614 slides. Z-stacks of the samples were imaged using a Leica high-resonance scanning SP5 confocal microscope  
615 with a 20x C-Apochromat 1.2 W objective.

616

#### 617 **Mouth opening after heptanol treatment**

618 Two-day starved *Hydra* were decapitated under a dissection scope using a scalpel and allowed to heal for 1  
619 hr in HM at room temperature. The heads, along with a whole *Hydra* were incubated in 0.04% 1-heptanol  
620 (Acros Organics, Cat#120362500). The solution was prepared by adding 4  $\mu$ L of heptanol to 10 mL HM.  
621 The whole *Hydra* polyps were pinched using forceps to ensure that body column contractions had been  
622 inhibited and therefore, gap junctions had been blocked (14). The incubated heads were then mounted on 75  
623 x 25 mm glass slides (Corning) in the heptanol solution and covered using 22x22 mm glass coverslips (Fisher  
624 Scientific) with layers of double-sided tape as spacers. Mouth opening was either spontaneous or induced  
625 using 0.5 mM quinine hydrochloride (Sigma-Aldrich) prepared in the 0.04% 1-heptanol solution. Images  
626 were recorded using the Olympus IX81 inverted microscope (Olympus Corporation) equipped with a  
627 10x/0.40 UPlanSApo objective and an Orca-ER charge-coupled device camera (Hamamatsu Photonics) using  
628 SlideBook software (Intelligent Imaging Innovations). Movies were analyzed to extract the timescale of  
629 opening (the d-parameter) as described in the “quantification and statistical analysis” section.

630

### 631 **QUANTIFICATION AND STATISTICAL ANALYSIS**

#### 632 **Mouth opening shape and area as functions of time**

633 Custom MATLAB scripts (MATLAB 2021a, Mathworks) were used to obtain the area of the mouth across  
634 several frames, as described in (1). Briefly, single channel images of the mouth were first binarized. A  
635 polygonal region was manually identified as the mouth in the first image of the sequence. Successive  
636 images were thresholded based on the pixel intensities in the polygonal region to identify the mouth. For  
637 each frame, once the mouth boundary was identified, the area of the mouth, major and minor diameters of  
638 the best fit ellipse, and the mean and standard deviation of the mouth radius were calculated.  
639 The areas were then normalized so that they range between 0 and 1. The normalized  
640 areas were plotted as a function of time and fit to the logistic equation below using linear least  
641 squares fit.

642

643

$$A(t) = a + \frac{b}{1 + e^{-\left(\frac{t-c}{d}\right)}}$$

644

#### 645 **Analysis of local contractions and mouth symmetry**

646 We binned the mouth opening movies into 100 ms intervals and quantified the relative angle between  
 647 successive tugging events (as defined in **Figure 1f,g**). Using a 2-sample Kolmogorov Smirnov test, we  
 648 found that the probability density of the angles is not significantly different from a uniform distribution. We  
 649 also tracked the relative fluctuations (over the azimuth) in the radius of the mouth at its maximum area.  
 650 This ratio is essentially a measure of the asymmetry of the mouth shape. The relative fluctuations were  
 651 calculated by dividing the standard deviation in the radius of the mouth by the average radius, measured  
 652 when the mouth was at its maximum area.

653

#### 654 **Analyzing mouth openings in microaspiration experiments**

655 Mouth openings were analyzed manually using FIJI. In each recorded video, the frame with the largest  
 656 mouth opening (the frame of interest) was first identified. To do so, the approximate frame of interest was  
 657 found by visual inspection. Then, the mouth areas in this frame and +/- 5 neighboring frames were  
 658 measured using the polygon tool. If the mouth area was largest in this frame of interest, it was selected for  
 659 further analysis. If not, the frame with the largest mouth area was selected as the approximate frame of  
 660 interest and the mouth areas in its neighboring frames were measured. This process was repeated until the  
 661 frame of interest was found.

662 Only videos in which heads did not appear damaged after microaspiration were included in analysis. For  
 663 movies in which multiple mouth openings occurred, the largest mouth opening frame was chosen for  
 664 analysis. The frame of interest from each video was independently analyzed by four researchers, two of  
 665 which were “blind” and knew nothing of the expected outcome for the shape of mouth opening in  
 666 microaspirated heads. All researchers had access to the raw images.

667 The frame of interest was analyzed as follows: A line was drawn connecting the center of the needles on  
 668 each side of the *Hydra* head (or for the control: from the center of one needle to the center of the mouth),  
 669 from which the angle to the image horizontal ( $\theta_1$ ) was recorded. The perimeter of the mouth was then  
 670 traced using the polygon tool, from which the ellipse major, the ellipse minor, and the angle of the ellipse  
 671 to the horizontal ( $\theta_2$ ) were recorded. The relative angle  $\theta_{Rel} = |\theta_2 - \theta_1|$  was calculated and recorded,  
 672 taking care to account for symmetry such that  $\theta_{Rel} \leq 90^\circ$ . The relative angle is plotted in **Figure 3f**. The  
 673 major to minor aspect ratios for the 13 microaspirated and 9 control heads were determined and were each  
 674 found not to differ significantly from a normal distribution (p-value = 0.18 (microaspirated), p-value = 0.66  
 675 (control), two-sample Kolmogorov Smirnov test against a uniform distribution MATLAB 2021a  
 676 (MathWorks)). Microaspirated heads and control heads were statistically compared by evaluating the p-  
 677 value from a two-sample one-tailed Student's *t*-test. The suction pressure (P) on the left (L) and right (R)  
 678 sides of the *Hydra* head was calculated by the formula  $P_{L/R} = \rho g \Delta h_{L/R}$ , where  $\rho$  is the density of water,  $g$   
 679 is the acceleration due to gravity, and  $\Delta h$  is the height difference between the water level in the syringe to

15

680 the bottom of the glass well containing the *Hydra* head. The cross-section areas of the needles were  
681 calculated from the recorded radii. Finally, the total suction force ( $F_{L/R}$ ) on each side of the hypostome was  
682 calculated by multiplying the suction pressure by the cross-section area of the needle.

683

684 **Analyzing mouth openings in innervated:nerve-free chimera**

685 Mouth openings in chimeras were analyzed manually using FIJI. The line tool was  
686 used to demarcate the segments of the mouth opening enclosed by the innervated (or nerve-free)  
687 half. The polygon tool was then used to enclose each segment of the mouth opening and  
688 obtain their areas. The areas of the nerve-free and the innervated segments of the mouth were  
689 individually normalized to their respective maximum areas, so the areas lay between 0 and 1.  
690 The normalized areas of the innervated and nerve-free halves were plotted against time and  
691 individually fit to the logistic equation to obtain the timescales of mouth opening  
692 (the 'd' parameter). The ratio of the d parameters for the A10 and HyBra halves was calculated  
693 for each chimera.