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Supplemental Information

Mechanical Properties of a *Drosophila* Larval Chordotonal Organ

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Supplementary Figures and Tables

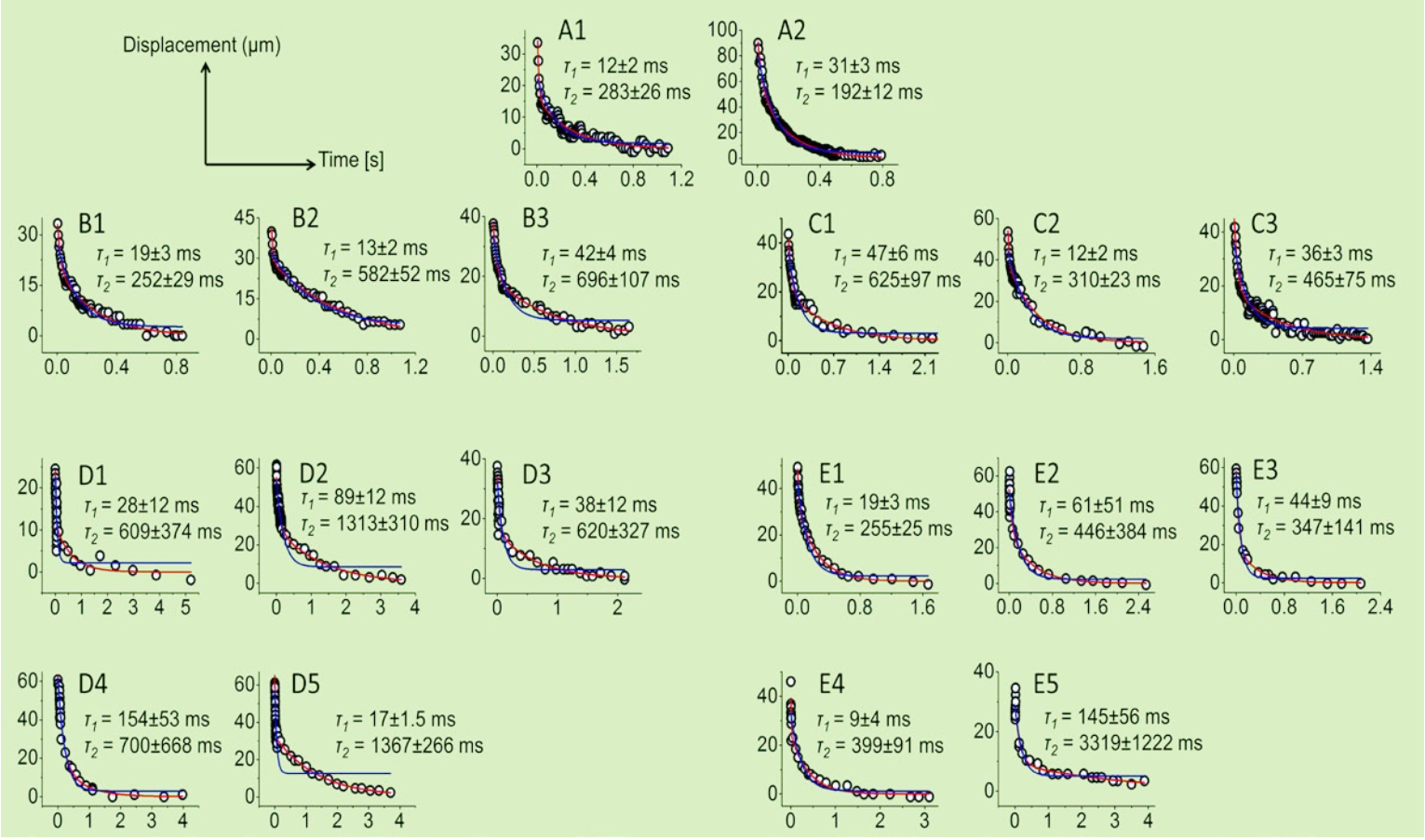

Fig. S1. Relaxation curves of laterally deflected lch5 organs for all the relaxation experiments in larval preparations with intact muscles, showing double-exponential fits (red curves) and single-exponential fits (grey curves). Reduced χ^2 values for single- and double-exponential fits are given in Table S1.

The maximum standard deviation of the error in the data points was approx. 6 µm.

A1, A2: Animal 1, lch5 organ 1
B1, B2, B3: Animal 2, lch5 organ 1
C1, C2, C3: Animal 2, lch5 organ 2
D1-D5: Animal 3, lch5 organ 1
E1-E5: Animal 4, lch5 organ 1

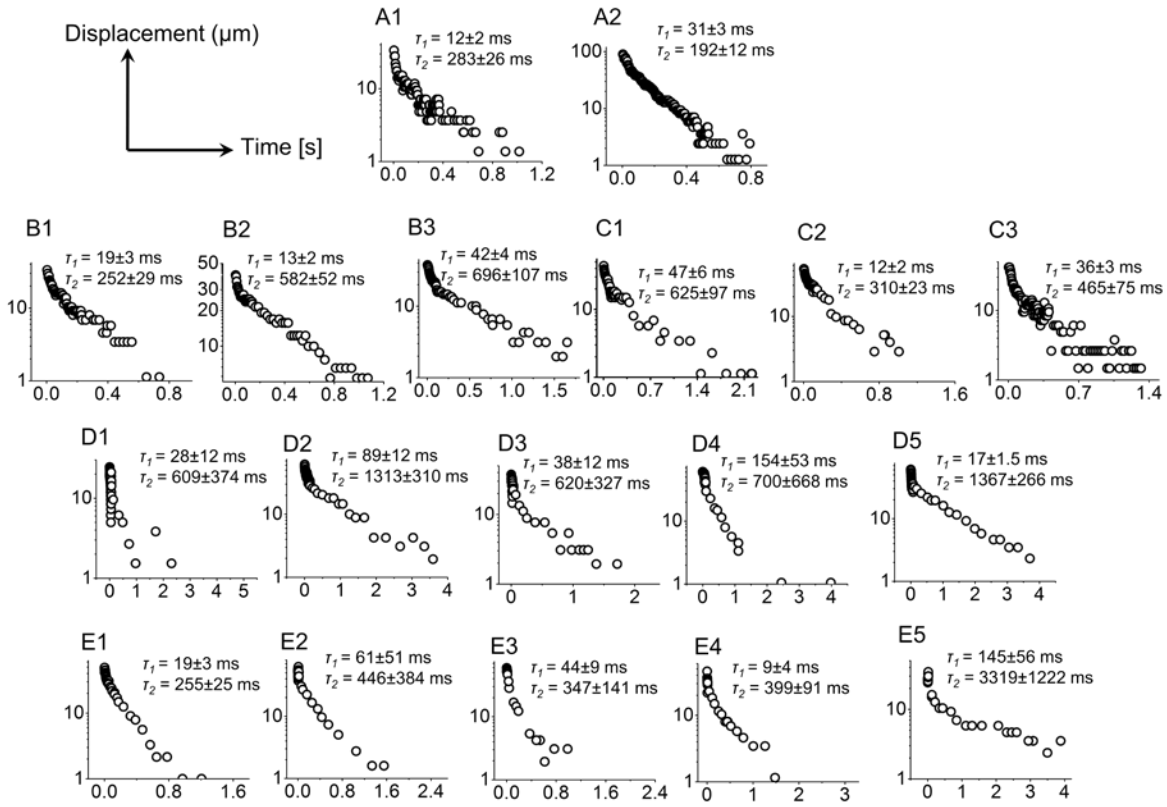

Fig. S2. Relaxation curves of laterally deflected lch5 organs for all the relaxation experiments in larval preparations with intact muscles, with y-axes converted to logarithmic scale. Reduced χ^2 values for single- and double-exponential fits are given in Table S1.

A1, A2: Animal 1, lch5 organ 1
B1, B2, B3: Animal 2, lch5 organ 1
C1, C2, C3: Animal 2, lch5 organ 2
D1-D5: Animal 3, lch5 organ 1
E1-E5: Animal 4, lch5 organ 1

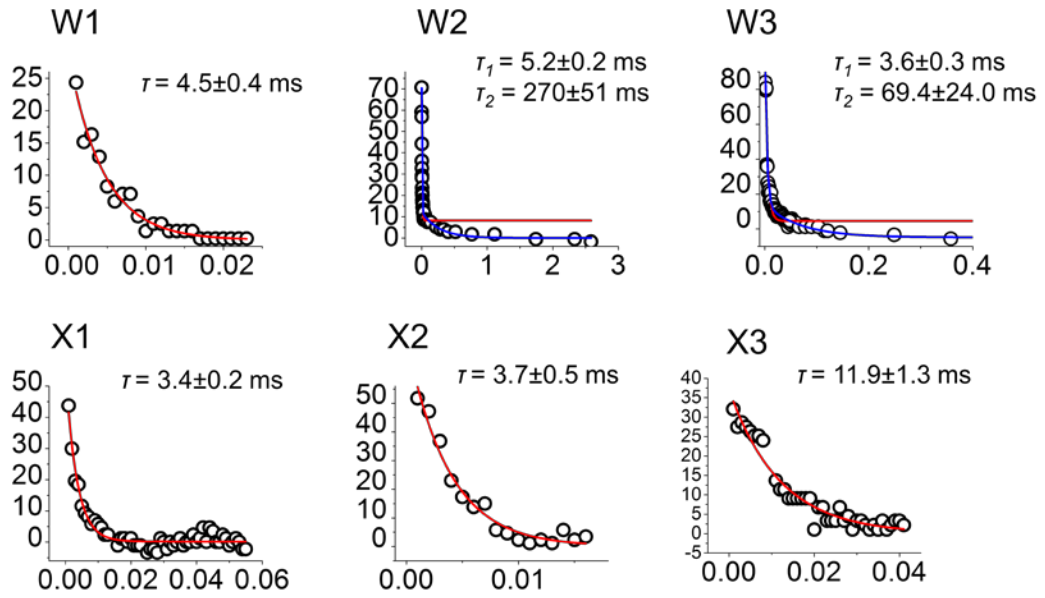

Fig. S3. Relaxation curves of laterally deflected lch5 organs for all the relaxation experiments in larval preparations with excised muscles, showing exponential fits. Red curves represent single exponential fits, and blue curves represent double exponential fits. In the graphs where double exponential fits are not shown, these fits either do not converge, or coincide with the single exponential fits. Reduced χ^2 values for single- and double-exponential fits are given in Table S1.

W1, W2, W3: Animal 1, lch5 organ 1
X1, X2, X3: Animal 2, lch5 organ 2

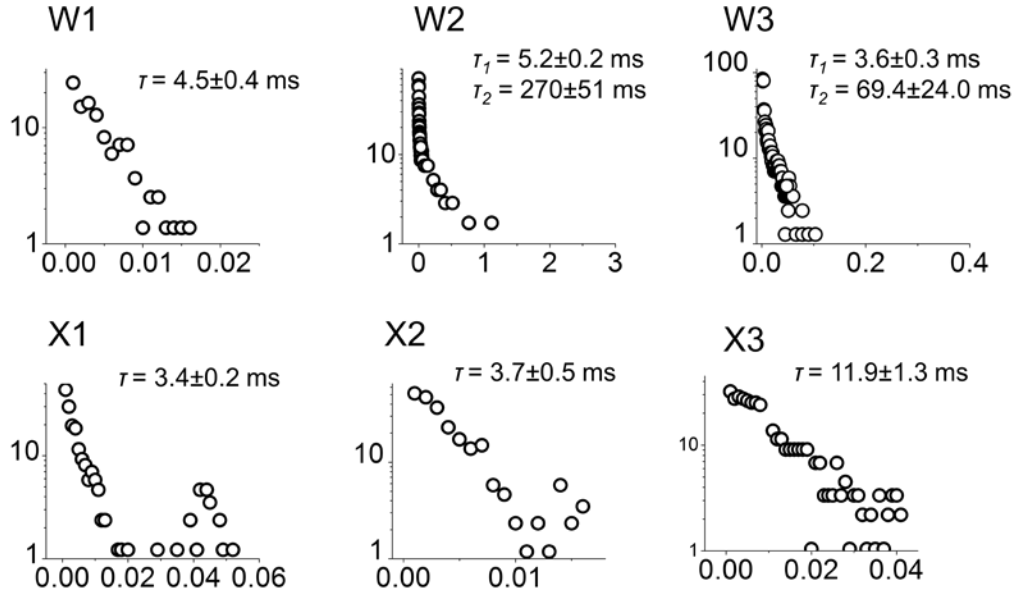

Fig. S4. Relaxation curves of laterally deflected lch5 organs for all the relaxation experiments in larval preparations with excised muscles, with y-axes converted to logarithmic scale. Reduced χ^2 values for single- and double-exponential fits are given in Table S1.

W1, W2, W3: Animal 1, lch5 organ 1
X1, X2, X3: Animal 2, lch5 organ 2

Experimental animal	Ich5 organ	Trial	Reduced χ^2 values	
			Single exponential fit	Double exponential fit
1	A	A1	4.73	1.74
		A2	7.42	2.44
2	B	B1	2.93	1.13
		B2	3.72	0.72
		B3	5.38	0.85
	C	C1	5.84	1.81
		C2	5.51	1.82
		C3	6.14	2.00
3	D	D1	6.62	5.91
		D2	5.89	3.45
		D3	8.53	6.82
		D4	9.14	8.58
		D5	21.38	4.74
4	E	E1	5.94	5.60
		E2	5.27	1.32
		E3	23.40	22.46
		E4	6.15	3.77
		E5	10.60	6.79

Table S1. Exponential fits of the relaxation curves of laterally deflected Ich5 organs. Quality of fit is judged by the χ^2 test. Reduced χ^2 values for double- and single-exponential fits for the data plots from relaxation experiments for Ich5 organs in larval preparations with intact muscles. These values indicate the relative goodness of fit. The model that has a lower χ^2 value is considered as a better fit for the data. The double-exponential model is seen to provide the better fit. In two cases (E1 and E3), the difference in the χ^2 values for single and double exponential fits is small, but nonetheless, the double-exponential model has the lower value.

Experimental animal	lch5 organ	Trial	Reduced χ^2 values	
			Single exponential fit	Double exponential fit
1	W	W1	1.15	1.05
		W2	8.56	1.35
		W3	64.79	66.59
2	X	X1	2.80	2.55
		X2	7.37	8.71
		X3	3.54	3.75

Table S2. Exponential fits of the relaxation curves of laterally deflected lch5 organs. Quality of fit is judge by the χ^2 test. Reduced χ^2 values for double- and single-exponential fits for the data plots from relaxation experiments for lch5 organs in larval preparations with excised muscles. The values indicate the relative goodness of fit. The model that has a lower χ^2 value is considered as a better fit for the data.

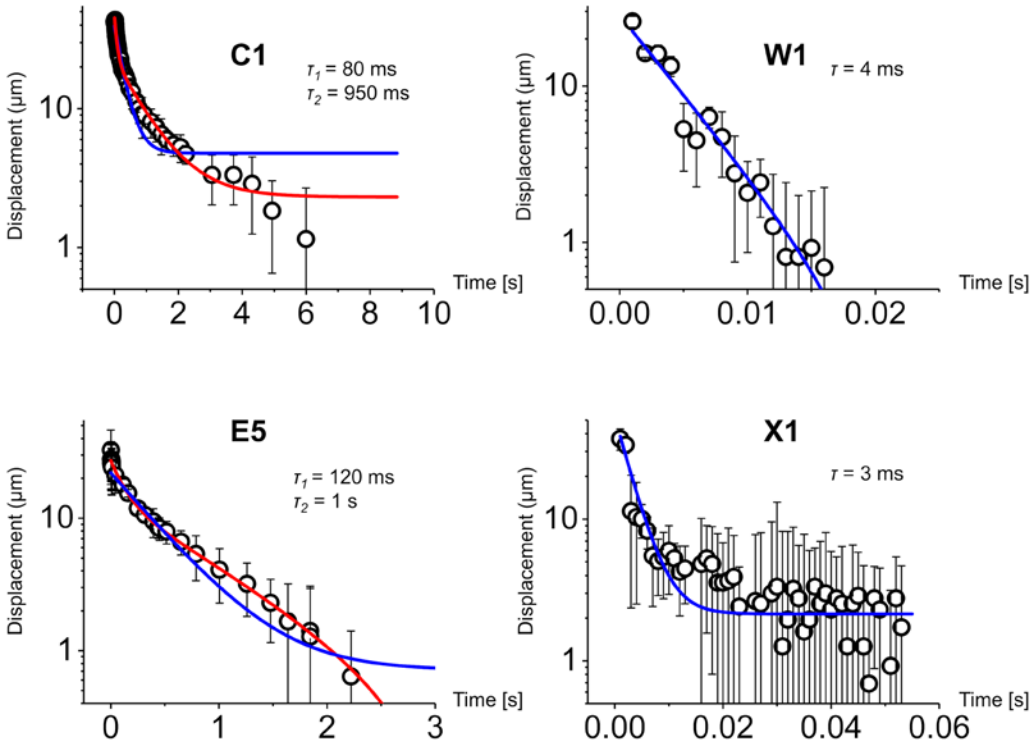

Fig. S5. Error analysis for four experiments: C1, E5 (muscles intact) and W1, X1 (muscles excised). Error bars represent twice the standard deviation of the Y-value.

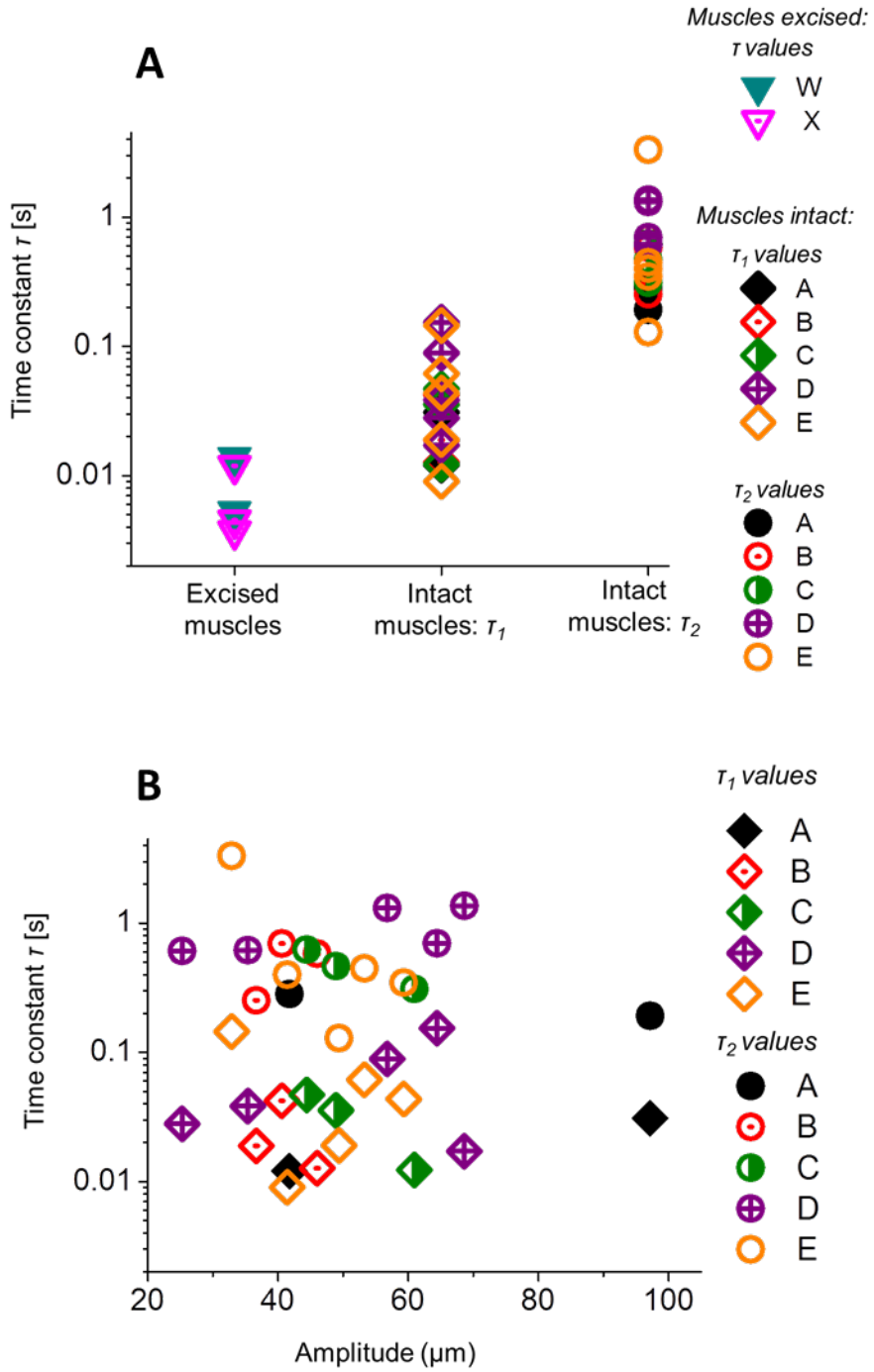

Fig. S6. Time constants for all relaxation experiments. W and X represent the larval preparations with muscles excised (time constants shown as inverted triangles), and A, B, C, D, E refer to the experiments on preparations with intact muscles (see fig. S1 and tables S1 and S2). In this case, diamonds represent the first time constant τ_1 and circles represent the second time constant τ_2. **(A)** Distribution of time constant values (refer to fig. 4C). **(B)** Plot of time constants versus amplitude for the preparations with intact muscles (refer to fig. 4D).

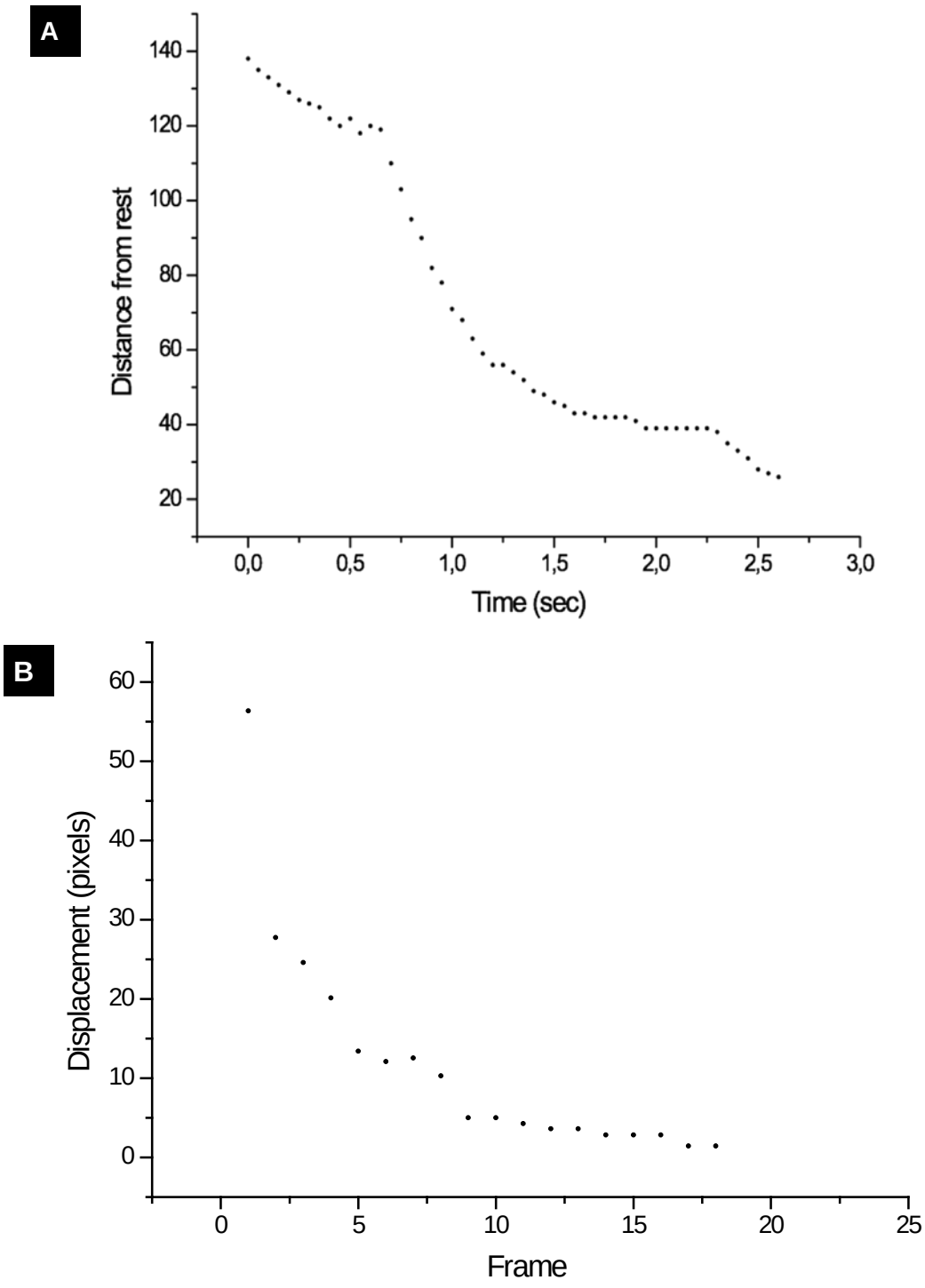

Fig. S7. (A) Incomplete disengagement of the lch5 organ from the tungsten needle, indicated by a plateau in the graph at around 0.5 sec. Displacement values are in pixels. **(B)** Data points missing from the displacement vs time plot, owing to low frame rate of the camera. In this plot, displacement is in pixels, and the X-axis represents the frame number of the video.

Supplementary Movies

Movie S0: Displacement of the muscles by the tungsten needle does not significantly deform the lch5 organ. The tungsten needle enters the sample from the top left corner. Scale bar: 50 µm.

Movie S1, deformation of the lch5 organ during lateral deflection by a tungsten needle: Needle placed near the middle of the organ to analyse the shape of the lch5 organ. Image laterally inverted for convenient viewing in comparison to other images. Arrows indicate the direction in which the scolopales and somata move upon being displaced by the needle. Scale bar: 50 µm.

CA: Cap attachment cell
LA: Ligament attachment cell
sc: Scolopales
n: Neuronal somata

Movie S2, deformation of the lch5 organ during lateral deflection by a tungsten needle: Another example of the same experiment as in M1. Arrows indicate the direction in which the scolopales and somata move upon being displaced by the needle. Scale bar: 50 µm.

CA: Cap attachment cell
LA: Ligament attachment cell
sc: Scolopales
n: Neuronal somata

Movie S3, relaxation of the lch5 organ after lateral deflection by a tungsten needle: Needle placed closer to the scolopales. Arrows indicate the direction in which the scolopales and somata move upon being released from the needle. Scale bar: 50 µm.

CA: Cap attachment cell
LA: Ligament attachment cell
sc: Scolopales
n: Neuronal somata

Movie S4, severing the cap cells: Retraction of dendrites and kink formation at the scolopale-dendrite junction upon severing the cap cells using a laser. The retraction is towards the bottom right. Scale bar: 15 µm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movie S5, severing the cap cells: Same experiment as in Movie S4. The retraction is towards the top left. Scale bar: 15 µm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movie S6, severing the cap cells: Same experiment as in Movie S4. Here only the dendrites and scolopales are visible. The retraction is towards the top left again. Scale bar: 15 μm.

Sc: Scolopales
De: Dendrites

Movie S7, severing the cap cells: Same experiment as in Movie S4. The retraction is towards the top right. Scale bar: 15 μm.

Sc: Scolopales
De: Dendrites

Movies S8-S9, severing the cap cells: Same experiment as in Movie S4. In S8 the dendrites are slightly extended due to application of the laser. In S9 the cap cells are severed, and the dendrites retract. The retraction is towards the bottom left. Scale bar: 15 μm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movie S10, severing the cap cells: Same experiment as in Movie S4. The retraction is towards the bottom left. It is incomplete owing to severing of only 3 cap cells out of 5. Scale bar: 15 μm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movie S11, severing the cap cells: Same experiment as in Movie S4. The retraction is towards the bottom left. Here, the scolopidia are seen to split, with the lower three retracting further than the upper two. This is possibly because the laser was aimed in between two scolopidia. Scale bar: 15 μm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movie S12, severing the cap cells: Same experiment as in Movie S4. The retraction is towards the top right. Scale bar: 15 μm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movie S13, severing the cap cells: Same experiment as in Movie S4. The retraction is towards the left. This video uses bright-field illumination. Scale bar: 15 μm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movies S14-S15, severing the dendrites: UV-Laser cutting of the dendrites leads to rapid retraction of the scolopales attached to the contracting cap cells. The dendrites were cut all at once, under 100X magnification. The scolopales retract towards the top right. Movie S14 depicts the experiment, and Movie S15 (40X magnification) shows the extent of retraction. Scale bar: 15 µm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movies S16-S17, severing the dendrites: Same experiment as in Movies S14-S15, under 40X magnification. The scolopales retract towards the top right. Scale bar: 15 µm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movies S18-S19, severing the dendrites: Same experiment as in Movies S14-S15. Movie S18 was recorded under 100X magnification, and Movie S19 was recorded under 40X magnification. The scolopales retract towards the top left. Scale bar: 15 µm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movie S20, severing the dendrites: Same experiment as in Movies S14-S15. Recorded under 40X magnification. The scolopales retract towards the top left. Scale bar: 15 µm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites

Movie S21, severing the dendrites: Same experiment as in Movies S14-S15. Recorded under 40X magnification. The scolopales retract towards the bottom right. The cap cells are faintly visible and are observed to retract. Scale bar: 15 µm.

Sc: Scolopales
De: Dendrites
C: Cap cells

Movie S22, severing the ligament cells: UV-Laser cutting of the ligament cells subsequent to cutting the cap cells. Axons are still attached to the neuronal cell bodies. The organ retracts slightly to the right. Scale bar: 15 µm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites
L: Ligament
A: Axons

Movie S23, severing the ligament cells: UV-Laser cutting of the ligament cells subsequent to cutting the cap cells. The organ retracts slightly to the right. This was carried out in a Sqh-GFP larva, with fluorescent cap cells and ligament (no fluorescence in the neuronal part). Scale bar: 15 µm.

C: cap cells
De: Dendrites
L: Ligament

Movie S24, severing the ligament cells: UV-Laser cutting of the ligament cells, followed by cutting the axons. When only the ligament cells are severed, there is negligible movement. When then the axons are also severed, the remaining organ retracts strongly and rapidly towards the cap cells. Scale bar: 15 µm.

N: Neuronal somata
Sc: Scolopales
De: Dendrites
L: Ligament
A: Axons

Ablation carried out in	**Movie**	**Retraction observed in**	**Retraction (in µm)**
Cap cells	S4	Dendrites	15
	S5		9
	S6		6
	S7		5
	S8		3 (slight extension)
	S9		10 (retraction)
	S10		6
	S11		15
	S12		8
	S13		9
			Average: 9
Dendrites	S14-S15	Cap cells (indicated by scolopale motion)	120
	S16-S17		134
	S18-S19		62
	S20		81
	S21		62
			Average: 92

Ligament (after ablating axons)	S22	Neurons	28
Ligament	S23	Ligament	7

Table S3. Quantification of the retraction of the lch5 organ observed in the laser ablation videos