The biomechanics of ultra-stretchable nerves

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Highlights
During oviposition the female locust extends her abdomen to 250% its original length.

The abdominal nervous system is unique in accommodating such extreme elongation.

We characterize and quantify the nervous system’s biomechanical properties.

We offer a valuable model for understanding pathologies related to nerve extension.

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The biomechanics of ultra-stretchable nerves

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SUMMARY

When digging in the ground during egg laying the female locust extends her abdomen to 2-3 times of its original length. How the abdominal nervous system accommodates such extreme elongation remains unknown. We characterized and quantified the system’s biomechanical response using controlled ex vivo elongation and force measurements. The microstructure of the nerves was studied using histology and high-resolution confocal microscopy. Although the nervous system of sexually mature females demonstrated fully reversible hyper-extensibility of up to 275%, the elongation observed in premature females and males was much more limited. The unique extension dynamics of the different groups were captured by their very different force-displacement curves. Confocal microscopy suggested that elongation is not owing to undulations of the nervous system structure. Thus, the exceptional resistance to deformation and rupture presents the female locust abdominal nervous system as a valuable model for understanding the functionality and pathology related to nerve extension and reversible elongation.

INTRODUCTION

The interest in the stretchability of nervous tissues and their potential relevance to injury repair goes back to the 18th century (Fontana and Gibelin, 1781). Typical stress-strain curves for peripheral nerves in the human body reveal that our nerves may experience the elongation of up to around 30% before they become irreversibly damaged (Topp and Boyd, 2006). Indeed, while stretch injuries are among the most devastating forms of peripheral nerve injury (Mahan et al., 2019), our understanding of nerve biomechanics is both conflicting and insufficient (Mahan et al., 2019; Topp and Boyd, 2006; Ommaya, 1968). There are very few exceptional cases of vertebrates (Vogl et al., 2015), and some invertebrates (Koike, 1987), that offer notable examples of the capacity of nerves to sustain tension and stretch following remarkable elongations. Detailed characterization of such examples and, moreover, insights into the underlying mechanisms, are extremely important if we are to fully understand the biomechanical aspects of nervous systems in health and disease.

A unique example of the extreme elongation of nerve tissue, critical for executing a key biological function, is offered by the locust. In the female desert locust, great stretchability is displayed when she extends her abdomen underground during oviposition digging (Vincent, 1976; Jorgensen and Rice, 1983), a first step in the egg-laying sequence, aimed at providing the eggs with safe and optimal conditions for hatching. Abdominal extension and elongation are achieved by means of dedicated structures at the tip of the abdomen that serve both to excavate into the substrate and to “anchor” the tip of the abdomen (Das et al., 2022). This more than 2-fold elongation of the locust abdomen is, of course, accompanied by longitudinal stress-induced elongation of the abdominal nervous system (ANS). This is the part of the insect ventral nerve cord (the functional equivalent of the vertebrate spinal cord) that serves to coordinate neural signaling from the brain to the body and vice versa, to integrate sensory input, and to control locomotor output. The insect ventral nerve cord is composed of a chain of ganglia (comprising five abdominal ganglia) connected longitudinally by commissures or connective nerves (Burrows, 1996). These connectives constitute bundles of axonal processes and their accompanying connective tissue, very similar to the major peripheral nerves of vertebrates (insect nerves, however, are unmyelinated).

Not only are the mechanical properties and the mechanism enabling extreme stretching of the locust ANS unknown, but the unique dynamics of this mechanism have also been little studied. Moreover, egg-laying is, naturally, a behavior exhibited only by sexually mature gravid females (several times within the life span of the female). Although the nervous system of the mature female locust is attributed a

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higher functional complexity in comparison to that in premature females or in males (Fuchs et al., 2006), the related changes in the biomechanics of the nervous system are still far from being fully understood.

In order to elucidate the unique mechanical properties that enable such extreme complexities, we set out to comparatively characterize the biomechanical response of the locust ANS to controlled applied stretch, at different life stages of the female (in relation to sexual maturation), as well as to compare it to that of male locusts. We found that only in the sexually mature females was fully reversible hyper-extensibility possible, while the elongation capacity of the premature females and of males was much lower. Preliminary exploration of the nerve tissue microstructure failed to support an undulatory folded structure as a major source of extensibility. We, therefore, suggest that such elongation is a result of the natural elasticity of the tissue, observed here only in the mature females. Our findings open the way for future studies related to the functionality of hyperextended nerves, as well as their pathology. They also offer important insights toward the development of bio-inspired stretchable and repairable materials.

RESULTS
Female locust subterranean oviposition
The locusts in our experiments were of one of the three groups (Figure 1): 1) young, premature females (PM), age = 6-9 days post adult emergence; 2) sexually mature females (SM), age ≥ 30 days post-moult to adult (the selected females were observed to be copulating with, or mounted by, sexually mature males); and 3) sexually mature males (M). Confirming ample previous reports (Vincent, 1976; Fuchs et al., 2006; Thompson et al., 2014; Das et al., 2022), we were able to observe extreme elongation of the female locust abdomen during oviposition. Figure 2 presents a sequence of images taken from a video, in which the female’s abdomen can be seen as more than doubled in length, with the segmented cuticular plates of the abdomen (appearing whitish) separated by a stretched intersegmental membrane (yellowish). The tip of the abdomen is indicated by white arrows.

Figure 2 depicts the extreme extension capacity of a mature female’s abdomen, followed by a remarkable full recovery after retraction. Retraction can be slow, when accompanied by egg laying and foam secretion (the careful laying out of the egg-pod). However, it can also be extremely swift in cases where oviposition failed owing to some interruption (e.g. by other locusts), or if the female decided that soil conditions were inappropriate (humidity or salinity). In such cases, full retraction was observed within less than a minute, or even within a few tens of seconds (Figure S1 in the supporting information).
Manual ex vivo extension of the abdominal nervous system

We conducted a set of preliminary experiments aimed at establishing the stretchability of the ANS. We measured the length of the intact abdomen, and the length of the ex vivo dissected ANS, before and after manually stretching it by holding it between two surgical forceps and gently extending until it ruptured. We compared our three experimental groups: young, premature females (PM), sexually mature females (SM), and males (Figure 3). The externally measured length of the abdomen did not differ between the two female groups in the resting state (31.3 ± 1.9 mm, N = 7, and 32.8 ± 1.4 mm, N = 9, for group PM and SM, respectively). Although the length of the ex vivo ANS at rest also did not significantly differ between the groups, the mean length after stretching was significantly different (Figure 3B; p < 0.0001, ANOVA ). The ANS of SM females could be stretched to an average of 214%, and up to 249% in extreme cases, compared to only 164% in most PM females, and up to 186% in an extreme case (Figure 3C). In males (N = 5), the abdomen length and length of the ANS at rest were similar to those in females (29 ± 1.7 mm), and the ANS could be stretched up to 47.8 ± 1.8 mm, corresponding to 140.5% of the original length. In all females, the ANS extension was not homogeneous along the entire ANS, as illustrated in Figure 3. Comparing the degree of stretch of the different connective sections (between consecutive ganglia), revealed that it ranged from 145% to 186% in PM females, and from 172% to 257% in all the SM ones.

Quantitative analysis of locust abdominal nervous system biomechanics and variations in mechanical strains within the abdominal nervous system

We next quantified the extensibility of the ANS in a controlled fashion: namely, in an aqueous medium under regulated temperature, applying controlled and consistent force, while monitoring time, length, and load (Figure 4). Examples of two representative experiments, stretching the ANS of a premature and a sexually mature female, are presented in Figures 4A and 4B, respectively. The images are extracted from video-recordings of the two experiments, from the initiation of extension (i) until shortly before and immediately following rupture of the ANS (iii and iv, respectively). The relative positions of the ganglia are indicated by the dashed red lines to better appreciate and compare the elongation.

The dynamics of the forces and extensions measured in our experiments with the locust ANS of sexually mature females (n = 7), premature females (n = 8), and mature males (n = 4) are summarized in Figure 5.
We were careful to start each experiment with the ANS in a loose (minimal strain) state. An initial length of 40 mm throughout satisfied this criterion. The averaged force-displacement curves are shown separately for each group (Figures 5A–5C). As is well demonstrated in the overlay of the averaged curves in Figure 5D, the mature females’ ANS could be stretched to a much greater length before rupturing. The extreme differences are clear in the comparisons of both length (mm) and degree of stretch (%), where 40 mm = 100%.

The fluctuations (or steps) in the force-displacement curves are possibly the result of sequential rupture of connective tissue, as well as of the nerve fibers comprising the nerve cords, during extension. Figure S2 in the supporting information shows representative force-displacement curves for each group.

Figure 6 summarizes the controlled stretching experiments of the overall ANS in terms of strain (Figure 6A), force at failure (Figure 6B), and normalized work to failure (Figure 6C). Similar to the data obtained by manual stretching (Manual ex vivo extension of the abdominal nervous system), a highly significant difference in the mean strain after stretching was observed between the two female groups ($p < 0.0001$, Student’s $t$ test), while the premature female and male groups did not differ much (Figure 6A). Interestingly,
the highest force at failure was recorded for the premature females (Figure 6B), whereas no significant difference in tensile forces was found between the mature male and mature female groups. The work to failure (Figure 6C), corresponding to the energy for complete rupture, is calculated according to the area under the force-displacement curves. After normalizing by the initial sample length, this was found to be maximum for the sexually mature females, and minimum for the males.

We next quantified the differential stretching of the ANS when divided into three sections, as illustrated in Figure 6D. The relative positions of the ganglia before and after elongation (Figure 4) indicate that the segment between the second and fourth ganglia experiences a larger elongation in comparison with the other segments (between the first and second, and fourth and fifth ganglia). In order to quantify these differences, we compared the elongation of three sections: Section 1, comprising ganglia 1 and 2, section 2, comprising ganglia 2, 3 and 4, and section 3, comprising ganglia 4 and 5 (Figure 6D). We compared elongations of each section in eight sexually mature female ANS, and found that the mid-region of the ANS was the most stretchable section.

Reversibility of abdominal nervous system extreme elongation
In our controlled ANS extension experiments described above, in two cases we reached the full extension potential enabled by the measuring device without rupture of the mature female ANS. In these cases, we allowed the system to relax progressively by means of controlled unloading (at a rate of 1 mm/s). In both cases, we observed full reversibility of the nerves to their original length after unloading (Figure 7). Notably, upon the retraction of the sample holder to its original position (panel V in Figure 7), the ANS did not appear to lose its tensile integrity and remained stretched between the grippers. Only beyond this point did we start to observe a curved-loose configuration (Figure 7vi).

Morphology of the abdominal nervous system
Figures 8A and 8B demonstrate the structural differences observed between the nerves (connectives) of premature and sexually mature females, respectively (overall, three experiments were conducted, each comparing dissected nerves from two females). Even prior to immunostaining and histology it was clear that the latter appears thicker. As can be seen this is owing to much more profound outer layers of connective tissue (nerve membranes). A slight undulation of the axonal processes is more apparent in the sexually mature female nerves. These undulations can account for approximately 20% of the extension during oviposition. However, we failed to observe any clear changes in the morphology of the sexually mature
female preparation upon stretching it to twice its original length prior to fixation (this was independently conducted in two experiments), seen in Figures 8C and 8D, respectively.

DISCUSSION
Stretchable and repairable materials underpin spectacular biological functions, from the consistent strains experienced by the pumping heart (Thompson, 1986) to the ability of an octopus to squeeze through small crevices (Liu et al., 2019). Highly stretchable biological materials have provided inspiration for engineering soft materials used in emerging technologies, from 3D printing of synthetic organs to the development of soft (Mahan et al., 2019) and stretchable materials, (Young, 1971; Ku et al., 2020; Wang et al., 2022; Vatankhah-Varnosfaderani et al., 2017) wearable devices (Sun et al., 2012) and robotics (Wu et al., 2020; Kim et al., 2013; Coyle et al., 2018; Whitesides, 2018). However, unwanted or uncontrolled tissue stretching can also lead to medical injuries, causing extreme and undesired morphological and functional changes (Wallin et al., 2018). From a clinical perspective, it is important to understand what level of external strain can be applied to soft tissues without causing rupture or damage (Deumens et al., 2010; Ommaya, 1968; Mahan et al., 2019; Bora et al., 1976). Hence, understanding how nature has been able to develop extendable organs may offer new knowledge for future medical treatments and the design of synthetic materials.

During a successful oviposition event, the sexually mature female locust extends her abdomen deep underground over the course of ~30 min. After reaching a desired depth, the eggs are deposited and the abdomen is then retracted, again over the course of ~30 min. This process is then repeated in the next oviposition event, overall suggesting the existence of controlled stretching and unstretching mechanisms. Retraction of the abdomen is much more rapid, however in a failed attempt, suggesting a purely elastic mechanism, similar to that of a rubber band (Budday et al., 2017a, 2017b; Ilton et al., 2018; Prado et al., 2020). The maximal ex vivo extension of the ANS of mature females following manual stretching, up to roughly three times the initial length, was in full accord with the abdominal extension, and markedly and significantly more than

Figure 5. Force-displacement curves calculated for the different experimental groups
(A–C) Solid lines denote the average curves. The colored background of each average curve denotes the SD (n = 7, 8, and 4 for the sexually mature females, premature females, and mature males, respectively).
(D) In scale overlay of the averaged curves.
that seen in the premature females. Although the strain rate was not controlled in these experiments, the residual strain suggested that the ANS is viscoelastic, as expected (for biological tissue).

Results of our controlled force measurements confirmed the sex and age dependency of the ANS extensibility, also adding clear differences in the force at failure, reinforcing the observation that the mature female ANS is much softer. The work to failure, determined as the energy needed in order for the tissue to rupture, was the highest for the mature females. When tested, the elongation of the ANS proved to be completely reversible. Such stretchability and, moreover, reversibility, i.e. retaining tension after large extensions, are uncommon in nerve tissues. In the case of the locust, this is a feature characterizing only the sexually mature female, and is not demonstrated by the male or the premature female ANS, suggesting the existence of some control by (hormonal) signals related to female sexual maturation.

Similar to our finding in the locust nerves, most nerve tissues in humans and other mammals exhibit ultra-soft behavior and high nonlinearity in uniaxial and combined multiaxial mechanical testing (Budday et al., 2017a, 2017b; Goriely et al., 2015; Miller and Chinzei, 2002; Budday et al., 2017a, 2017b). The biomechanics of brain tissues are broadly explained by the Ogden hyperelastic model for isotropic incompressible materials (Mihai et al., 2015, 2017; Brewick and Teferra 2018; De Rooij and Kuhl, 2016). This model captures the hyperelastic behavior of soft biological tissues undergoing large deformations. In addition, owing to the high water content in such tissues, they display a viscoelastic behavior (Safa et al., 2021; Lu et al., 2006). The inclusion of viscoelasticity in the hyperelastic model - namely, the model including both elastic and time-dependent elements, improves the agreement with our experimental results. The mechanical properties of nerve tissue have therefore been best described by adopting hyper-viscoelastic material models (Hosseini-Farid et al., 2019).

In addition to the biomechanical properties of the tissue itself, reports in the literature of hyper-elongation of nerves can be attributed to the following reversible structural conformations: a) undulation or zigzagging of the tissue; or b) stretchability of the nerve sheath (in the case of mammals). An undulating structure of nerve tissues has been reported for a variety of species, including both mammals and non-mammals.
such as fish and birds (Clarke and Bearn, 1972). However, extreme stretchability of more than 200% is rare. In mammals, for example, nerves that experience hyper-extension of more than twice their original length are found in the tongue of the rorqual whale. There, stretchability is enabled through a dedicated geometric design, in which the unstretched nerves are folded like an accordion (Vogl et al., 2015). When the tongue stretches during feeding, the folds straighten out and thus allow reversible extensions, adding up to 115% to their original length, without damage to the actual tissue. The nerve fibers are concentrated in a small core of the surrounding nerve’s protective sheath, covered with collagen and elastin biopolymers, while also being highly folded. The elasticity of the biopolymers, combined with the accordion-like folding of both the nerve and nerve protective sheath, enable the application of high strain on the biopolymers themselves, and a consequent overall high elongation of the rorqual whale’s tongue nerves.

Figure 7. Reversible elongation of nerves in mature female locusts
Consecutive snapshots taken from a video-recording of an experiment exemplifying reversibility following unloading. Red arrows indicate the direction of the holder motion. Green dashed lines are a guide to the eye, indicating the state of the ANS.
Another example of extreme elongation of nervous tissues, aided by an undulating-folded geometry has been reported in the sea slug *aplysia* (Koike, 1987). In the *aplysia* nerve, which is easily elongated up to ~5 times its resting length, the relaxed nerve bundle demonstrates zigzagging of the axons and numerous folds in the axon protective membrane. The zigzagging course of the axon is suggested to be responsible for the first phase of elongation, in which the axons’ actual length does not change but takes a straight course upon nerve extension. This, however, can explain elongations of up to 1.2-1.5 the original tissue length only. The second phase appears to be caused by the actual extension of the axons and can be explained by the reduction of extensive wrinkling of the membrane. Notably, this biomechanical process takes place without impairing nerve functionality: namely, the ability to transmit electrical messages along the axons in the nerve. To the best of our knowledge, however, this hypothesis has not been fully confirmed by additional studies.

Based on the reported examples noted above, we expected the ANS of sexually mature female locust to present an extreme undulated-folded structure of both the axonal bundles and accompanying membranes and connective tissue. Surprisingly, however, while the nerves of the sexually mature female possess more highly developed accompanying connective tissue, compared to that in the premature ANS, only a very moderate undulation of the nerves was observed. Hence the stretchability of the locust nerves does not appear to be enabled by an undulated geometry. The tentative mechanism should therefore be sought at the level of the biomechanical properties of the tissue. To the best of our knowledge, the biomechanics, specifically stretchability, of the insect nerve-associated connective tissue have not been investigated or reported to date (note that insect nerves are not encased in myelin).

**Conclusions**

The abdominal nervous system of mature female locusts can elongate up to approximately 250% of their original length. This remarkable hyper-extension of the nerve system is found only in sexually mature females, while premature females and males exhibit much smaller elongations. Moreover, this stretchability is fully reversible within the physiological range of extension rates and can be repeated several times during the lifetime of the mature female locust. Although extreme elongations of nervous tissues are usually attributed to undulated structures or extensibility of the nerve sheath in the case of mammals, a histological study of the locust nervous system did not reveal any significantly undulating structures. Namely, sexually mature females exhibit very slight undulation that cannot explain the hyper-extension of the ANS tissue.
Limitations of the study

In light of the above findings, it is important to further characterize the biomechanics of the unique hyper-extension of the sexually mature female locust abdominal nervous system. Emulating the mechanism of reversible stretching of the locust nerves may conduce to promoting new models of tissue regeneration and repair, as well as the structural design and development of soft robotic systems with a large degree of stretchability and multiple degrees of freedom.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105295.

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AUTHORS CONTRIBUTIONS

R.D.: experiments, formal analysis, investigation, methodology, visualization, writing. A.A.: conceptualization, experiments, investigation, supervision, validation, writing. M.G.: experiments, formal analysis, writing. A.I.: experiments, formal analysis, writing. E.P.: supervision, resources, validation. B.-E.P.: conceptualization, investigation, resources, supervision, validation, writing. All authors gave final approval for publication and agreed to be held accountable for the work performed herein.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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### STAR★METHODS

#### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Chemicals, peptides, and recombinant proteins | | |
| Sodium chloride | Bio-Lab Ltd | Cat No 001903059100, CAS[7647-14-5] |
| Potassium chloride | Merck | K31302936951, CAS [7447-40-7] |
| Calcium chloride | Sigma-Aldrich | 017013002 CAS [10043-52-4] |
| Sodium hydroxide | Bio-Lab Ltd | Cat No 001908029100, CAS[1310-73-2] |
| Hepes | Bio-Lab Ltd | Cat No 000804235600, CAS[7365-45-9] |
| PFA | Electron Microscopy science | Cat No 15714, CAS[3291471] |
| PBS | Gibco | Cat No 14200-067, CAS[11530486] |
| Methanol | Bio-Lab Ltd | Cat No 001368052100, CAS[0010005760] |
| Bovine Serum | Biological Industries | Cat No 041271A, CAS[2137767] |
| Triton | Sigma | Cat No 93443, CAS[9036195] |
| Fluorescein Phalloidin (Invitrogen) | Thermo fisher | Cat No A12379, CAS[2515909] |
| VectaShield | Vector laboratories | Cat No H-1000, CAS[ZH1108] |
| Sylgard | GES-global environmental | Cat No 41201841, CAS[243184950500] |
| Software & and Algorithms | | |
| Fiji | https://imagej.nih.gov/ij/ | V.2.0.0 |
| Origin 2020 | Origin Lab | https://www.originlab.com/ |
| Other | | |
| Mechanical Testing Bench | TA Instruments | https://www.tainstruments.com/testbench-systems/ |
| Nikon Ti microscope equipped with a Yokogawa CSU X-1 spinning disc | Andor | https://andor.oxinst.com/ |
| Andor iXon897 EMCCD camera | Andor | https://andor.oxinst.com/ |
| Andor IQ3 software | Andor | https://andor.oxinst.com/ |

#### RESOURCE AVAILABILITY

##### Lead contact
Further information and requests for resources should be directed to and will be fulfilled by the lead contact Dr. Bat-El Pinchasik (pinchasik@tauex.tau.ac.il).

##### Materials availability
This study did not generate new unique reagents.

##### Data and code availability
Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

#### METHOD DETAILS

##### Experimental model and subject details
Locusts were obtained from our desert locust, Schistocerca gregaria, colony at the School of Zoology, Tel Aviv University. The colony is maintained in highly crowded conditions, under 12:12, L:D regime, at 30°C during the night, and up to 36°C during the day, ~50% humidity. Care was taken to maintain age-synchrony in the breeding cages such that adult emergence of all insects in a cage was within ±3 days.
**Video recording of female oviposition**

In order to observe the process of extension and elongation of the female locust abdomen during oviposition, several sexually-mature females observed to have been mounted by males (prior to or following copulation) were transferred from their cage in our breeding colony to a small metal cage with a glass front. They were denied access to any substrate suitable for oviposition for several days, before allowing them to attempt oviposition in a 2 cm wide and 15 cm deep wet-soil-filled chamber, adjacent to the glass wall of the cage (below the cage floor level). A video camera was positioned overnight to capture any digging and oviposition activity.

**Sample preparation for mechanical testing**

The locust ANS (Figure 1C), comprising the five abdominal ganglia and their major connectives, was dissected out of the insect under a stereo-microscope, following quick decapitation and a longitudinal dorsal cut along the body. In order to access the ANS we first removed the digestive tract, reproductive system, fat, and air sacs as needed. The state of development of the reproductive system (e.g. the presence of mature eggs; Figures 1A and 1B) was noted in order to confirm the status of the different experimental groups. The third thoracic ganglion with a piece of ventral cuticle was left intact on the rostral end of the dissected preparation, and similarly, on the caudal end, the ovipositor valves were kept intact in order to allow the pinning and later clamping of the preparation without damaging the ANS (Figure 1C). The preparation was pinned onto a Sylgard coated Petri dish under chilled physiological solution (locust saline (Abrams and Pearson, 1982; Ayali et al., 2002)) for further cleaning of trachea and fat tissue that are present along the nerve cord. Locust saline contained 147 mmol L\(^{-1}\)NaCl, 10 mmol L\(^{-1}\)KCl, 4 mmol L\(^{-1}\)CaCl\(_2\), 3 mmol L\(^{-1}\)NaOH, 10 mmol L\(^{-1}\)HEPES, pH 7.2–7.4. While this process was kept to a minimum, to avoid possible mechanical damage to the ANS, care was also taken to ensure that any remaining connective tissue or loose parts of the fat sleeve surrounding the connectives would be transected in multiple locations. This was in order to ensure that the connective tissue would not be available to participate in bearing longitudinal stress or load during the experiments. The entire process, from initiating the dissection to conducting the experiment, lasted no more than 30 min (usually much less).

**Mechanical testing**

The delicate nerve preparations were carefully mounted using the polymeric grips of the mechanical testing bench (TA Instruments, USA). The preparation was clamped gently by the grips (see description of the gripping points above in 2.3), leaving the entire ANS submerged within the saline bath (locust saline) of the testing system throughout the experiment. The ANS was mounted in a straight (devoid of curve or bend), but unstretched manner (zero load). The grip clamping the rostral end was immobile while that clamping the caudal end could move and generate extension at the calculated physiological speed of abdominal elongation during oviposition (0.03 mm/s). Time, length, and tensile force were constantly monitored and recorded by the load cell of the system, together with an HD video-recording (Panasonic DC-S1 with Sigma 24–70 mm F2.8 DG DN lens, 1280 × 720P, from 40 cm distance). The entire process of ANS extension was captured, until rupture. In the rare cases (2 out of 20) where extreme extension (to the limit of the instrument) did not result in rupture, extension was followed by controlled retraction, at a rate of 1 mm/s, until the ANS was totally loose (revealing a clear curving and bending of the connectives). We chose a retraction rate that was somewhat faster than the extension rate to reflect the fact that in natural conditions retraction may be either gradual or rapid (in cases of failed oviposition; See results below). The force versus displacement curves obtained from these experiments were used for further calculations.

**Histology**

The ANSs were dissected as above and maintained in chilled locust saline until fixation (up to 60 min). For the purpose of immunostaining and histology, samples were prepared from a section of the dissected preparation (comprising the second abdominal ganglion and a section of the paired connectives), and thoroughly cleaned of all non-nervous tissue. Samples were fixed in 4% PFA for 16 h at 4°C, then washed in PBS and permeabilized with ice-cold MeOH at −20°C for 5 min, blocked, and further permeabilized with 10% fetal bovine serum and 1% Triton for 2h. Samples were agitated and actin was stained with Fluorescein Phalloidin (Invitrogen) at 10 µg/mL for 1h. Finally, samples were mounted with VectaShield (Vector Laboratories). Cover slides were sealed with nail polish until use.
Confocal images were captured using a Nikon Ti microscope equipped with a Yokogawa CSU X-1 spinning disc and an Andor iXon897 EMCCD camera controlled by Andor IQ3 software. Image analysis was performed using FIJI ImageJ V.2.0.0.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Statistical significance of the difference between any two data groups was demonstrated using Student’s t test. ANOVA was used for multiple comparisons. For all the experimental results shown, the error bars indicate the variation in the data and the details of the statistical tests are presented in the figure legends.