Drosophila Neurons Actively Regulate Axonal Tension In Vivo

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ABSTRACT Several experiments have shown that mechanical forces significantly influence the initiation, growth, and retraction of neurites of cultured neurons. A similar role has long been suggested for mechanical forces in vivo, but this hypothesis has remained unverified due to the paucity of in vivo studies of neuronal mechanical behavior. In this study, we used high-resolution micromechanical force sensors to study the mechanical response of motor neurons in live *Drosophila* embryos. Our experiments showed that *Drosophila* neurons maintained a rest tension (1–13 nN) and behaved like viscoelastic solids (i.e., with a linear force-deformation response followed by force relaxation to steady state) in response to sustained stretching. More importantly, when the tension was suddenly diminished by a release of the externally applied force, the neurons contracted and actively generated force to restore tension, sometimes to a value close to their rest tension. In addition, axons that were slackened by displacing the neuromuscular junction contracted and became taut in 10–30 min. These observations are remarkably similar to results from in vitro studies and suggest that mechanical tension may also strongly influence neuronal behavior in vivo.

INTRODUCTION

In recent years it has become increasingly evident that mechanical stimuli play an important role in the differentiation, growth, development, and motility of cells. Cells sense and respond to cues from their mechanical microenvironment as well as externally applied mechanical stimuli. For example, the lineage of stem cells is altered by the stiffness of the substrate on which they are grown (1), locomotion of epithelial cells and fibroblasts is regulated by substrate stiffness (2), and cell growth and development are dependent on substrate compliance (3).

Neurons in particular have been shown to be highly sensitive to a variety of mechanical inputs. Unlike most other cell types, in vitro neurons prefer to grow on soft substrates (4) and extend more branches on soft substrates than on stiff ones (5). Several studies have shown that neurites actively respond to mechanical forces. For example, Bray (6) showed that neurites undergo apparently normal elongation when towed with an appropriately paced motor. More recently, Pfister et al. (7) demonstrated that axonal elongation (up to several centimeters) can be induced by mechanical tension, and these axons retain their electrophysiological functions (8). The intimate link between tension and axonal elongation is underscored by the fact that the elongation rate of PC-12 neurites, chick sensory neurons, and chick forebrain neurons all follow a robust linear relationship with applied tension (9–11). In addition, experiments have also shown that tension applied to the margins of neuronal cell bodies can initiate neurites that exhibit all the typical characteristics of spontaneously initiated (growth cone-mediated) neurites (6,10). Based on these observations and the evidence that advancing growth cones pull on the neuronal cell body, Heidemann et al. (12) suggested that under both in vivo and in vitro conditions tension acts as a proximate stimulus and regulator of axonal elongation.

The prominent role of tension in neuronal function is further underscored by the observation that a sudden reduction/loss of tension results in retraction of previously stable neurites in vitro. For example, chick sensory neurons subjected to neurite slackening undergo retraction and recover their tension, in many cases to a level greater than the initial value, within a period of 60–90 min (9). Similar behavior is also seen in chick forebrain neurites, but to a much lesser degree (11). Based on these observations, it has been suggested that a similar mechanism may underlie the retraction of axons from neuromuscular junctions (13–15), which results in a pattern of innervation in which only one motor neuron synapses with each skeletal muscle fiber. In fact, experiments have provided evidence that branches of developing axons that experience a buildup of mechanical tension stabilize and, in the process, cause the retraction of other branches and axon collaterals (16). Of interest, application of mechanical forces above a threshold on the growth cone has also been shown to cause neurite retraction in a Ca^{+2}-dependent manner (17).

As outlined above, numerous studies have revealed the important role of mechanical tension in the initiation, development, elongation, and retraction of neurites in vitro. A similar role has been long suggested for mechanical forces in vivo. Weiss (18) first suggested that the final phase of elongation in peripheral neurons after the axon synapses with its target is mediated by the tension applied by the moving target. Van Essen (19) hypothesized that tension in axons may underlie many aspects of morphogenesis of the brain, especially the cortical regions of the brain. For example, he suggested that the folding of the cerebral cortex is due to the tension exerted by axons that connect relatively

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distant regions of the brain, and that the folding minimizes the communication time between interconnected brain regions. Unfortunately, many of these hypotheses remain unverified because studies detailing the in vivo mechanical behavior of neurons have been rather limited.

However, some recent experiments (20) have provided new evidence of the role of mechanical forces in the functioning of neurons in vivo. These experiments have shown that vesicle clustering in the presynaptic terminal of the neuromuscular junction in *Drosophila* embryos is dependent on mechanical tension in the axons. Vesicle clustering disappears with loss of mechanical tension and is regained upon restoration of tension. In addition, an increase in tension appears to increase the vesicle density at the synapse, suggesting that mechanical tension could be a signal to modulate synaptic plasticity in vivo.

If mechanical tension indeed modulates synaptic plasticity, one would expect neurons to respond to stimuli that alter the tension in the axons. To verify whether this is the case, we examined the mechanical behavior of axons in live *Drosophila* embryos. In particular, we investigated two main questions: 1), Do *Drosophila* axons have a rest tension, and, if so, what is its magnitude? 2), Do *Drosophila* neurons regulate their tension when subjected to mechanical perturbation? To answer these questions, we used high-resolution micromechanical force sensors to systematically deform the axons and measured their force response simultaneously. Our measurements revealed the following:

1. Axons have a rest tension in the range of 1–13 nN.
2. In response to fast deformation, axons behave like elastic springs, showing a linear force-deformation response that is followed by force relaxation to a steady-state value after 15–30 min.
3. When the applied deformation is sufficiently large, the axons adopt a slack appearance upon removal of force. However, the axons tauten and build up tension, sometimes to a level close to their rest tension, within a period of 15–60 min.

These observations clearly show that neurons actively regulate tension in axons in vivo. Furthermore, our observations of neuronal mechanical behavior in vivo are remarkably similar to those made in previous in vitro studies, suggesting that mechanical forces could also prominently influence neuronal growth and function in vivo.

MATERIALS AND METHODS

Culture of *Drosophila* embryos

Transgenic *Drosophila* (elav′-GAL4/UAS-gap::GFP) expressing green fluorescent protein (GFP) in all neuronal membranes (Fig. 1) were used for the experiments. For embryo harvesting, the *Drosophila* were cultured on standard grape agar plates at ~25°C. Embryonic dissection was carried out on glass coverslides as previously described by Budnik et al. (21). Briefly, the embryos were dechorionated with a 50/50 bleach and water solution for 2 min and then rinsed with deionized water. Embryos of the correct age (16–18 h after egg laying) were placed on double-sided tape, flooded with insect saline solution, and then devitellinized before they were placed on the glass surface. The embryos were oriented such that the ventral nerve cord was closest to the glass surface, and a glass dissection needle was used to make a dorsal incision. The incision was made from posterior to anterior along the embryo to remove the guts and lay the body walls down flat. Then the axons of the aCC motor neuron and the RP2 motor neuron, which comprise the intersegmental nerve (22), were isolated by gently removing other nearby sensory and motor neurons as well as the fat cells and muscle fibers around them. The neuromuscular junctions (NMJs) of the aCC and RP2 neuron were not damaged during this process. After isolation, in some cases the axon of the RP2 neuron was excised near its NMJ, leaving only the axon of the aCC neuron intact. In most cases, both axons were intact and we measured their combined response. For dissection materials, glass slides were incubated with 10% (3-aminopropyl)triethoxysilane to improve embryo adhesion, and glass microneedles were created using a Sutter Instruments (Novota, CA) laser-based micropipette/fiber puller.

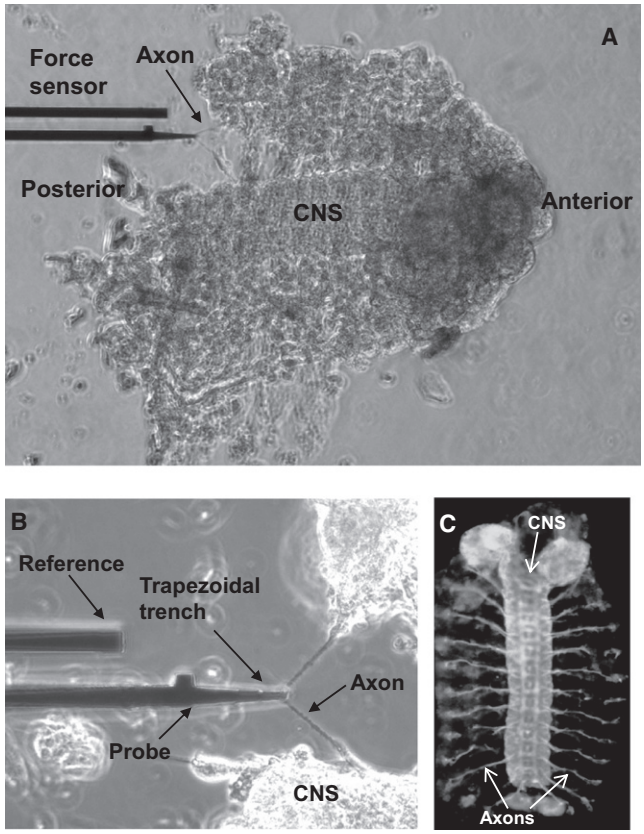

FIGURE 1 (*A*) Phase-contrast image showing a dissected embryo and the force sensor. As shown in the figure, axons close to the posterior of the embryo were usually isolated for the experiments. (*B*) A higher-magnification image of an axon being deformed by a force sensor. A trapezoidal trench was cut into the force sensor probe using focused ion beam milling to grip the axon. The principle of operation of the force sensor is explained in Fig. 2. (*C*) Fluorescence image of the *Drosophila* embryo expressing GFP in all neuronal membranes.

Deformation of axons and force measurement

The isolated axons of *Drosophila* neurons were deformed with the use of micromechanical force sensors and their force response was monitored simultaneously. All experiments were performed within 3 h after embryo

dissection. The operation of force sensors has been described in detail elsewhere (23). Briefly, the sensor consists of a rigid probe attached to a series of flexible beams. When a force is applied on the probe, the flexible beams deform in response to it. The deflection of the beams is measured by the relative displacement of the probe with respect to a fixed reference. The force on the probe is then calculated by multiplying the stiffness of the beams with the measured deflection. Once the force on the probe is known, the tension in the axon is calculated using a simple force balance as shown in Fig. 2. The movement of the force sensors was controlled by means of an x-y-z piezo actuator (NanoPZ PZC200; Newport, Irvine, CA). Live imaging of the axon under the applied deformation was carried out on an inverted microscope (IX81; Olympus, Nashua, NH). The time-lapse images were analyzed using MATLAB (The MathWorks, Natick, MA) to measure the deformation and force on the individual axons. The length of the axons in the axon contraction experiments was measured using the NeuronJ plug-in of ImageJ software (U.S. National Institutes of Health, Bethesda, MD).

RESULTS

We investigated the mechanical behavior of the axons by studying their response to systematic stretching experiments, which consisted of the following steps:

Step 1. The axons were loaded within a period of 1–2 min to a predetermined level of stretch (usually <50% of the axon length) using the force sensor.

Step 2. The force sensor was held fixed and the time evolution of axonal force was recorded over a period of 10–15 min.

Step 3. The force sensor was quickly unloaded (within 1–2 min) to release the force on the axon.

These three steps together constitute one complete deformation cycle. The mechanical behavior of 14 axons, each from a different embryo, were examined in this manner. Twelve of the 14 axons were subjected to at least two deformation cycles.

Axonal response is linear during loading

During fast loading (step 1), a linear relationship between axonal force and applied deformation was found in all the axons. In effect, the axons behaved like elastic springs when subjected to sudden changes in force. The stiffness of the axons, given by the slope of the force-deformation curve, varied from embryo to embryo with values ranging from 0.2 nN/μm to 1.2 nN/μm. In addition, individual axons also showed variation in stiffness from one cycle to the next. As discussed below, the stiffness of the axons appeared to have a direct correlation with the extent of force relaxation in the previous deformation cycle, with larger force relaxation leading to lower axonal stiffness in the subsequent cycle and vice versa. The response of two representative axons during the first loading is shown in Fig. 3. From our visual observation, axons that were thicker usually seemed to have higher stiffness, but an attempt to correlate axonal diameter with stiffness was hindered by the presence of fat cells around the axons in some of the experiments.

To verify whether axons maintain a rest tension, we extrapolated the force-deformation curve of each axon during the first loading to zero deformation. The extrapolation yielded a positive force value for 13 out of 14 axons (Fig. 3 *C*), confirming that the axons maintain a rest tension in vivo. In similarity to the stiffness values, there was a fairly large variation in the rest tensions of the axons, with values ranging from 1 nN to 13 nN. The presence of a rest tension, we note, is consistent with the taut appearance of the axons. By extrapolating the force-deformation curve to zero force, we found that the stretch in the axons in their resting state was ~5–15%.

Axons show force relaxation after loading

When the force sensor was held fixed after loading (step 2), the force in the axons decreased over time. An initial fast decay in force was followed by a more gradual decrease to a steady-state value over a period of 15–30 min in all the experiments. As illustrated in Fig. 2, when the axonal force is reduced, the length of the axon continuously increases. Fig. 4 shows the decay in force and the corresponding increase in length over time of the same axon

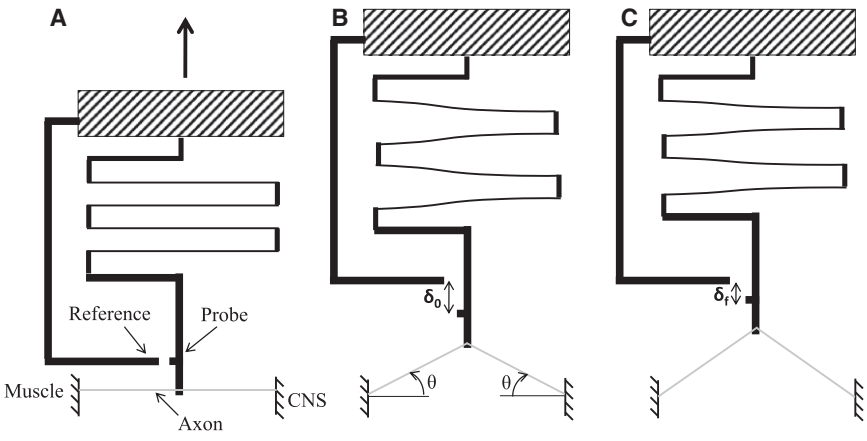

FIGURE 2 Schematic of the experiment used to measure the mechanical response of *Drosophila* axons. (*A*) Initial configuration of the force sensor and the axon. In this configuration, the force sensor is engaged to the axon but is not exerting any deformation. (*B*) In the first step, the axon is deformed by moving the force sensor away from the axon. The deflection of the probe (δ_0) with respect to the reference gives a direct measure of the force (F) acting on the probe. The tension (T) in the axon is then calculated from the force balance as $T = F/2 \sin \theta$. (*C*) When the force sensor is held fixed after loading, the tension in the axon relaxes. During this process the force on the probe reduces and the length of the axon increases. Note that the change in axon length is directly coupled to the deflection of the probe (δ_f).

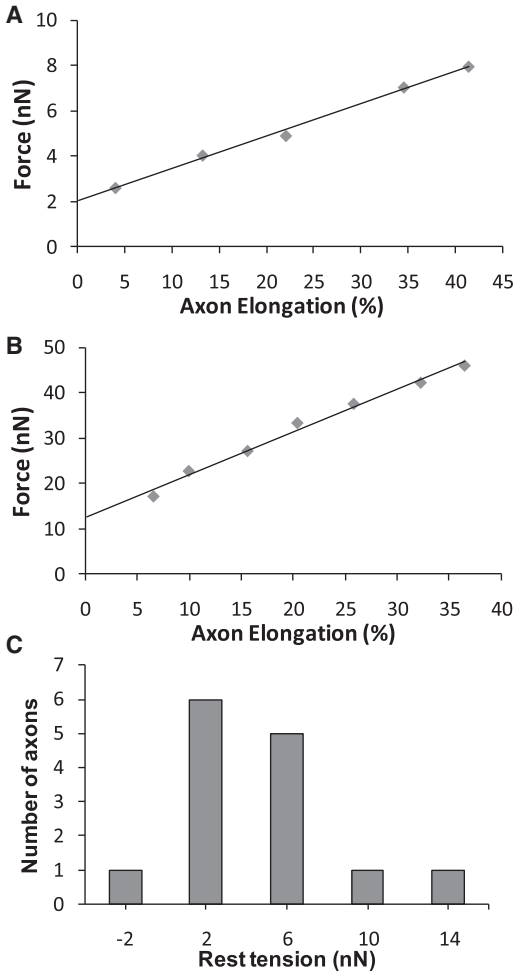

FIGURE 3 (*A* and *B*) The force-deformation response of two axons from different embryos during loading. Extrapolation of the force-deformation curve to zero deformation results in a positive force value in the axons, indicating the presence of a rest tension. (*C*) Histogram of axonal rest tensions.

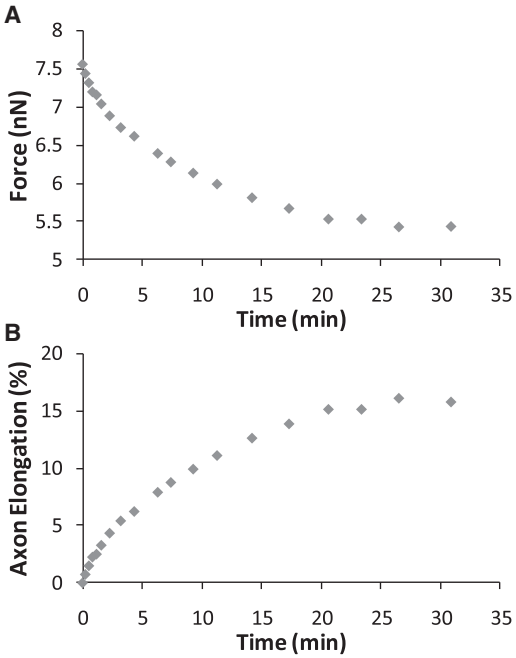

FIGURE 4 (*A*) Force relaxation in the axon whose loading response is shown in Fig. 3 *A*. (*B*) Elongation of the axon during force relaxation. The plot of axon elongation over time mirrors the relaxation in force, since the axon length and the probe deflection are coupled.

shown in Fig. 3 *A*. As is evident from Fig. 4, the axonal length can increase substantially during force relaxation. It should be noted that the increase in axon length for a given amount of force relaxation depends on the force sensor stiffness because of the coupling between the force sensor deflection and axonal length. For a given reduction in force, the increase in axon length is large when the force sensor stiffness is small, and vice versa. However, the extent of force relaxation in different axons, and consequently their increase in length, did not show any correlation with the stiffness of the force sensor used to measure their response (see Fig. S1 in the Supporting Material).

As with the rest tension and stiffness, the axons also showed considerable variation in the extent of their force relaxation. The extent of force relaxation ((initial force - steady-state force)/initial force) ranged from 30% to nearly 90% (Fig. 5 *A*). Of interest, the force relaxation in the axons had no correlation with either the initial force or the deformation imposed on the axons (Fig. S1). However, the extent of relaxation appeared to affect the stiffness of the axons in the subsequent loading. Axons that showed low relaxation typically exhibited higher stiffness during the next loading, whereas the opposite was true for axons that underwent large relaxation (Fig. 5 *B*). The axons that exhibited a large relaxation in force (>75%) noticeably thinned during the process, but the thinning was not uniform and was usually restricted to a particular region of the axon (Fig. 5 *C*).

Axons contract and build up tension after unloading

After unloading (step 3), the axons showed two types of behavior. In 12 of the 14 experiments the axons developed a slack appearance after the first unloading, i.e., they were free of tension. In the other two experiments the axons immediately regained their taut appearance upon unloading. In the 12 axons that became slack after unloading, the force sensor was held fixed and the response of the axons was recorded. All 12 axons reduced their length and visibly straightened over time. After becoming taut, five axons showed a measurable buildup of force that reached a steady value after ~15–60 min. In the other axons, the force buildup could not be measured due to limitations in the sensitivity of the force sensors. Of interest, in three of the five axons where the force buildup could be measured, the steady-state value of the force was similar to the rest tension, whereas it was lower than the rest tension in the other two cases.

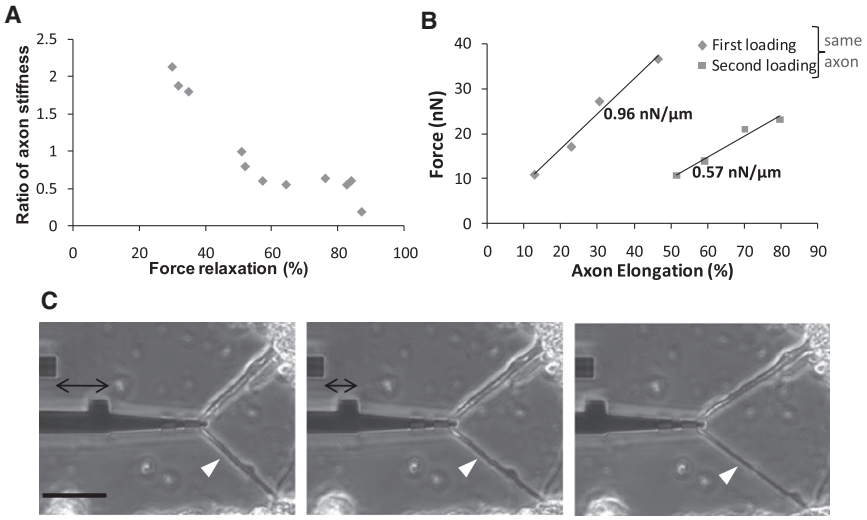

FIGURE 5 (*A*) Ratio of the stiffness of the axons during the first and second loadings plotted as a function of their force relaxation after the first loading. Axons that show a large force relaxation show diminished stiffness during the second loading, and vice versa. (*B*) Force-deformation response for the first and second loadings of an axon that underwent a large force relaxation after the first loading. (*C*) Thinning of the axon shown in *B* during force relaxation after the first loading. The reduction in diameter is especially pronounced in the lower half of the axon, as indicated by the arrowheads. The decrease in gap (indicated by double-headed arrows) between the reference and the probe shows the large decrease in force during relaxation. Scale bar = 35 μm.

The increase in tension in two axons after unloading is shown in Fig. 6. The final tension in the first axon (Fig. 6 *A*) was 1.77 nN (rest tension: 2 nN), whereas in the second axon (Fig. 6 *B*) it was 2.77 nN (rest tension: 4.21 nN). At the end of the force buildup, the lengths of the first and second axons were respectively 10.2% and 47.6% larger than their initial lengths. Therefore, for both axons, although the final (equilibrium) length was larger, the force was smaller than the initial rest tension. This was the case for all five axons in which we could measure the force buildup. In contrast, for a standard viscoelastic solid in equilibrium, a larger length would always correspond to a higher force. Thus, unlike their elastic behavior and force relaxation response, the force generation of axons is not characteristic of standard viscoelastic solids. The fact that the final axonal tension never exceeds the rest tension suggests that the force buildup is a calibrated response of neurons to mechanical perturbation. In other words, neurons appear to actively regulate the tension in the axons in vivo.

Axons contract at a constant rate in the absence of tension

In the experiments described above, the contraction of axons occurred under a condition of steadily increasing tension because the axon length and the deflection of the force sensor were directly coupled. To check whether axonal tension affects the dynamics of contraction, we studied the free contraction of axons. In these experiments, initially taut axons were made slack and the change in axon length was monitored over time. Two different approaches were used to make the axons slack. In the first approach, a microneedle was used to deform the axon to a predetermined level of stretch, and the needle was held fixed to allow the axon to relax. The needle was then moved to its initial position to release the force on the axons, thereby making the axons slack.

Six axons were examined in this manner and all of them showed contraction after slackening. Of interest, in all the axons a linear relationship was found between axon length and time, i.e., the axons contracted at a constant velocity. However, there were some significant differences between the axons. In three of the axons only a single, slow contraction phase (velocity: 1–3 nm/s) that lasted 10–15 min was observed. In the other three axons, two phases of contraction were seen: a initial fast contraction phase (velocity: 6–45 nm/s) that terminated within 5 min, followed

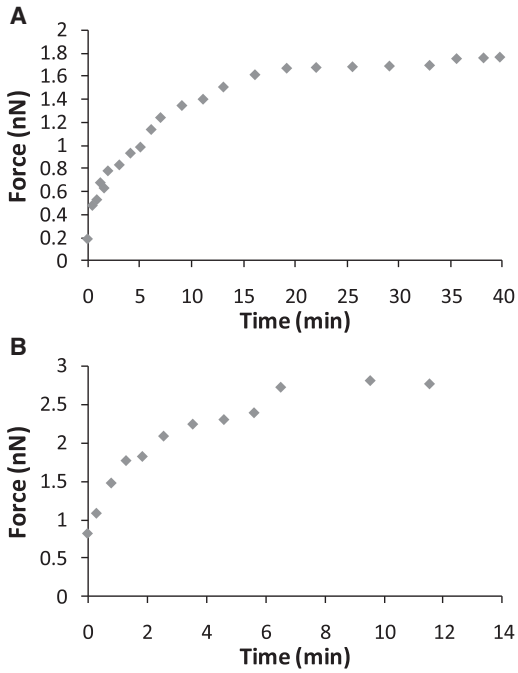

FIGURE 6 Force buildup in two axons after unloading. Part *A* corresponds to the axon whose loading curve is shown in Fig. 3 *A*. Note that the tension in the axon after force buildup (1.77 nN) was close to its rest tension (2 nN). The tension in the axon shown in *B* after force buildup (2.77 nN) was lower than its rest tension (4.21 nN).

by a slow contraction phase (velocity: 1–3 nm/s) that extended from 10 to 80 min. The contraction of two axons—one showing a single phase and the other showing two phases—is shown in Fig. 7.

In the second approach, we moved the neuromuscular junctions of axons toward the central nervous system (Fig. 8) using a micromanipulator, in the process making the axons slack. This method had the advantage that it imposed no external force on the axons, but still allowed us to study the response of the neurons to a sudden loss of tension. All of the four axons examined by this method also contracted and straightened, often within a period of a few minutes. More importantly, the extent of contraction exceeded 40% of the initial axon length in some cases, which is well above the stretch in the axons in their resting state (5–15%). This provides further evidence that the contraction is an active response of the neurons to the loss of tension.

DISCUSSION

Our experiments show that *Drosophila* motor neurons maintain a rest tension in vivo and actively restore tension after being subjected to mechanical perturbation. The results clearly suggest that neurons regulate their tension in vivo. The force measurements further reveal that *Drosophila* neurons behave like viscoelastic solids under sustained stretching, i.e., they show a linear force-displacement response to fast stretching and exhibit force relaxation when the applied stretch is held constant.

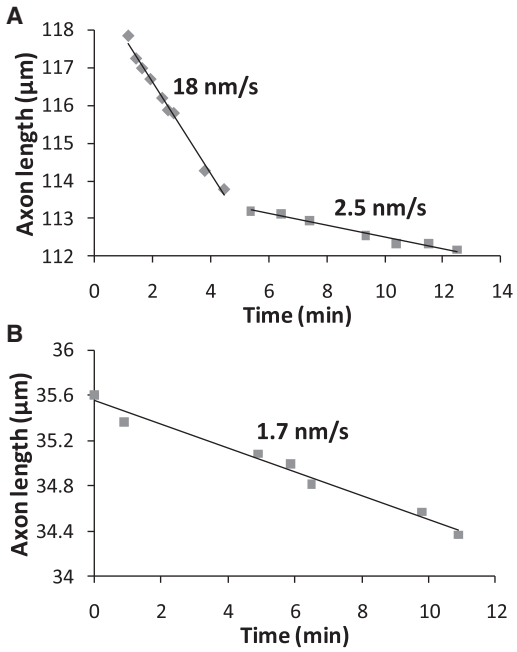

FIGURE 7 Free contraction of axons over time. (*A*) An axon showing two phases of contraction. Note that the fast contraction rate (18 nm/s) is more than seven times the slow contraction rate (2.5 nm/s). (*B*) An axon showing a single, slow contraction phase.

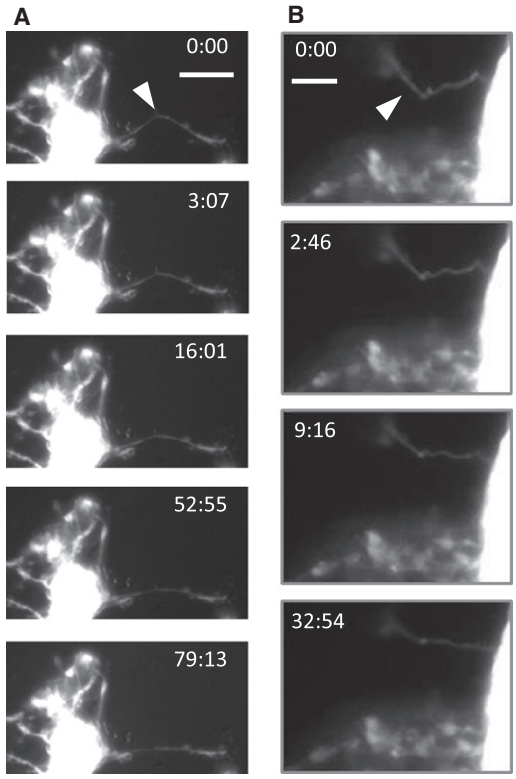

FIGURE 8 Fluorescence images of the contraction of two axons (indicated by arrowheads) over time. (*A*) The left panel shows the contraction of an axon that was deformed by a microneedle. (*B*) The right panel shows an axon whose neuromuscular junction was moved toward the central nervous system. Note that the contraction of this axon is nearly 40% of its original length. Both scale bars = 40 μm.

These observations are in remarkable agreement with previous in vitro studies of cultured neurons. Dennerll et al. (9,24), for example, showed that both PC12 neurites and chick sensory neurons maintain a rest tension and have a linear relationship between force and length change when subjected to rapid distensions. These neurites also exhibited a viscoelastic response that is well described by the classical viscoelastic model of a stiff spring in series with a Voigt element comprised of a softer spring in parallel with a dashpot. In addition, PC-12 neurites that had become flaccid after stretch release shortened and became straight within a period of 5–15 min. This shortening was accompanied by an increase in tension to a value close to their rest tension (24). Similarly, chick sensory neurons were also observed to actively generate tension in response to slackening, often to a value larger than their initial rest tension. Thus, the presence of a rest tension, viscoelastic behavior, and active force generation in response to loss of tension appear to be common elements of neuronal mechanical behavior both in vivo and in vitro.

Furthermore, tension appears to stimulate growth in *Drosophila* axons in a similar manner as it does in vitro. In a recent work, Lamoureux et al. (25) showed that

in vitro axonal growth proceeds through a combination of steps, i.e., lengthening by viscoelastic stretching and intercalated addition of material. During viscoelastic stretching, a noticeable thinning of the axons was observed, but over a period of several hours the axons eventually regained their thickness by the addition of material. As shown in Fig. 5 *C*, some *Drosophila* axons that exhibit large force relaxation also become noticeably thin during the process. The relaxation is accompanied by significant lengthening of the axon, which we interpret as the first stage of axonal growth. The decrease in their diameter also explains the reduction in their stiffness (Fig. 5 *A*) during the next loading. On the other hand, *Drosophila* axons that show a relatively low force relaxation exhibit an increase in stiffness even though they also undergo a modest increase in length. Although this may seem counterintuitive, the observations of Lamoureux et al. (25) provide a possible explanation for this increase in stiffness. They showed that addition of material to the axon, and consequently an increase in axon diameter, can precede the lengthening of axons in both spontaneously growing (growth cone-mediated) and towed axons. Such an addition of material, caused in our case by the externally applied force, would explain the increase in stiffness seen in some of the axons.

As noted above, the in vitro mechanical behavior of neurons has been studied extensively, and several models, both qualitative and quantitative, have been proposed to describe it. For example, Dennerll et al. (24) proposed a qualitative model in which the axonal response to tension is divided into three distinct phases. They suggested that when tension falls below a lower threshold, axons actively generate tension by contracting, whereas when tension exceeds a higher threshold, axons elongate to reduce tension. For intermediate values of applied tension, axons behave like passive viscoelastic solids. Recently, Bernal et al. (26) modeled axons as viscoelastic solids but added an extra element to mimic the action of molecular motors. They showed quantitatively that their extended model could predict several features of axonal contraction, at least for small deformations.

Although the macroscopic mechanical behavior of neurons is reasonably well understood, the cytoskeletal elements that underpin their behavior have yet to be unambiguously identified. In typical neurons, the axon cytoskeleton comprises a cortical actin network attached to the plasma membrane that runs parallel to a network of neurofilaments in which bundles of microtubules are embedded (27–29). The viscoelastic behavior of axons is assumed to result from the elastic interactions and dissipation between these different cytoskeletal components. Among these components, the cortical actin network has been shown to be necessary for the elastic response of axons since their disruption by actin depolymerizing agents (such as cytochalasin D) significantly reduces the axon stiffness (24,30). Through similar pharmacological interventions, microtubules have been shown to play a mainly structural role, undergoing assembly/depolymerization during growth/retraction without having any effect on the elastic behavior or viscoelastic response of axons (9). However, the role of intermediate filaments (neurofilaments), which comprise a significant part of the axonal cytoskeleton, is still virtually unknown. Of interest, of the three major cytoskeletal elements, only intermediate filaments have been shown to sustain large deformation and forces (31–33).

In this context, our results showing that the behavior of *Drosophila* neurons is similar to that of other neurons become especially relevant. Because *Drosophila* is one of the most studied organisms, the organization and functioning of its nervous system is known in great detail. Furthermore, a myriad of sophisticated tools, including a wide array of genetic tools, are available to manipulate and probe them (34). Therefore, the molecular aspects of the mechanical behavior of *Drosophila* neurons can be examined in a degree of detail that is not possible with other types of neurons. Such detailed molecular investigations have the potential to unambiguously clarify the roles of different cytoskeletal elements in axonal response to tension. It is also worth noting that although there are many similarities in the behavior of *Drosophila* neurons and other types of neurites, there are significant differences in their cytoskeletal structures. For example, although the *Drosophila* axonal shaft is devoid of neurofilaments, it contains other filamentous networks (35). The use of sophisticated molecular and genetic tools may be able to reveal what role, if any, these networks play in the mechanical response of neurons.

Although many questions remain about the cytoskeletal elements responsible for the viscoelastic behavior of *Drosophila* axons, their contraction behavior is consistent with the action of molecular motors. Experiments on cultured neurons have shown that axon contraction is mediated by tensile forces generated by the actomyosin contractile machinery. In the absence of tension, myosin motors can slide parallel F-actin filaments, and in the process shorten the axon (36). During the free contraction of the *Drosophila* axons, there is no external force on the motors, and hence their velocity (and consequently the overall contraction rate) is constant (Fig. 7). However, the reason for the existence of two different contraction rates in some of the axons is unclear.

To summarize, in this work we examined the mechanical behavior of axons in live *Drosophila* embryos using high-resolution micromechanical force sensors. Our experiments show that *Drosophila* neurons maintain a rest tension and actively regulate axonal tension in vivo. They also show passive viscoelastic behavior in response to applied deformation. These results are almost in exact agreement with the in vitro behavior of PC12 neurites and chick sensory neurons, and suggest that mechanical tension may strongly influence neuronal behavior in vivo. Given the

vast knowledge of the organization and functioning of *Drosophila* neurons, and the genetic tools available to manipulate them, our results provide a platform to examine the molecular aspects of neuronal response to tension in detail.

SUPPORTING MATERIAL

One figure is available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)01176-8.

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