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The Role of Mechanical Tension in Neurons

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ABSTRACT

We used high resolution micromechanical force sensors to study the *in vivo* mechanical response of embryonic Drosophila neurons. Our experiments show that Drosophila axons have a rest tension of a few nN and respond to mechanical forces in a manner characteristic of viscoelastic solids. In response to fast externally applied stretch they show a linear force-deformation response and when the applied stretch is held constant the force in the axons relaxes to a steady state value over time. More importantly, when the tension in the axons is suddenly reduced by releasing the external force the neurons actively restore the tension, sometimes close to their resting value. Along with the recent findings of Siechen et al (Proc. Natl. Acad. Sci. USA **106**, 12611 (2009)) showing a link between mechanical tension and synaptic plasticity, our observation of active tension regulation in neurons suggest an important role for mechanical forces in the functioning of neurons in vivo.

INTRODUCTION

The influence of mechanical forces/microenvironment on various cell processes such as motility, growth and differentiation has become increasingly clear over the last two decades [1, 2]. In particular, neurons have been shown to respond to a variety of mechanical inputs. For example, in vitro experiments on different neuronal cells have revealed that new axons can be initiated by externally applied tension [3, 4] and that existing axons grow when tension that exceeds a threshold is applied [5, 6]. Experiments have also shown that a buildup of mechanical tension in a developing axonal branch stabilizes it and causes the retraction of other axon branches and collaterals [7].

A recent experimental study by Siechen et al [8] on live Drosophila embryos has revealed an important new facet of the role of mechanical tension in neurons. These experiments show that mechanical tension is necessary for accumulation of neurotransmitter vesicles in the pre-synaptic terminal of Drosophila motor neurons. Furthermore, an increase in tension enhances the accumulation of the vesicles. This connection between tension and vesicle accumulation suggests that neurons are likely to regulate their tension in vivo. To test this hypothesis, we studied the *in vivo* mechanical response of Drosophila axons using high resolution micromechanical force sensors. Our experiments reveal the overall mechanical behavior of axons and provide direct evidence that neurons regulate their tension in response to mechanical perturbations.

EXPERIMENTAL DETAILS

Transgenic *Drosophila (ELAV-GAP/ GFP)* embryos expressing green fluorescent protein (GFP) in neuronal membranes were used for the experiments. Flies were transferred from fly stock into a culture container sealed with grape jelly-coated petri dish and maintained under controlled temperature (25 C) and humidity (~60%). After 20-24 hours in the culture container, eggs hatched on the petri dish were washed with 50% diluted bleach, filtered and rinsed with deionized (DI) water for 2 minutes. Under fluorescent light, eggs at correct age were separated and placed on a double sided tape affixed to a glass cover slide for dissection. The cover slide was functionalized with *3-Amino propyltrie thoxysilane* (APTES) solution to improve the adherence of the embryos to the surface during dissection. Under saline solution, egg membranes were pierced using glass micro needles and the embryos were extracted and placed on the cover slide. Embryos were oriented such that the ventral nerve cord was closest to the glass surface. Dorsal incisions were made and the embryos were flattened out against the glass. The gut and fat cells around the axon of interest were gently scraped off using the needle until a single axon was exposed and isolated.

The isolated axons were deformed using micromechanical force sensors and their force response was monitored simultaneously. The fabrication and working of the force sensors have been described elsewhere [9]. The movement of the force sensors was controlled using an x-y-z piezo actuator. Live imaging of the axon under the applied deformation was carried out using an inverted Olympus microscope. The time-lapse images were analyzed using ImageJ to measure the deformation and force on the individual axons.

The mechanical properties of the axons were investigated by studying their response to systematic stretching experiments, which comprised of the following steps:

1. In the first step, the axons were loaded within a period of 1-2 minutes to a predetermined level of stretch (usually less than 40% of the axon length) using the force sensor.
2. The force sensor was then held fixed and the time evolution of axonal force was recorded over a period of 10-15 minutes.
3. After force relaxation to a steady state, the force sensor was quickly (within 1-2 minutes) backed up to release the force on the axon.

RESULTS AND DISCUSSIONS

During fast loading (step 1) a linear relationship between axonal force and applied deformation was found in all the axons. In effect, the axons behave like elastic springs when subject to sudden changes in force (Fig. 1). The stiffness of the axons, given by the slope of the force-deformation curve, varied from embryo to embryo with values ranging from 5 nN/μm to 50 nN/μm. To verify whether axons maintain a rest tension, we extrapolated the force deformation curve during loading to zero deformation. The extrapolation yielded a positive force value for each of the axons, confirming that the axons maintain a rest tension in vivo.

When the force sensor was held fixed after loading, 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 10-15 minutes in all the experiments. The steady state value of the axonal force was typically larger when the force applied during loading was larger. Figure 2 shows the force relaxation in a typical axon over time.

When the force sensor was backed up to reduce the force on the axon in step 3, the force-deformation response of the axon was again linear but the slope of the curve showed some variation from loading. In some of the cases, the stiffness of the axon during unloading was higher than the loading stiffness where as in other cases it was lower. There was also variability in the response of the axons after the external force was completely removed. Several axons immediately regained their taut appearance after unloading. On the other hand, some of the axons had a slack appearance after unloading, indicating a lack of tension. However, these axons actively contracted over time and in the process built up the tension. The tension buildup in one of the axons after unloading is shown in Fig. 3. As evident from the figure, the force build up mirrors the force relaxation in the axon - an initial fast increase is followed a more gradual approach to a steady state over time. The similarity between the time evolution of force relaxation and force build up suggests that the same biological mechanism may underlie both phenomena. More importantly, this force buildup in the axons after unloading cannot be accounted by a standard viscoelastic model, that is, this restoration of tension in the axons represents an active biological response of the neurons. In other words, neurons actively regulate the tension in the axons in vivo.

As mentioned earlier, recent experiments [8] have shown that tension is necessary for the accumulation of neurotransmitter vesicles at the pre-synaptic terminal of motor neurons. When the axon is severed using laser axotomy, thereby releasing the tension, the density of vesicles reduces more than threefold compared to intact axons. However, when tension is resupplied by pulling the severed end with a micropipette the vesicle accumulation increases significantly. Furthermore, increasing the tension in an axon by applying an external force increases the vesicle accumulation to more than two times that of unstretched axons, which shows a direct relationship between mechanical tension and clustering of neurotransmitter vesicles. Our experiments provide further support, albeit indirectly, to the notion that tension plays a vital role in neuronal function by demonstrating that neurons actively regulate the tension in the axons in vivo.

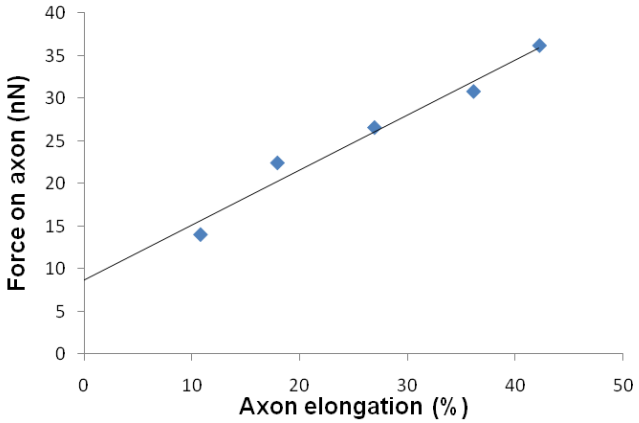

Figure 1: Force-deformation response of a typical axon to fast stretching. Extrapolation of the curve to zero axon elongation results in a positive force, which is the rest tension in the axon.

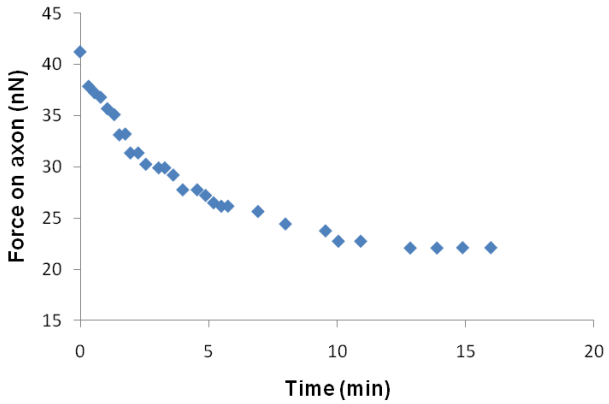

Figure 2: Force relaxation in an axon over time. An initial fast decrease in force is followed by a more gradual reduction to a steady state value over a period of 10-15 min.

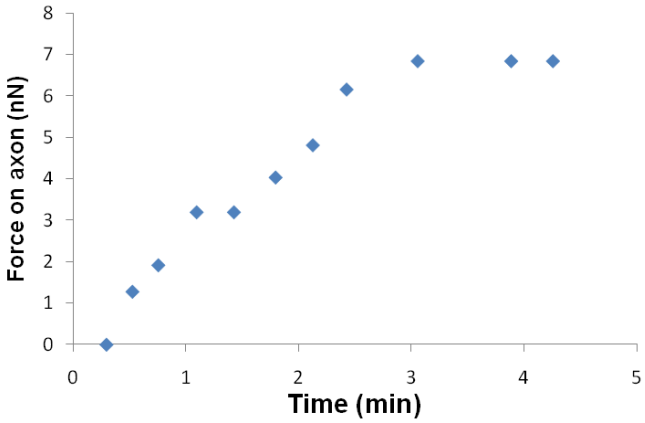

Figure 3: Force buildup in an axon over time after the external force has been released.

It is well known that the release of neurotransmitters clustered at the synapse is essential for neurotransmission and that the amount of neurotransmitters released for a given action potential depends on how frequently the synapse had been used. This usage dependent release of neurotransmitters, referred to as synaptic plasticity [10], is believed to be the basis for learning and memory formation in animals. The findings of Siechen et al. [8] show that mechanical tension influences the accumulation of neurotransmitters in the synapse and our experiments strongly suggest that neurons regulate their tension in vivo. Together, these results raise the possibility that neurons may use mechanical tension to modulate synaptic plasticity and that tension may be essential for learning and memory formation in animals.

CONCLUSIONS

The mechanical behavior of neurons in live Drosophila embryos was studied using high resolution micromechanical force sensors. Drosophila axons behave like viscoelastic solids in response to externally applied force but, in addition, also exhibit active force generation to

restore tension after mechanical perturbation. These results show that neurons regulate the tension in the axons and suggest that tension may be critical for neuronal function *in vivo*.

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