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Mechanical Tension Modulates Local and Global Vesicle Dynamics in Neurons

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Abstract—Growing experimental evidence suggests that mechanical tension plays a significant role in determining the growth, guidance, and function of neurons. Mechanical tension in axons contributes to neurotransmitter clustering at the *Drosophila* neuromuscular junction (NMJ) and is actively regulated by neurons both *in vitro* and *in vivo*. In this work, we applied mechanical strain on *in vivo Drosophila* neurons and *in vitro Aplysia* neurons and studied their vesicle dynamics by live-imaging. Our experiments show that mechanical stretch modulates the dynamics of vesicles in two different model systems: (1) The global accumulation of synaptic vesicles (SV) at the *Drosophila* NMJ and (2) the local motion of individual large dense core vesicles (LDCV) in *Aplysia* neurites. Specifically, a sustained stretch results in enhanced SV accumulation in the *Drosophila* NMJ. This increased SV accumulation occurs in the absence of extracellular Ca^{2+}, plateaus after approximately 50 min, and persists for at least 30 min after stretch is reduced. On the other hand, mechanical compression in *Aplysia* neurites immediately disrupts LDCV motion, leading to decreased range and processivity. This impairment of LDCV motion persists for at least 15 min after tension is restored. These results show that mechanical stretch modulates both local and global vesicle dynamics and strengthens the notion that tension serves a role in regulating neuronal function.

Keywords—Cell mechanics, Subcellular, Live-imaging, Vesicle tracking, *Drosophila*, *Aplysia*.

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INTRODUCTION

Neurons are the key rapid signal generating and processing components of the nervous system. They generate and transport vesicles containing neurotransmitters and neuromodulators used in communication with other cells by exocytic release of vesicle contents. Neurotransmitter filled synaptic vesicles (SV) cluster at the synapse prior to release for signaling a postsynaptic cell. Previous studies of vesicle clustering have focused primarily on biochemical signaling.[35] But recent investigations show that mechanical tension contributes to clustering of neurotransmitter vesicles at the presynaptic terminal.[46] Furthermore, the modulation of presynaptic terminal machinery is critical in synaptic plasticity.[24] This suggests a functional role for mechanical tension in neuronal signaling.

While *in vivo* studies of mechanical tension in neurons are fairly recent, the presence of tension in cultured neurons has been known for several decades. Mechanical tension was observed to exist in neurons in cell culture many decades ago. One of the first observations of *in vitro* axonal tension was made in 1945 by Weiss, who suggested that axons straighten along their length, possibly due to an "elastic tension."[52] Decades later, Bray[7] showed that growth cones exert mechanical tension and that the direction of tension determines growth direction. Subsequently, it was discovered that growth cones pull on their underlying substrate to induce neurite growth.[30]

Growing evidence of the mechanical sensitivity of neurons has led to many recent *in vitro* studies that highlight how mechanical forces affect neuronal behavior. For instance, axons undergo continuous

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elongation under mechanical tension and exhibit normal subcellular structures and electrophysiology.[37,38,48] Coupling of the cytoskeleton to the underlying substrate mediates cytoskeletal polymerization and growth cone steering,[49,50] and tension generated along the axon may serve as a signal for branch pruning.[3,18] Mechanical tension has been shown to exist in *in vivo Drosophila* motor neurons and this tension is actively regulated.[40] In an effort to explain the mechanical sensitivity of neurons, mathematical models have been developed to explain force generation, elongation, and axonal transport.[4,13,32–34] For in depth reviews of the role of forces in neuronal growth see Franze *et al.*,[20] Franze and Guck,[19] and Suter and Miller.[51]

While many recent studies have focused on neuron growth, guidance, and structure, studies of the effect of mechanical tension on neuronal signaling are limited. Previous studies have shown an immediate and reversible increase of neurotransmitter release due to muscle stretch in frogs.[9,16,23] Chen *et al.*[9] hypothesized that stretch enhancement of neurotransmitter release was derived from either elevated intracellular Ca^{2+} concentrations or by increased sensitivity to Ca^{2+} in the nerve terminal. They later showed that Ca^{2+} influx was not necessary for stretch enhanced neurotransmitter release but the magnitude of the effect was attenuated in the absence of extracellular Ca^{2+}. As a result they postulated that tension on integrins mechanically transduces changes in molecular conformations causing increased sensitivity to Ca^{2+}.[10] Stretch enhanced neurotransmitter release occurred on a millisecond time scale and was reversible after stimulation was removed. However, recent experiments have suggested that tension induced SV accumulation occurs over a much larger time scale.[46] Additionally, the effect of tension on the global behavior of vesicle accumulation naturally leads to the question: does tension affect the local behavior of individual vesicles?

We investigated three main questions: (1) Does stretch enhanced SV accumulation occur in the absence of extracellular Ca^{2+}, and what are the time dynamics? (2) Does decreased tension in intact neurons affect SV accumulation? (3) Does mechanical tension affect local vesicle dynamics?

Our experiments show: (1) Increased mechanical tension enhanced SV accumulation at *in vivo Drosophila* NMJ's in the absence of extracellular Ca^{2+} and accumulation saturated after approximately 50 min of elevated tension. This effect persisted for at least 30 min after axonal tension was reduced. (2) Compressive strain along *Drosophila* motor neuron axons did not affect SV accumulation. (3) Decreased tension in neurites of cultured *Aplysia* neurons disrupted motion of large dense core vesicles (LDCV), including the local range and processivity, and this effect persisted for at least 15 min after tension was restored. A significant difference between the *in vivo Drosophila* and the *in vitro Aplysia* neurons studied here was that the former had a neuromuscular synapse, while the latter had not formed any synapse and had a growth cone as their neurite terminal.

MATERIALS AND METHODS

Stretching Experiments

In this study we used a stretching system for high-resolution live-imaging of cells and tissues under applied mechanical strain.[2] The system applied a static deformation to a stretchable polydimethylsiloxane (PDMS) cell culture substrate. The substrate consisted of two different thicknesses, an outer thick region (1.2 mm) to provide mechanical stability and an inner thin region (170 μm), which served as a cell culture well. The thin region allowed imaging through the PDMS substrate with high-resolution optics. When the substrate was stretched in one direction, it experienced a Poisson contraction in the orthogonal direction. Thus, a cell aligned along the stretch direction would be under tensile strain, and a cell aligned perpendicular to the stretch direction would be under compressive strain. In this study, cells experienced up to 20% tensile strain and up to 8% compressive strain. The substrate deformation was characterized experimentally by digital image correlation and computationally by the finite element method and it was found that the mechanical strain of the cell culture surface was uniform over greater than 95% of the surface area.[2]

We investigated the change in vesicle dynamics in neurons in response to mechanical tension by using a three step experiment:

1. *Control*: Cells were attached to the cell culture substrate in their natural undeformed state.
2. *Applied deformation*: Mechanical stretch or compression was applied along the axis of the neuron.
3. *Deformation removed*: The deformation applied in step 2 was completely reversed by unstretching the substrate back to its original state.

Vesicle dynamics were observed by live-imaging as described below for all stages of the experiment.

Drosophila Embryo Motor Neurons

Drosophila embryonic *in vivo* motor neurons were used in this study to investigate SV dynamics. They are amenable to live-imaging and are known to have structurally plastic neuromuscular junctions (NMJ).[47] To study SV dynamics, transgenically modified *Drosophila* (eve-GAL4;UAS-sytEGFP) were used to

express synaptotagmin–GFP.[15] Synaptotagmin is an integral vesicle membrane protein which can be tagged with GFP to serve as a SV marker in living synapses.[53] *Drosophila* were cultured on standard grape agar plates under ambient light at 25 °C. Embryonic dissection was carried out on silane functionalized PDMS substrates, which was described in detail in previous studies.[2,8] Briefly, embryos were dechorionated in 50/50 bleach and water solution for 2 min. Embryos of the correct age (~16 h after egg laying) were placed on the substrate and the PDMS reservoir was flooded with Ca^{2+} free PBS. Ca^{2+} free solution minimized muscle twitching in the embryos. Such twitching caused imaging artifacts. Glass micro-needles were used to devitellinize and dissect the embryo such that the motor neuron axons were parallel to the PDMS surface. The motor neuron axons were attached to the cell body at one end, and to the NMJ at the other, and thus the axon was not anchored along its length. Since the embryo was adhered to the PDMS surface, substrate deformation induced mechanical strain along the length of the axon. Sample sizes for experiments were: control ($n = 10$), stretch ($n = 8$), compress ($n = 8$).

Aplysia Neurons

Aplysia californica pedal ganglion neurons were used for tracking local vesicle dynamics because this *in vitro* system had large neurites that allow high-resolution imaging of LDCVs.[22] *Aplysia* neurons were isolated and cultured as in previous studies.[42] Briefly, *A. californica* were obtained from the National Resource for Aplysia (University of Miami/RSMAS, Miami, FL) and maintained in a 14 °C tank of circulating artificial sea water until use. Neurons were mechanically dissociated from the *Aplysia* CNS after a 30–60 min incubation in 1% protease. Cells were plated on polylysine coated PDMS substrates overnight at room temperature and then at 14 °C for 24–48 h in artificial sea water antibiotic solution. Experiments were conducted with cells from multiple animals ($n > 10$) and multiple vesicles ($n > 11$) were tracked for each cell.

Light Microscopy and Image Processing

All images were collected on a Zeiss LSM 710 laser scanning confocal microscope using a 40× (1.2 NA) water immersion objective lens (Carl Zeiss, Germany). All imaging parameters (e.g., laser power, pixel dwell time, pinhole size, gain, etc.) were kept constant for a given set of experiments. Image analysis was carried out in MATLAB (The MathWorks, Natick, MA) or ImageJ.[1]

For *Drosophila*, fluorescent images of SVs were captured as Z-stacks at approximately 10 min intervals. It was assumed that the amount of SVs present is proportional to the fluorescence intensity of the synaptotagmin–GFP signal. Thus, the amount of SVs was quantified using the fluorescent intensity calculated from the collapsed Z-stack. The average intensity in a 2.5 μm by 2.5 μm region at the presynaptic terminal was used to quantify the SVs at the NMJ.

For *Aplysia*, differential interference contrast (DIC) image sequences of LDCVs were captured at a rate of 2.5 frames per second. Videos were captured approximately every 5 min. Dynamics of LDCVs were tracked using an algorithm for precise particle tracking by polynomial fitting with Gaussian weight in MATLAB.[41] Note that this method of particle tracking allows particle displacements to be tracked with nanometer precision.

RESULTS

Increased Axonal Tension Induces Synaptic Vesicle Accumulation at the In Vivo Drosophila NMJ

Global accumulation of SVs was investigated in motor neurons of *Drosophila* embryos.[28,47] The tension in *Drosophila* axons was modulated by conducting stretch and compression experiments. Under stretch, axonal tension was increased and leads to increased SV accumulation at the presynaptic terminal of the NMJ. Under compression, axonal tension was decreased and no significant change in SV accumulation was observed.

Figure 1 shows representative images of axons under mechanical strain where the color map represents the fluorescent intensity, blue indicates low synaptotagmin and red indicates high. A control axon is shown in Fig. 1a from a dissected embryo on the PDMS surface in the absence of applied deformation. The zoomed-in inset at the right shows a 10 μm by 10 μm region near the presynaptic terminal of the NMJ. A 2.5 μm by 2.5 μm region in the synapse was used to quantify synaptic vesicle accumulation. Figures 1b and 1c show a stretched and compressed axon, respectively. Note that the stretched axon is straight (increased tension) and the compressed axon is squiggly (decreased tension). In the stretch experiments on axons, a static tensile strain was applied and held for 90 min to increase tension, after which the stretch was removed and the substrate strain was reduced to zero. In the compression experiments, a static compressive strain was applied and held for 60 min to decrease axonal tension, after which the deformation was reversed and the substrate strain was reduced to zero. All experiments with *Drosophila* motor neurons were carried out in the absence of extracellular Ca^{2+}. The overall duration of the experiments was maintained at

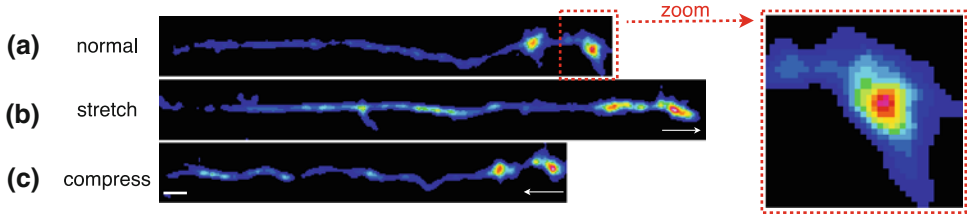

FIGURE 1. Axons of *Drosophila* motor neuron under mechanical strain where the color map represents the fluorescent intensity, blue indicates low synaptotagmin and red indicates high. (a) A control axon is shown on the PDMS surface. The inset on the right shows a 10 μm by 10 μm region near the presynaptic terminal where a 2.5 μm by 2.5 μm region was used to quantify synaptic vesicle accumulation. (b) An axon is shown stretched by substrate deformation, notice the straightness of the axon (increased tension). (c) An axon is shown compressed by substrate deformation, notice the axon is squiggly (decreased tension). Note that images in (a) and (b) show the same axon, while the image in (c) is a different axon (scale bar = 5 μm).

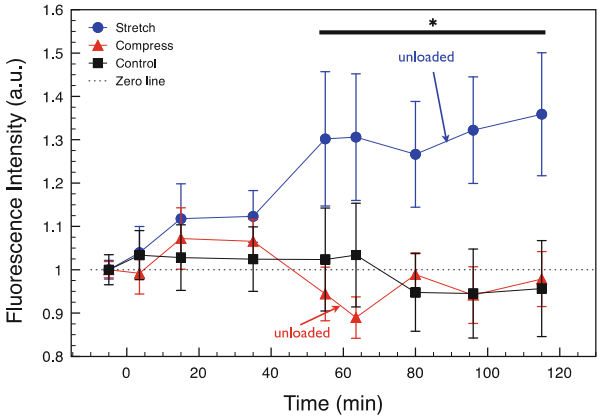

FIGURE 2. Synaptic vesicle accumulation (in terms of fluorescent intensity) at the *Drosophila* NMJ as a function of time. Control samples (n = 10) show no significant change in synaptic vesicle accumulation. When axons are stretched (n = 8), increased accumulation by 30 ± 15% is observed after approximately 50 min and the effect persists for at least 30 min after stretch is removed. In compressed axons (n = 8), no significant change occurs in accumulation during compression or after it is removed. Statistical significance evaluated by Student's t-test ($p < 0.01$) and denoted in figure by * (error bars = SEM).

approximately 2 h to avoid complications with embryo aging. In stretch experiments, the tensile strain was held for 90 min to investigate if accumulation continued to increase with time. Then the substrate was unloaded and the axon was observed for 30 min to investigate if stretch enhanced accumulation persisted after axonal strain was decreased. For compression experiments, the compressive strain was held for 60 min because it was known the axon would shorten and rebuild its rest tension well within 60 min.[40] Then the substrate was unloaded, stretching out the axon to restore its original length, and the axon was imaged for an additional 60 min to observe the effect of stretch from the shortened state.

The time dynamics of vesicle accumulation are shown in Fig. 2. When the axons were subjected to sustained stretch, the tension increased and we observed a significant ($p < 0.01$) increase in SV accumulation of 30 ± 15% after approximately 50 min as shown in Fig. 2. The increased SV accumulation saturated by 50 min. Then after 90 min of static stretch, the substrate was undeformed and the axon was unstretched. In this state of decreased axonal tension, SV accumulation persisted for at least 30 min (end of experiment). Therefore, we observed that SVs accumulated during the stage of increased axonal stretch in the absence of extracellular Ca^{2+}, the increase saturated after approximately 50 min, and this effect persisted after axonal strain was decreased by unloading the substrate. When static substrate compression was applied, the tension in the axon was decreased. During this stage of decreased axonal tension the accumulation of SVs seemed to fluctuate but did not significantly differ from the control as shown in Fig. 2. When the substrate was undeformed, the axonal tension increased due to stretching it back out to its original length. In this stage the SV accumulation also did not significantly differ from the control case.

Representative images of the synaptic regions of the *Drosophila* NMJ throughout the experiment are shown in Fig. 3. Here, variations in SV accumulation can be observed qualitatively by fluorescence intensity of the SVs in the presynaptic terminal. In the control case, the amount and distribution of SVs remains approximately the same throughout the experiment. Under stretch, the SV accumulation increased significantly by 60 min and remained at the elevated level until 120 min (end of experiment), even though the stretch was released at 90 min. Under compression, fluctuations in SV accumulation are observed, however, they are not statistically significant.

Decreased Neurite Tension Disrupts Vesicle Motion in In Vitro Aplysia Neurites

To investigate the effect of tension on local dynamics of individual LDCVs we used *in vitro Aplysia* neurons because they have large growth cones, which

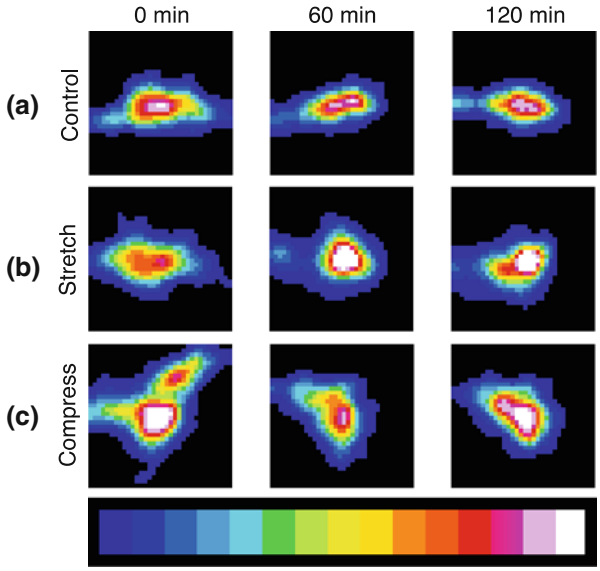

FIGURE 3. Snapshots of representative *Drosophila* presynaptic terminals during the experiment. Blue indicates low fluorescence intensity of SV and white indicates high intensity. (a) Control samples show no significant change in vesicle clustering throughout the entire experiment. (b) Increased tension by stretch showed increased accumulation in the 60 min frame and this effect persists until the end of the experiment at 120 min (stretch was removed at 90 min). (c) Decreased tension by compression showed fluctuations in vesicle accumulation, but the amount of vesicles was similar to that of the control throughout the experiment. (Each image is 10 μm × 10 μm).

are easily visualized,[17] are known to generate high levels of tension,[49] and have large neuropeptide containing LDCVs.[12,26,43] The tension in *Aplysia* neurites was modulated by conducting stretch and compression experiments. Under stretch, neurite tension was increased and no discernable difference in the LDCV velocity distribution could be detected throughout the course of the experiment. Under compression, neurite tension was decreased and LDCV motion was disrupted.

Figure 4 shows the velocity distributions of LDCV motion from the stretch and compression experiments where the vertical axis is the relative frequency and the horizontal axis is the vesicle velocity. When the neurite was stretched, the velocity distribution of LDCVs did not change significantly (Figs. 4a and 4c). Similarly, upon unloading the substrate, there was no observable difference in the velocity distribution of LDCV motion (Fig. 4d). This experiment shows that an increase in neurite stretch did not significantly affect the velocity distribution of LDCVs in *Aplysia* neurites. On the other hand, when neurite tension was decreased (by compression), then the velocity distribution narrows significantly ($p < 0.01$) and the peak at zero velocity increased as shown in Figs. 4e–4f. The magnitude of this effect increased as compression is held static (Fig. 4g). This indicated that the motion of the LDCVs decreased and they spend more time not moving (zero

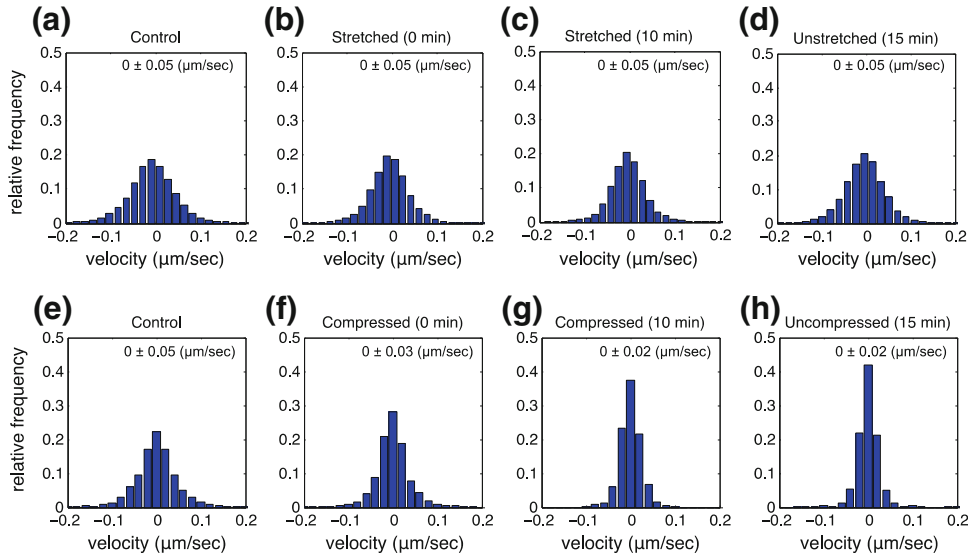

FIGURE 4. Histograms showing the velocity distribution of LDCVs in *Aplysia* neurons. (a–d) When *Aplysia* neurons are stretched, the velocity distribution of the LDCVs remains the same throughout the time-course of the experiment. Mean ± standard deviation are indicated in the charts and were not found to be statistically different. This shows that increased tension in *Aplysia* neurites does not affect the velocity distribution of LDCVs. (e–h) When *Aplysia* neurons are compressed, the velocity distribution of the LDCVs narrows in width and increases in magnitude throughout the experiment. Mean ± standard deviation are indicated in the charts and velocity distributions were found to be statistically different (Student's *t*-test, $p < 0.01$). This shows that decreased tension in *Aplysia* neurites leads to disrupted motion of LDCVs.

velocity). Upon stretching from the compressed state, the neurite length was restored. In this case the velocity distribution remained narrow for at least 15 min (end of experiment) as shown in Fig. 4h. Videos of vesicle motion are shown for a control (see Movie S1 in the Supporting Material), immediately after compression (Movie S2), 10 min thereafter (Movie S3), and 15 min after re-stretching to original length (Movie S4).

To illustrate this effect on compressed neurites a representative plot of position vs. time for a LDCV (Fig. 5) shows that in the control case a vesicle moved back and forth over a large range (*black*). When the neurite was compressed, LDCV motion immediately decreased (blue). After 10 min of static compression the vesicle motion decreased even further (pink). LDCV motion was not restored even 15 min (end of experiment) after the neurite's original length was restored (see supplementary videos). Figure 6 shows the effect of mechanical compression on LDCV range and the largest processive movement. Here, range refers to the range of vesicle motion and the largest processive movement is the longest distance of continuous vesicle motion as shown in Fig. 6a. The range of LDCV motion started out around 550 nm, decreased to 200 nm immediately after compression, and decreased further to 140 nm after an additional 10 min of compression. LDCV range did not recover and remained around 140 nm after removal of compression for 15 min (Fig. 6b). Similarly, before stimulation LDCVs exhibited an average largest processive movement of approximately 380 nm, which decreased to 140 nm immediately after compression and further decreased to 80 nm after 10 min of static compression. Vesicle processivity does not recover and remains around 80 nm even 15 min after the neurite is restored to its initial length (Fig. 6c).

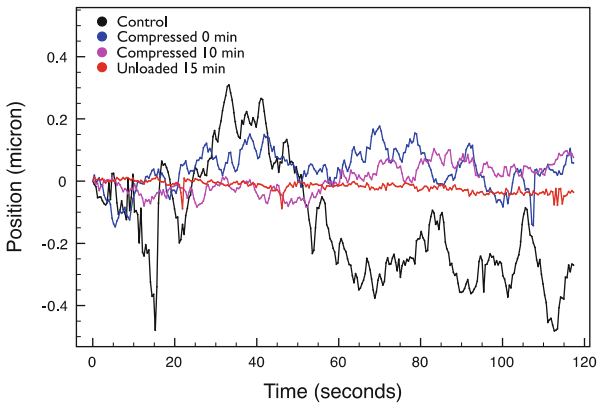

FIGURE 5. Motion of an individual *Aplysia* vesicle as a function of time. Vesicles in control samples show a large motion back and forth about a central region. Immediately after compression is applied the vesicle motion decreases dramatically (blue) and continues to decrease while compression is held (pink) for over 10 min. Even after removal of compression (neurite tension is restored), vesicle motion does not recover (red) for over 15 min. In this plot the motion of one representative vesicle is shown.

DISCUSSION

Our results show that mechanical tension affects both global and local vesicle dynamics. Increased axonal tension (by mechanical stretch) led to increased SV accumulation at the *in vivo* NMJ of *Drosophila*. Decreased neurite tension (by mechanical compression)

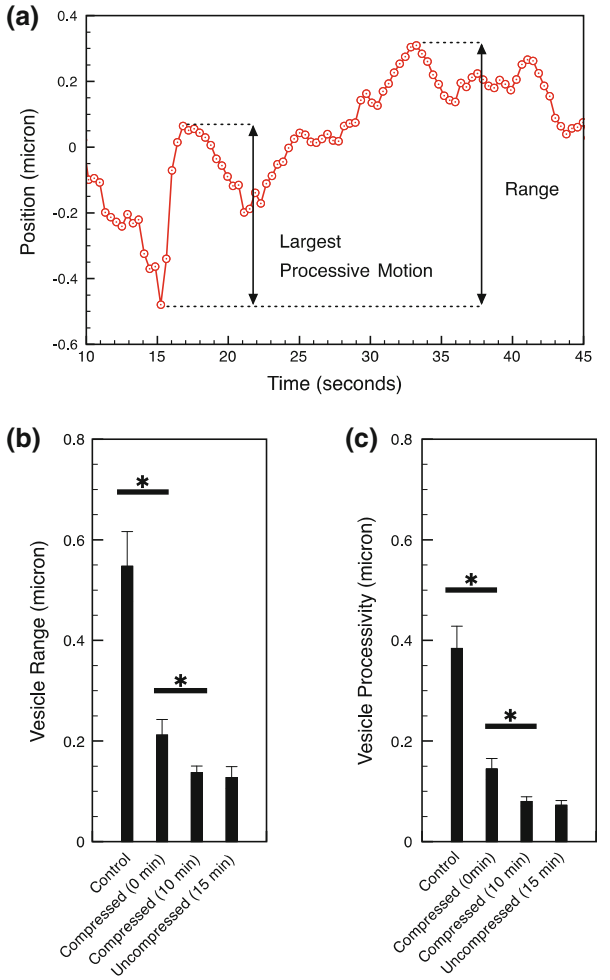

FIGURE 6. Range and processivity of vesicles in *Aplysia* neurons. (a) A representative plot showing the range and largest processive motion of a single vesicle. (b) Vesicle range of motion was approximately 550 nm in the control samples. Mechanical compression caused an immediate decrease to 200 nm and the range continued to decrease to 140 nm. (c) In control samples the largest processive motion of vesicles was approximately 380 nm. This decreased to 140 nm immediately after compression and continued to decrease to 80 nm. In both cases, the effect persists for over 15 min after compression was removed. Statistical significance evaluated by Student's *t*-test ($p < 0.05$) and denoted in figure by * (error bar = SEM).

disrupted LDCV motion in *in vitro* neurites of cultured *Aplysia* neurons.

SV Accumulation

Increased axonal stretch resulted in enhanced SV accumulation at the *in vivo Drosophila* NMJ. This accumulation occurred in the absence of extracellular Ca^{2+}, saturated after approximately 50 min of sustained stretch, and persisted for at least 30 min after the stretch was removed.

When *Drosophila* neurons are stretched the axonal tension increases linearly with applied deformation, and when the deformation is held fixed the tension relaxes to a steady state value in about 25 min. This steady state tension is usually larger than the rest tension.[40] Thus, when the axons are subjected to sustained stretching, they experience an increased level of tension for an extended period of time. It has been hypothesized that actin acts as a structural scaffold to cluster vesicles at the synapse and transport them to the active zone.[11,14] It has also been shown that tensile strain promotes polymerization of both actin and microtubules.[2,36,39] Thus, it is possible that increased axonal tension promotes polymerization of actin scaffolding in the cytomatrix of the synapse, providing more sites for actin-synapsin based tethering of vesicles.[6,11] As mentioned earlier, the time scale of this vesicle accumulation is orders of magnitude greater than that of stretch induced neurotransmitter release.[9,10,16,23] The amount of time required for polymerization of actin scaffolding structures could explain the slow time scale observed for tension induced SV accumulation. Equivalently, scaffolds would not immediately depolymerize upon decreased tension, which may explain the persistent SV accumulation after stretch is removed. Also due to active regulation of axonal tension, the neuron will rebuild tension shortly after it is reduced.[40] This suggests a possible mechanism for neurons to utilize axonal tension to modulate synaptic structure.

In contrast to stretching, compression of *Drosophila* neurons has much less effect since the axons actively generate force in response to a loss of tension. This force generation restores axonal tension to a value close to its rest tension in less than 15 min.[40] Thus, when axons are compressed they are subjected to reduced tension only for a short period of time because they are actively shortening to build axonal force. This short perturbation in axonal tension may not be adequate to alter SV accumulation, and thus no effect is observed in the compression experiments. It is, however, worth noting that while a temporary reduction in axonal tension does not alter SV accumulation, a permanent removal of tension through axotomy leads to the dispersion of SVs from the NMJ.[46] These experiments further suggest if neurons do use axonal tension as regulatory signal, then their ability to rapidly rebuild tension may be a mechanism for maintaining synaptic structure.

When the compression was removed, axons were stretched back to their original length causing an increase in tension above the rest tension. In this case we still do not see a significant change in synaptic vesicle accumulation. One possible reason is that there exists a threshold that must be crossed to induce a neural response. Dennerll *et al.*[13] proposed a three stage model for neural response to axonal tension. They suggest there exists an intermediate regime of axonal tension where neurons respond to deformations with passive viscoelasticity. When tension is decreased below this regime, neurons regulate tension by actively shortening to build force. When tension is increased above this regime, neurons will grow in length to decrease axonal force. In our experiments, when the compression was removed the axons were stretched back out to their original length. This deformation back to its original length may be insufficient to elicit a stretch enhancement of SV accumulation. Unfortunately, the current experimental setup does not allow us to stretch the compressed axons beyond their original length.

Our experiments with *Drosophila* showed stretch enhancement of SV accumulation occurred in the absence of extracellular Ca^{2+}. Previous experiments by Siechen *et al.*[46] showing tension induced (stretch $<$ 10%) SV accumulation were conducted in the presence of extracellular Ca^{2+}.[46] We observed a similar qualitative response in the absence of extracellular Ca^{2+}. However in comparison to the study by Siechen *et al.*,[46] where the increase in accumulation was close to 300%, the magnitude of increase in our experiments is much smaller (30%). This suggests the attenuated stretch enhancement observed in our experiments could be due to reduced levels of extracellular Ca^{2+}. Previous experiments demonstrated that the stretch enhancement of neurotransmitter release is attenuated in the absence of extracellular Ca^{2+} in frogs and still occurs when internal stores of Ca^{2+} are buffered.[10] Chen *et al.*[10] suggests the stretch enhancement does not occur *via* secondary messenger pathways or chemical modification but rather could be mediated by mechanical coupling *via* integrin mechanosensors. Thus, it seems that extracellular Ca^{2+} may contribute to the magnitude of stretch enhanced SV accumulation, but is not necessary for it to occur. This suggests that the mechanism of stretch enhanced SV accumulation is not completely dependent on extracellular Ca^{2+}.

Disruption of Local LDCV Dynamics

Local dynamics of LDCVs in cultured *Aplysia* neurites was disrupted immediately after neurite tension was reduced (by compression). Vesicle range and processivity continued to decrease as compression was held fixed, and remained low for at least 15 min after compression was removed. Interestingly, increased neurite tension does not seem to affect the LDCV velocity distribution. This may suggest that neurons are able to maintain normal LDCV dynamics under mechanical stretch, such as occurs during motion or natural growth of an organism.[25] On the other hand, this may also suggest LDCV dynamics could be disrupted when neurite tension is lost as occurs in axonal branch pruning and retraction.[5,29,31]

When *Aplysia* neurites are compressed, any preexisting tension decreased immediately. Reduced neurite tension could affect vesicle dynamics in many ways including altering: microtubule depolymerization, microtubule binding proteins, or molecular motor function. It has been shown that microtubule depolymerization leads to disrupted vesicle transport in *Aplysia*.[27] Depolymerization of microtubules could occur due to the mechanical compression along the neurite.[39] Additionally, if microtubules exhibit buckling,[21] this may promote mechanical severing of the microtubule binding protein Tau which leads to disrupted vesicle transport[44] due to polar mismatching or discontinuities.

When neurite tension was restored by reversing substrate compression, vesicle motion did not recover. This suggests that a structural change has occurred that persists after reversal of substrate deformation. Shemesh *et al.*,[45] found that microtubule polar mismatching or discontinuities that lead to disrupted vesicle transport are structurally stable. Therefore, in our experiments, any microtubule reconfiguration due to compression along the neurite may remain after the deformation is reversed. Thus, vesicle motion would remain disrupted even after neurite tension is restored.

Vesicle Transport and Accumulation

Transport and accumulation of vesicles appear related given the collective changes in the spatiotemporal organization of vesicles with stretch. Here we discuss a hypothesis linking the transport of vesicles along the axon to their accumulation at the synapse as shown schematically in Fig. 7. In its natural resting state (Fig. 7a), an axon has microtubules (green) running along its length, which are crosslinked (black) together to form a network. Vesicles (blue) are attached to microtubules *via* molecular motors (brown) that transport them along the axon. Some of these vesicles may attach to actin scaffolding (red) and accumulate at the synapse. When the axon is compressed along its length (Fig. 7b), tension in the axon is reduced as shown by its wavy morphology. Vesicle transport is disrupted, perhaps due to microtubule depolymerization. Thus, vesicles previously located at the synapse would remain trapped in the actin scaffolding. When the axon is stretched (Fig. 7c), tension in the axon is increased and vesicle transport is not disrupted. Increased tension may induce actin polymerization at the synapse allowing more vesicles to attach to the scaffold at the synapse leading to increased accumulation. This putative mechanism between vesicle transport and accumulation may explain an increase in vesicle accumulation under stretch and no effect under compression, although further studies are certainly needed to validate the proposed relationships between mechanical perturbation, actin polymerization, and vesicle movement/accumulation.

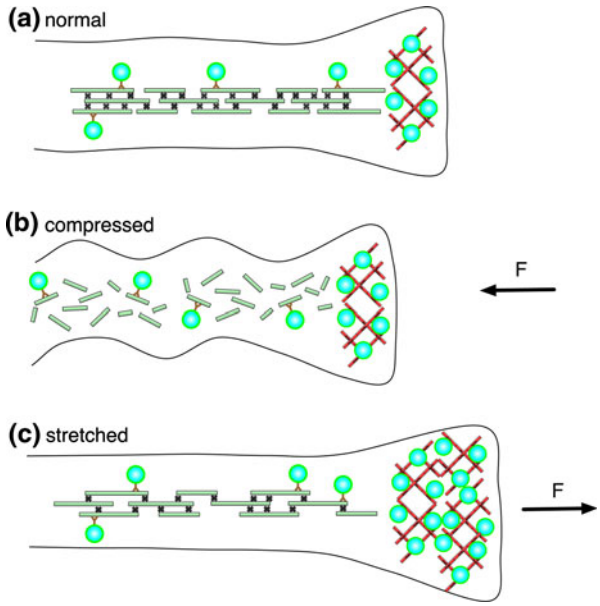

FIGURE 7. A connection between vesicle transport and accumulation. (a) A schematic diagram of an axon in its normal resting state. Microtubules (green) extend along the axon and are crosslinked (black) together to form a network. Vesicles (blue) are attached to molecular motors (brown) that transport them along the microtubule network and some accumulate in the actin scaffolding (red) at the synapse. (b) Microtubules depolymerize under compression leading to disrupted vesicle transport while maintaining normal vesicle accumulation at the synapse. (c) A stretched axon exhibits increased vesicle accumulation at the synapse due to tension induced actin polymerization creating more vesicle binding sites.

CONCLUSION

Our results show that mechanical stretch affects vesicle dynamics at the local scale of individual LDCV

motion as well as the global scale of SV accumulation at the synapse. Increased axonal stretch induced enhanced SV accumulation at the NMJ of *Drosophila* motor neurons, which saturated after approximately 50 min. This increase in vesicle accumulation occurred in the absence of extracellular Ca^{2+} and persisted for at least 30 min after axonal stretch was decreased. Mechanical compression disrupted LDCV dynamics in *Aplysia* neurites, including range and processivity. This decreased LDCV motion occurred immediately after compression was applied, and persisted for at least 15 min after compression was removed. These results strengthen the hypothesis that mechanical tension could serve as a mechanism to regulate neuronal function including vesicle transport and synaptic plasticity. The underlying molecular mechanisms that link mechanical tension and vesicle dynamics warrant further investigations.

ELECTRONIC SUPPLEMENTARY MATERIAL

The online version of this article (doi: 10.1007/s12195-012-0223-1) contains supplementary material, which is available to authorized users.

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