The Mechanical Sensitivity of Vesicle Dynamics

in In Vivo and In Vitro Neurons

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ABSTRACT

Axonal tension exists in neurons and may be involved in neuronal signaling. We study the effect of mechanical strain on the dynamics of vesicles in in vivo Drosophila motor neurons and in vitro Aplysia neurons. Neurons are stretched or compressed while observing vesicle dynamics by high-resolution live-imaging. In response to mechanical stretch we observe the dynamic accumulation of synaptic vesicles at the in vivo neuromuscular junction (NMJ) after approximately 50 min. Vesicle accumulation at the NMJ persists for at least 30 min after stretch is removed. In response to compression we observe disruption of vesicle dynamics in in vitro growth cones. Range and processivity of vesicle motion decrease immediately after applied compression and do not recover for at least 20 min after compression is removed. Through live-imaging this study shows that mechanical stretch promotes vesicle clustering in in vivo synapses, and compression impedes vesicle transport in in vitro growth cones.

Keywords: neuron, vesicle, mechanical strain, in vitro, in vivo

1 INTRODUCTION

Neurons are the basic communication element of the nervous system. They transport neurotransmitter filled vesicles to the synapse where they cluster prior to release for signaling a target cell. The more frequently a neuron is used, the higher its efficiency and this is partly due to increased vesicle clustering at the synapse. This usage dependent efficiency is known as synaptic plasticity and it is crucial to the formation of memory and learning in animals. Previously, vesicle clustering was believed to be a primarily biochemical phenomenon. However, it has recently been discovered that mechanical tension contributes to clustering of neurotransmitter vesicles at the presynaptic terminal [1]. This suggests a possible role for mechanics in neuronal signaling.

The first evidence of mechanical tension in in vitro neurons came in 1979, when D. Bray [2] found that growth cones of cultured neurons can exert mechanical tension, and that the direction of tension determines growth direction. Ten years later in 1989, Dennerll et al. [3] found that the magnitude of tension influenced neuronal elongation and retraction, and Zheng et al. showed tension could initiate neuritogenesis [4]. It has been suggested that tension may play a role in the morphogenesis and compact wiring of the in vivo nervous system [5]. Recently it has been shown that in vivo motor neurons of Drosophila embryos are also naturally under tension and maintain axonal tension when mechanically perturbed [6]. The mechanical micro-environment is known to regulate cell function [7] and it is important to study these processes in neurons to understand the role of mechanics in neuronal function.

Here, we study the effect of mechanical strain on subcellular dynamics in two different model systems: (1) In vivo motor neurons of the Drosophila melanogaster embryo and (2) In vitro neurons of the Aplysia californica. We show that mechanical strain affects local vesicle dynamics as well as global vesicle clustering.

2 MATERIALS AND METHODS

2.1 Stretching System

In this study we utilized a stretching system which allowed simultaneous high-resolution live-imaging and application of mechanical strain [8]. Briefly, the system uses a deformable polydimethylsiloxane (PDMS) cell culture substrate capable of applying mechanical strain to cells and tissues up to 45%. The strain field was characterized experimentally and computationally to show uniformity for greater than 95% of the cell culture surface. A standard mechanical stage (Newport Inc.) was used to apply a static deformation to the substrate, which transferred mechanical strain to cells adherent to the culture surface.
2.2 Drosophila Embryos

The *Drosophila melanogaster* is a convenient model system for studying neuronal function *in vivo*. They have long been used for studying synaptic development and neurotransmission and are known to have structurally plastic neuromuscular junctions (NMJ) [9]. *Drosophila* (eve-GAL4;UAS-sytEGFP) embryos expressing green fluorescent protein (GFP) in membrane bound synaptotagmin were used for this investigation (Source: Chiba Lab). *Drosophila* embryo samples were prepared similarly as previous studies [8]. Briefly, embryos were dechorionated in 50/50 bleach and water solution for 2 min. Embryos of the correct age (16 hr after egg laying) were fillet dissected on the APTES functionalized PDMS surface. Experiments were completed within 3 hours of embryonic dissection.

2.3 Aplysia Cell Culture

*Aplysia* neurons are a convenient model system for studying subcellular dynamics in neurons because their large neurite structures are highly amenable to live-imaging [10]. *Aplysia californica* neurons were harvested as previously published [11]. Briefly, neural tissue was enzymatically digested and cells were dissected using sharp tungsten needles or a glass suction pipet. Cells were plated on pretreated polylysine coated PDMS substrates for 24-48 hours before experiments.

2.4 Microscopy and Image Analysis

Images were collected with a Zeiss LSM 710 laser scanning confocal microscope using a 40x (1.2 NA) water immersion objective lens (Institute for Genomic Biology, Urbana, IL). All imaging parameters (e.g. laser power, pixel dwell time, pinhole size, gain, etc.) were kept constant for a given set of experiments.

For *Drosophila*, fluorescent images of synaptic vesicles were captured as Z-stacks at approximately 5 min intervals. Here it is assumed that the amount of synaptic vesicles present is proportional to the fluorescence intensity of the synaptotagmin-GFP signal. Thus the amount of synaptic vesicles was quantified using the fluorescent intensity calculated from the collapsed Z-stack. The average intensity in a 2.5 micron square region at the presynaptic terminal was used to quantify the synaptic vesicles at the neuromuscular junction (NMJ).

For *Aplysia*, differential interference contrast (DIC) image sequences of large dense core vesicles (LDCV) were captured at a rate of 2.5 frames per second. Videos were captured approximately every 5 minutes. Dynamics of LDCVs were tracked using an algorithm for precise particle tracking by polynomial fitting with Gaussian weight in Matlab [12].

3 RESULTS

3.1 Stretch Induced Accumulation of Synaptic Vesicles

We observed synaptic vesicle accumulation at the NMJ of *Drosophila* motor neurons in response to applied mechanical stretching. Figure 1 shows the fluorescence intensity of synaptic vesicles at the NMJ region versus the time course of the experiment. For control experiments neurons were imaged at identical intervals as other conditions in the absence of externally applied mechanical perturbation. The black data points in Figure 1 shows that over the course of the experiment, the amount of synaptic vesicles stays approximately constant.

To investigate the effect of increased mechanical tension in the axons of *in vivo* neurons, the stretching system was used to stretch the entire *Drosophila* embryo by 20%. This results in a tensile strain in the motor neuron axons of 20%. The mechanical deformation was applied at time 0 min and was held constant for 90 min. The deformation was then reversed to the original configuration and the sample was observed for an additional 30 min in the unstretched state. The effect of mechanical stretching on synaptic vesicle accumulation at the NMJ is shown by the blue data points in Figure 1. Approximately 50 minutes after stretching, there is a 30% increase in the amount of synaptic vesicles at the NMJ. The increase in amount of synaptic vesicles persists for at least 30 minutes after the stretch has been removed.

![Figure 1: Fluorescence intensity of synaptic vesicles vs. time at the neuromuscular junction of Drosophila neurons. The control (black) data shows there is no significant change in synaptic vesicle accumulation in the absence of mechanical perturbation. When the axons are stretched (blue) they show a significant accumulation after ~ 50 min, which persists after mechanical unloading. When compressed (red),](image-url)
synaptic vesicle accumulation does not differ from the control.

To investigate the effect of mechanical compression in the axons of in vivo neurons, the stretching system was used to compress the entire Drosophila embryo by 8%. This results in a compressive strain along the direction of the axons by 8%. This temporarily removes any previously existing tension in the axon. The compression was applied at time 0 min and held constant for 60 min. The deformation was then reversed to the original configuration and the samples were observed for an additional 60 min. As shown in Figure 1 by the red data points, mechanical compression along the axon does not have a significant effect on accumulation of synaptic vesicles at the NMJ.

3.2 Compression Induced Disruption of Vesicle Motion

We observed disruption of vesicle dynamics in growth cones of Aplysia neurons in response to applied mechanical compression. Figure 2 shows the position of LDCVs in the growth cone versus time. For control experiments the neurons were imaged in the absence of mechanical stimulation. The black data points in Figure 2 show that in control samples, vesicles have a relatively large range of oscillatory motion moving up to 500 nm from their central position. Vesicles in the control samples also exhibited processive motions of approximately 380 nm on average.

To investigate the effect of mechanical compression on vesicle dynamics in neurites of in vitro neurons, we applied 8% compressive strain along the axis of Aplysia neurites. Static compression was applied for 15 minutes and then the deformation was reversed back to the original configuration. Vesicle dynamics was observed during the 15 min of static compression, and 20 min after the deformation was reversed. Immediately after compression was applied, the motion of vesicles decreased significantly as is shown by the blue data points in Figure 2. Their range of motion fell to less than 300 nm from their central location and vesicle processivity decreased to approximately 150 nm. After 10 min of static compression (pink), vesicle range decreased below 200 nm and processivity further decreased below 100 nm. The effect of retarded vesicle dynamics persisted for at least 20 min after the mechanical compression was removed. It should be noted that throughout the experiment, the same vesicles are being tracked, and thus the observed effect is not due to variation in dynamics between different vesicles.

4 DISCUSSION

4.1 Synaptic Vesicle Accumulation

Accumulation of synaptic vesicles at the presynaptic terminal is integral to the process of usage dependent tuning of synaptic efficiency (e.g. synaptic plasticity). This process is part of the formation of memory and learning in animals and involves intricate biochemical signaling cascades. Here we have observed the accumulation of synaptic vesicles in response to mechanical stretching. This connection suggests a role for mechanical forces in neuronal function as recent reviews have highlighted [13-14].

It has been hypothesized that actin may serve as a scaffold for clustering of vesicles at the presynaptic terminal [15] and it has also been reported that mechanical tension promotes F-actin polymerization [16]. From previous studies it is known that upon stretching the Drosophila axon, the force instantaneously increases and decays exponentially to a steady-state tension (greater than the initial rest tension) after approximately 25 min [6]. Therefore when stretching the axon, it is subjected to increased tensile forces for an extended period of time. Thus it is possible that mechanical tension in neurons modulates the polymerization of F-actin in the cytomatrix of the synaptic region. Therefore when tension is increased by stretching, the amount of F-actin may increase, leading to increased clustering of vesicles at the NMJ. In
addition it is also known that neuronal membrane expansion (as can be forced by stretching) is mediated by vesicle exocytosis [17]. This effect, coupled with possible changes in gene expression due to mechanical deformation [18] could promote vesicle synthesis to allow membrane expansion and possibly cause increased vesicle accumulation at the synapse.

In contrast to stretching, when compression is applied along the neuronal axon, the natural rest tension is temporarily removed. From previous studies it has been observed that Drosophila motor neurons will build axonal tension to a value close to their natural rest tension in as little as 10 minutes [6]. Therefore this short perturbation of axonal force may not be adequate to affect F-actin polymerization or vesicle synthesis. This may be the reason that no effect on vesicle accumulation is observed when mechanical compression is applied along the axons.

Currently, the mechanism of tension induced vesicle accumulation at the NMJ of in vivo Drosophila motor neurons is not well understood. The authors are currently investigating the effect of tension on cytoskeletal polymerization as well as vesicle synthesis.

4.2 Disruption of Vesicle Dynamics

In vitro experiments with Aplysia neurons show that mechanical compression applied along neurites results in decreased range andprocessivity of vesicle motion. The significant decrease in vesicle dynamics may be due to depolymerization of microtubules.

It has been shown that extracellular calcium influx into the cell can cause microtubule depolymerization and thus impaired vesicle transport [19]. When mechanical compression is applied along the neurite axis, a lateral stretching is induced perpendicular to the direction of the neurite due to Poisson expansion [8]. As a result, it is possible that stretch activated ion channels are opened allowing a flux of extracellular calcium into the neurite. If the increased calcium concentration causes microtubule depolymerization then vesicle transport could be disrupted.

It is also possible that the mechanical compression itself is causing microtubule depolymerization. Previous reports have shown that compressive force applied along the microtubule axis results in depolymerization [20]. Additionally if microtubule bundles exhibit buckling [21] this may promote severing of the microtubule binding protein Tau, which is known to lead to microtubule depolymerization and disrupted vesicle dynamics [22].

5 CONCLUSION

Vesicle dynamics in both in vivo and in vitro neurons are sensitive to mechanical perturbations. Mechanical forces affect local dynamics of single vesicles on the time scale of seconds, as well as global accumulation of synaptic vesicles at the NMJ over 1-2 hrs. Our results show that neurons are highly sensitive to mechanical perturbations, and axonal tension may play a role in neuronal function. Further studies are necessary to uncover the mechanotransduction pathways involved in force modulated vesicle behavior.

REFERENCES