

P16 – Using Optical Tweezers to Study the Elementary Events Underlying Force Generation in Neuronal Lamellipodia

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The propulsion of the leading edge of neuronal lamellipodia is a complex process in which the polymerization of actin filaments towards the cell membrane is a major component [1,2]. This process is at the origin of force generation in neurons. By using optical tweezers, we have characterized the dynamics by which lamellipodia of Dorsal Root Ganglia neurons exerted force on encountered obstacles such as silica beads. To determine the displacements produced by elementary events behind force generation, the stiffness of the optical trap was kept as low as $0.015 \text{ pN}\cdot\text{nm}^{-1}$, so that the underlying motion could occur in an unhindered fashion [3]. At such a low stiffness the bead held in the optical tweezers necessarily fluctuate with large amplitude possibly masking the underlying biological events. Because of the presence of adhesion forces, beads in close contact with a lamellipodium could seal on its membrane so that the standard deviation of Brownian fluctuations could be reduced by 10 times. In several experiments, the bead remained within 300 nm from the center of the optical trap where the voltage sensitivity of the detector and the trap stiffness is constant. Under these conditions, if the lamellipodium pushed the bead, discrete jumps could be detected. Jumps were detected using an algorithm based on nonlinear diffusion filtering [4]. Briefly, the original signal was smoothed in order to obtain a smooth piece-wise (regularized) trace where the discrete jumps were enhanced and then detected. These jumps occurred within 1 ms and had an amplitude varying from 5 to 20 nm. When the lamellipodium retracted, pulling the beads with it, no discrete events were observed. These discrete events were not observed in the presence of Latrunculin A, a blocker of actin polymerization or when neurons were fixed with paraformaldehyde. These jumps show that force generation in lamellipodia is a discontinuous process in which bursts of actin polymerization and depolymerization alternate continuously. In future we will explore changes in the characteristics of these jumps by pharmacologically altering the membrane rigidity to understand the role of the membrane in this process.

References

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Abstract

The propulsion of the leading edge of neuronal lamellipodia is a complex process in which the polymerization of actin filaments towards the cell membrane is a major component [1,2]. This process is at the origin of force generation in neurons. By using optical tweezers, we have characterized the dynamics by which lamellipodia of Dorsal Root Ganglia neurons exerted force on encountered obstacles such as silica beads. To determine the displacements produced by elementary events behind force generation, the stiffness of the optical trap was kept as low as 0.015 pN nm^{-1} , so that the underlying motion could occur in an unhindered fashion [3]. At such a low stiffness the bead held in the optical tweezers necessarily fluctuate with large amplitude possibly masking the underlying biological events. Because of the presence of adhesion forces, beads in close contact with a lamellipodium could seal on its membrane so that the standard deviation of Brownian fluctuations could be reduced by 10 times. In several experiments, the bead remains within 300 nm from the center of the optical trap where the voltage sensitivity of the detector and the trap stiffness is constant. Under these conditions, if the lamellipodium pushed the bead, discrete jumps could be detected enhanced and then detected. These jumps occurred within 1 ms and had an amplitude varying from 5 to 20 nm. When the lamellipodium retracted, pulling the beads with it, no discrete events were observed. These discrete events were not observed in the presence of Latrunculin A, a blocker of actin polymerization or when neurons were fixed with paraformaldehyde. These jumps show that force generation in lamellipodia is a discontinuous process in which bursts of actin polymerization and depolymerization alternate continuously. In future we will explore changes in the characteristics of these jumps by pharmacologically altering the membrane rigidity to understand the role of the membrane in this process.

1. Change of noise during push and pull

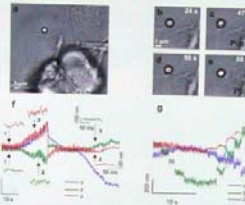
Lamellipodium protruding from the DRG neuron soma.

The bead is trapped in front of the lamellipodium leading edge by an IR laser (a).

Push. Successive high resolution frames of the lamellipodium during a push (b-c). At 24 s the bead is in its equilibrium position in front of the leading edge of the lamellipodium (b). Subsequently, the lamellipodium grows and pushes the bead (47 s) displacing it both laterally and axially (c). The cross indicates the equilibrium position of the optical trap.

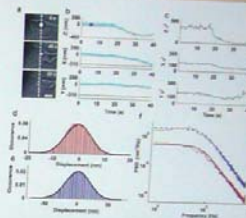
Pull. The lamellipodium retracted, pulling the bead back into its equilibrium position (56 s). Then, it continues retracting (88 s) pulling further the bead. The cross indicates the equilibrium position of the optical trap.

Increase of noise during push and decrease of noise during pull. The three components of the bead displacement (x , y , and z), (f), insets highlight the increase of noise during the push (2), the decrease of noise during pull (3) with respect to the amplitude of the Brownian fluctuations of the trapped bead in the equilibrium position (1) for the axial (z) and one of the lateral components.



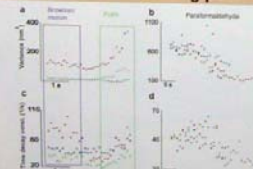
No increase of noise after treatment with Latrunculin A. The three components of the bead displacement (x , y , and z) recorded while moving the laser beam trapping the bead towards the neuron, after treatment with 100 nM Latrunculin A (g).

2. Adhesion properties



High resolution frames showing lamellipodium adhering to the trapped bead (a). When silica bead seals on the membrane it is pulled downwards by lamellipodium (at 40 s), the Brownian noise affecting displacement recordings is drastically reduced on the three components x , y and z (b). In several experiments, the variance of lateral and axial fluctuations of displacement far from the lamellipodium was about 100 and 500 nm^2 , respectively, but during adhesion it could decrease by 10 times (c). In several occasions, the bead was attracted towards the lamellipodium (upper trace in (b)) and its axial position z decreased by 100-300 nm and, simultaneously, Brownian fluctuations greatly reduced and often their variance σ_z^2 decreased to less than 5 nm^2 (upper trace in c). After adhesion the bead was still in the harmonic potential of the optical trap and its position fluctuations could be fitted with a Gaussian curve with reduced amplitude of fluctuations (d) as compared to bead in Brownian motion (e). Also power spectral density (PSD) of bead positions after adhesion could be fitted with single Lorentzian (red trace in f) but with a reduced rolloff frequency as compared to PSD during Brownian motion.

3. Increase of noise during push



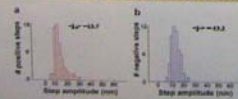
Variance

During the establishment of adhesion the amplitude of Brownian fluctuations was decreased and when the lamellipodium pushed the bead, the variance of recorded displacements increased (a). When the motility of the lamellipodia was completely suppressed by fixing the cells with paraformaldehyde and trapped bead was moved towards it, exactly mimicking displacement when the bead is pushed by the lamellipodium decrease in variance was observed (b), suggesting that increased noise has biological origin.

Autocorrelation

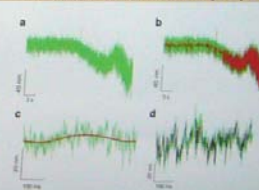
The autocorrelation function $\langle \delta x(t) \delta x(t+\tau) \rangle$ could be fitted by an exponential function $e^{-\tau/\alpha}$. The value of α (time decay constant) increased during the pushing phase (c) as compared to adhesion phase. In contrast when push was mimicked with fixed cells value of α was found to be decreasing as the bead was displaced from the center of the trap (d).

5. Distribution of jumps



Many discrete jumps could be detected during the pushing phase with a variable amplitude. The distribution of the amplitude of detected positive ($+$) and negative jumps ($-$) is reproduced in (a) and (b), respectively. The amplitude of these jumps varied from 5 up to 20 nm, and had a mean value $\langle + \rangle$ and $\langle - \rangle$ equal to 13.7 and -13.2 nm respectively.

4. Detection of discrete jumps



Discrete jumps with a varying amplitude were detected using well known tools from signal processing. We used a nonlinear diffusion filtering [4] in which two parameters, σ and γ , are used to fix a threshold for the detector of jumps (minimal length detected: 5 ms) and for their bandwidth (up to 5 kHz). This procedure allowed the original data (a) to be approximated with a smooth piece-wise function (red trace in (b)) interrupted by discrete jumps or discontinuities (black trace in (d)). Almost no jumps were detected during the Brownian motion and adhesion phase (c), but jumps became evident only when the exerted force increased and lamellipodia pushed the bead (d).

6. Conclusions and perspectives

Our results show that force generation in DRG lamellipodia is characterized by the presence of discrete jumps occurring in a millisecond window, and with an amplitude varying from 5 to 20 nm, corresponding to forces up to 200 pN. These jumps could be the exclusive elementary events underlying force generation due to bursts of actin polymerization alternating with bursts of depolymerization.

Future work will consist of performing a statistical analysis of these jumps, and to explore changes in the characteristics of these jumps by pharmacologically altering the membrane rigidity to understand the role of the membrane in the force generation process. Moreover, we envisage to propose a theoretical model which explains our experimental observations regarding elementary events underlying force generation in neuronal lamellipodia.

References

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