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Force generation in lamellipodia is a probabilistic process with fast growth and retraction events

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Bold notations \mathbf{x} , \mathbf{v} , and \mathbf{F} indicate vectorial quantities and x , v and F indicate either the modulus or a component of these vectors.

Abstract

Polymerization of actin filaments is the primary source of motility in lamellipodia and is controlled by a variety of regulatory proteins. The underlying molecular mechanisms are only partially understood and a precise determination of dynamical properties of force generation is necessary. Using optical tweezers we have measured with millisecond temporal resolution and pN sensitivity the force-velocity (Fv) relationship and the power dissipated by lamellipodia of dorsal root ganglia (DRG) neurons. When force and velocity are averaged over 3-5 s, the Fv relationships can be flat. On a finer time scale, random occurrence of fast growths and sub-second retractions become predominant. Maximal power dissipated by lamellipodia over a silica bead with a diameter of 1 μm is 10^{-16} W. Our results clarify the dynamical properties of force generation: i - force generation is a probabilistic process; ii - underlying biological events have a bandwidth up to at least 10 Hz; iii - fast growths of lamellipodia leading edge alternate with local retractions.

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Introduction

Neurons are among the most specialized cells in living organisms and are capable to self organize in complex networks, formed by billions of individual cells, at the basis of higher brain functions. In order to develop a network, neurons protrude to form neurites, highly motile structures exploring the environment in search of the appropriate chemical cues necessary for the formation of the correct synaptic connections (1-3). The neurite's search is guided by growth cones (4-6) located at their tip, formed by an extended lamellipodium from which thin filopodia emerge (7). Filopodia tips can move at a speed up to $0.8\text{-}1\ \mu\text{m s}^{-1}$ and their motility is at the basis of the efficient formation of neural networks. Due to its enormous importance, this system has been the object of intense experimental and theoretical investigation. The primary source of motility in growth cones is the polymerization of actin filaments (8, 9), a process controlled by a variety of regulatory proteins (10). The addition of actin polymers to actin filaments in close contact with the membrane pushes the cellular membrane forward exerting a protrusive force (11, 12). The overall dynamics regulating this process is not yet clear and mathematical modeling provides a way to link known molecular events to force generation (1). A key outcome of these models is represented by the so-called Fv relationships, describing how the force F exerted by the actin filament network is related to the velocity v of their growing ends (8, 13-17). Fluctuations of contacts between the tip of actin filaments and the surrounding membrane is an essential feature of Brownian ratchet models (8, 13, 14) leading to Fv relationships in which v decreases exponentially with increasing values of F . In autocatalytic models (17, 18) when an obstacle is encountered the actin network, due to the action of controlling proteins, originates new branches, so that the velocity v can remain constant with increasing values of F . The experimental investigation of the molecular events underlying force generation in growth cones requires a precise measurement of Fv relationships with high temporal resolution and sensitivity. Previous determinations of the Fv relationships (19) with an Atomic Force Microscope (AFM) cantilever (20, 21) had a limited time resolution and were obtained either in vitro or in migrating keratocytes exerting forces in the nN range.

In this work, by using optical tweezers (22-24), we provide an experimental characterization of Fv relationships in neuronal growth cones with a millisecond resolution and pN sensitivity. This experimental technique enabled us to determine the three components of the force $\mathbf{F} = (F_x, F_y, F_z)$ exerted by a lamellipodium from rat dorsal root ganglia (DRG) and of the velocity $\mathbf{v} = (v_x, v_y, v_z)$ of its leading edge. From these vectorial quantities we have derived several properties of force generation in lamellipodia that have important biological consequences. We find that force generation in lamellipodia is an intrinsically multi-scale process. At a temporal resolution of 3-5 s, the exerted force can increase, maintaining a constant velocity, where the Fv relationships are almost flat. At a millisecond resolution, a much more complex behavior is observed, with random occurrence of fast growths and sub-second retractions. Our results show that autocatalytic models (15, 17, 18) of force generation are correct in a mean approximation. At a higher temporal resolution the network of actin filaments evolves in a much more complex manner that can be characterized only probabilistically. Fast forward motions consuming up to 10^4 molecules of $\text{ATP s}^{-1}\ \mu\text{m}^{-2}$ alternate with local catastrophes, whose duration has a power law distribution. These results provide a precise characterization of the dynamics of force generation in lamellipodia, necessary to understand the biochemical events underlying force generation.

Results

DRG neurons isolated from P10-P12 rats were plated on poly-L-lysine-coated glass coverslips, positioned on the stage of an inverted microscope used for imaging and measuring forces (see Methods). After 1 or 2 days of incubation, neurites emerged from the DRG soma and their motion was analysed. Filopodia and lamellipodia moved rapidly exploring the three dimensional (3D) space in all directions, which, in some occasions, could have a tip velocity as high as $1 \mu\text{m s}^{-1}$. DRG lamellipodia were imaged with Atomic Force Microscopy (Fig.1a) and the height of their leading edges varied from 45 to 660 nm (Fig.1b). Silica beads of $1 \mu\text{m}$ diameter were trapped with a 1064 nm infrared (IR) laser tweezers and positioned in front of the leading edge of a lamellipodium (Fig.1e). When the centre of the bead is located at about 800 nm above the coverslip, a thick lamellipodium can push the bead (Fig.1c). Visual inspection of lamellipodia indicates the existence of four stereotyped behaviours (25): (i) the lamellipodium grows underneath the bead without displacing it (Fig.1d); (ii) the bead seals to the cell membrane and when the lamellipodium retracts the bead is pulled away from the trap; (iii) the lamellipodium grows underneath the bead displacing it upwards and eventually moving it in a “shovel-like” manner (26); (iv) the lamellipodium pushes the bead forward exerting a force in the direction of its growth (Figs.3a and b). Often, two or more of these stereotyped behaviours were observed in the same experiment. In the example illustrated in Fig.1f, initially the lamellipodium pushed the bead upwards by some hundreds nm (at 68.2 s) and the bead returned into the equilibrium position inside the trap following lamellipodium retraction (at 94 s). After a few seconds, the lamellipodium grew under the bead and, because of the presence of adhesion forces, the bead sealed to the lamellipodium membrane. Finally, when the lamellipodium retracted, it dragged away the bead from the trap (after 100 s). Force velocity relationships were computed only from those experiments in which the lamellipodium pushed the bead and then retracted (events of type iv). In all experiments, the growth cone behaviour was followed with video imaging and the displacement of the bead $\mathbf{x} = (x, y, z)$ was measured with a high temporal resolution using a Quadrant Photo Diode (QPD). The z axis is perpendicular to the coverslip and parallel to the IR laser beam used for optical trapping. By determining the trap stiffness $\boldsymbol{\kappa} = (k_x, k_y, k_z)$, \mathbf{F} was obtained as $(-x k_x, -y k_y, -z k_z)$ (23, 27).

At a low temporal resolution the force can increase with an almost constant velocity and the Fv relationships can be flat.

Figure 1 near here

When lamellipodia pushed the bead upwards, they exerted forces up to 20 pN. In the case of the experiment shown in Fig.1, when the bead displacement was low pass filtered at 0.2 Hz (green trace in Fig.1g) corresponding to a temporal averaging over a time window of 3-5 s, the computed velocity v_z had little oscillations around an almost constant value. From the smoothed values of F_z and v_z , the Fv relationship (green trace) shown in Fig.1h was obtained. The Fv relationship - following an initial rise - resulted almost flat, indicating that the lamellipodium can increase the exerted force while the velocity of its leading edge remains almost constant. Nearly identical results were obtained when Fv relationships were computed from the modulus of \mathbf{F} and not from a single component (F_z).

An almost flat Fv relationship was observed in other 7/95 experiments but not in all of them. As it will be discussed later, force generation is not a deterministic event but a probabilistic process. The observation that, in some experiments, Fv relationships filtered at 0.2 Hz are flat, indicates that the overall dynamics assumed by autocatalytic models capture basic properties of force generation. These models predict that when the underlying system of actin filaments and controlling proteins have the

time to self-reorganize, v becomes independent of F . Almost flat Fv relationships were obtained averaging the values of F and v in a time window of 3-5 s, which could be the time required by the underlying system of actin filaments to reorganize properly, as predicted by these models.

At a higher temporal resolution the velocity oscillates and transient periods of negative velocities are observed.

Figure2 near here

Averaging a temporal series over a time window of 3-5 s corresponds to smoothing the data with a low pass filter with a bandwidth up to 0.2 Hz. This is a strong assumption, and, in order to determine if this cut-off frequency is appropriate, we investigated the bandwidth of biological events underlying force generation. We computed and compared the power spectrum density $PSD_{noise}(f)$ of forces measured far from any neuron (red inset in Fig.2a) - originating from Brownian fluctuations and instrumental noise - and the $PSD_{push}(f)$ of forces measured when the leading edge of the lamellipodium pushed the bead (blue inset). $PSD_{noise}(f)$ and $PSD_{push}(f)$ are very similar and almost indistinguishable for $f > 30$ Hz, but at frequencies below 1 Hz the energy of $PSD_{push}(f)$ is at least 30 times larger than that caused by Brownian collisions. The analysis of $PSD_{noise}(f)$ and $PSD_{push}(f)$ in 14 experiments indicates that the bandwidth of biological events underlying force generation in DRG lamellipodia extends up to 10 Hz. Therefore, events occurring on a time scale of 100 ms cannot be neglected and force generation must be analysed at a temporal resolution higher than in previous investigations. In some experiments, we also observed 5-30 nm jumps of bead position occurring in less than 1 ms, which could constitute the elementary events underlying force generation (26).

We computed Fv relationships from the experiment of Fig.1g after smoothing at 0.2 (green trace in Fig.1h), 1 (pink trace in Fig.1h) and 10 Hz (Fig.1j). When data were smoothed at 1 and 10 Hz the velocity oscillated around an almost constant value of 200 nm s^{-1} reaching occasional peak values up to $1-10 \text{ } \mu\text{m s}^{-1}$. In the majority of the experiments (88/95) the shape of measured Fv relationships was not constant. Two examples are shown in Fig.1 and Fig.3. In some experiments (14/95) the lamellipodium pushed the bead and then retracted repetitively. Also in these cases the maximal force and the shape of Fv relationships measured at different times varied. These results suggest that force generation in lamellipodia is an inherently probabilistic process and does not follow a deterministic mechanism. In order to characterize this probabilistic dynamics we attempted to determine *average* Fv relationships $\langle Fv \rangle$. Forces measured in the individual experiments were normalized to the maximal exerted force F_{max} , defined as the maximal force beyond which the lamellipodium leading edge does not advance and the velocity is consistently negative for at least 10 s. This procedure was repeated for data filtered at 0.2, 1 and 10 Hz. The three average Fv relationships obtained in this manner exhibited the same overall behaviour (Fig.2b), with the velocity increasing together with the force, up to about 30 nm/s , and remaining approximately constant up to F_{max} . Thus, even if in the single events the Fv relationship can vary significantly, the average Fv relationship is flat, consistent with autocatalytic models.

Having observed the existence of periods during which lamellipodia leading edges have negative velocities, we asked whether these periods occurred at random times or occurred more frequently near the maximal measured force F_{max} . We computed the probability distribution of velocities being less than $-3\sigma_v$ at fixed forces F/F_{max} , $p(v < -3\sigma_v | F/F_{max})$, where σ_v is the standard deviation of Brownian fluctuations at a given bandwidth (see next section and Fig.3). This probability distribution was estimated from the 95 experimentally determined Fv relationships. The value of $p(v < -3\sigma_v | F/F_{max})$ varied between 0.05 and 0.1 (Fig.2c) and was nearly identical when it was derived from

Fv relationships computed at 0.2, 1 and 10 Hz bandwidth. This result indicates that retractions of the lamellipodium leading edge are not triggered by a strong load but their occurrence is random.

Statistical properties of Fv relationships were characterized by measuring the distribution of time intervals (Δt) with a positive (Fig.2d) and a negative velocity (Fig.2e), representing the ON and OFF events of the lamellipodium leading edge. Detected ON and OFF events were seen using a bandwidth of 0.2 Hz. The distributions of the ON and OFF events obtained at the a bandwidth of 0.2 Hz do not have an exponential behaviour but exhibit a power law distribution of the type $\Delta t^{-0.7}$ (see straight lines in Figs.2d and e) over almost two log units, suggestive of a growth characterized by avalanches (28).

In some experiments, the lamellipodium (Fig.3a) pushed the bead causing a pure lateral displacement (Fig.3b) so that only F_x and F_y changed appreciably (Figs.3c and d), whereas F_z remained constant (Fig.3e). Transient retractions of the lamellipodium leading edge caused the appearance of knots i.e. those periods with a negative velocity in the Fv relationships (Figs.3g and h). Because of the limited spatial and temporal resolution of the CCD camera used, these transient retractions could not be confirmed by video imaging (Fig.3b). Therefore, we asked whether they could originate from numerical artefacts and noise fluctuations. Indeed, the numerical computation of derivatives from noisy data is ill-conditioned (29) and negative velocities could be produced by the specific method used to compute the velocity from the displacement. Therefore, we compared two alternative methods to obtain the velocity from the displacement, such as Gaussian filtering and Linear regression. In Gaussian filtering the velocity is obtained from the displacement by its convolution with the derivative of a Gaussian function with a given cut-off frequency, while in the Linear regression method (see Methods) the velocity is obtained from the displacement and a linear interpolation of the data on a window containing W data points. In these two methods the time scale is given by the cut-off frequency of the Gaussian function and by the number of points W in the window, respectively. As shown in Fig.3f (compare green and black traces) the Fv relationships computed by the two methods from F_y had the same shape and number of knots.

Figure3 near here

However, as shown in Figs.3g and h, the number of knots in the Fv relationships computed for both the F_x and F_y components, increased when the bandwidth of Gaussian filtering increased from 0.2 to 1 Hz (green and pink traces respectively). As numerical differentiation is very sensitive to noise and it amplifies its high frequency components, we investigated at what extent the knots, are caused by Brownian fluctuations. We computed Fv relationships from force measurements obtained far from the lamellipodia. The obtained velocity was Gaussian distributed around 0, with a standard deviation σ_v increasing with the bandwidth of Gaussian filtering, depending also on the trap stiffness (Fig.3i). Periods with a negative velocity, less than $-3\sigma_v$, could not be ascribed to Brownian fluctuations and all negative velocities exceeding $-3\sigma_v$ lines (green and red dotted lines in Figs.3g and h) were caused by interactions with the lamellipodium: the $-3\sigma_v$ line was crossed several times and more often at larger bandwidths.

Lamellipodia dissipate power per unit area up to 10^{-16} W μm^{-2} during force generation.

Having determined the Fv relationships and estimated the maximal exerted pressure, we asked how much mechanical work and power lamellipodia exert on encountered obstacles such as beads. In several occasions we have observed that the lamellipodium leading edge (Fig.4a) pushed the bead in an elaborated, non-linear way (Fig.4b) so that its motion was not a simple displacement in one preferred direction. In these experiments F_x , F_y , F_z change almost independently, reaching their

maximum amplitude at different times (Fig.4c). In these cases, the bead motion is not a simple upward axial motion as in Fig.1, but the bead moves along a trajectory that often changes its direction (see black trace in Fig.4f). The knots in the Fv relationship described in the previous section are a consequence of these changes of direction. In order to investigate more quantitatively the nature of these events, it is useful to monitor the vectors \mathbf{F} and \mathbf{v} , with their modulus and direction. The power dissipated by the lamellipodium is the scalar product $\mathbf{F}\cdot\mathbf{v}$. The amplitude of the instantaneous velocity depends on the bandwidth used for filtering the data and $\mathbf{F}\cdot\mathbf{v}$ reaches values up to 4×10^{-18} W, when \mathbf{v} is computed at a bandwidth of 0.2 Hz but up to 10^{-16} W at a bandwidth up to 10 Hz (Fig.4d).

The analysis of the angle ϕ between \mathbf{F} and \mathbf{v} provides useful information to understand the mechanics of collisions between beads and lamellipodia: when ϕ is close to 0 the lamellipodium pushes the bead and develops a positive work, and when ϕ is close to π the lamellipodium retracts. When the angle ϕ is close to $\pi/2$ lamellipodia do not perform any work. A negligible work is performed primarily in two occasions: firstly, when the lamellipodium exerts a force comparable with that caused by Brownian collisions with the surrounding medium molecules; secondly, when the bead slides over the lamellipodium and \mathbf{F} becomes orthogonal to \mathbf{v} and no work is generated. The angle ϕ was determined as $\phi = \text{Arccos}(\mathbf{F}\cdot\mathbf{v} / |\mathbf{F}| |\mathbf{v}|)$ (Fig.4e). When the modulus of \mathbf{F} was larger than 2 pN, ϕ was usually close to either 0 or to π (Fig.4h), indicating that \mathbf{F} and \mathbf{v} are parallel or antiparallel with an opposite versus.

Figure4 near here

In contrast, when the modulus of \mathbf{F} is smaller than 2 pN (Fig.4g) the value of ϕ is most of the time close to $\pi/2$. A sudden change of the bead motion like those shown in Fig.4f could be caused either by a momentary sliding of the bead over the lamellipodium or by a transient retraction of the lamellipodium leading edge. The position of the lamellipodia was followed by video imaging with a CCD camera (see Figs.4a and b) and we could verify by visual inspection that the bead was always in contact with the lamellipodium leading edge. In addition, these two mechanisms can be easily distinguished observing the work: if the bead slides over the lamellipodium no work will be done and ϕ will remain close to $\pi/2$. Instead, if the lamellipodium transiently retracts, the work done by the lamellipodium will be negative and ϕ will remain close to π . With this procedure, we verified that periods with negative velocities analyzed in Fig.2 and Fig.3 were indeed associated to values of ϕ close to π and therefore were not caused by an occasional sliding of the bead but by transient retractions of the lamellipodium leading edge.

Discussion

The present manuscript provides a precise characterization of force generation in DRG lamellipodia with millisecond time resolution and pN sensitivity. Previous measurements made with the cantilever of an AFM were restricted to a temporal resolution in the second range and were obtained in migrating keratocytes producing forces in the nN range (21). By using optical tweezers we measured force generation in DRG growth cones and we could characterize several physical properties of the molecular network underlying force generation. As shown in Fig.2, relevant biological events occur on a time scale of less than 100 ms and different dynamical properties are seen at a time scale of 3-5 s. Our results show that: i - force generation is not a deterministic mechanism but follows a probabilistic process; ii - underlying dynamical events occur on different time scales varying from 100 ms to 5 s; iii - fast growths alternate to local retractions of the lamellipodium leading edge. These

results shed a new light on the biochemical network controlling force generation in neuronal growth cone lamellipodia (10, 30, 31).

Physical properties of force generation. The maximal force exerted by pushing lamellipodia on a bead with a diameter of 1 μm was about 20 pN (24). In some experiments this force clearly stopped the lamellipodium growth and could be identified as the stall force F_{stall} , i.e. the force that is able to block protrusion. As very often lamellipodia retract spontaneously, in most experiments F_{stall} was expected to be larger than the maximum force that was measured, F_{max} . The contact area between pushing lamellipodia and beads was determined by the analysis of video images of the event under examination. For all frames i corresponding to a detectable force measured with the QPD, we determined the arc Γ_i of the bead in close contact with the leading edge of the lamellipodium and the corresponding angle $2\theta_i$ on the bead center, as shown in red in Figs.5a-c.

Figure5 near here

Then the contact surface at frame i , $S_c(i)$, is assumed to be equal to the corresponding spherical cap of the bead. Simple geometrical formulae indicate that $S_c(i) = 2 \pi (1 - \cos \theta_i) r^2$, where r is the bead radius. Fig.5d reproduces the time evolution of the estimated value of S_c when a lamellipodium pushed a bead. The value of S_c varied from 0.25 to 1.57 μm^2 (Fig.5e). Therefore, the maximal pressure exerted by DRG lamellipodia was 20-80 pN μm^{-2} . The maximum power per unit area exerted by lamellipodia was estimated to be $1-4 \times 10^{-16}$ W μm^{-2} . The hydrolysis of one molecule of ATP provides energy of about 10^{-19} J (32) and, if this energy is converted into work with an efficiency of 60%, the hydrolysis of about $0.25-1 \times 10^4$ s $^{-1}$ of ATP per μm^2 is necessary to produce the measured power. The number of actin filaments in keratocyte and fibroblast lamellipodia has been estimated to be of the order of 100 per μm^2 (21). Therefore, the number of elementary motors per μm^2 is likely to be of the order of 100, where each elementary motor consumes approximately 25 to 100 ATP per second.

Fv Relationships. When position and force were filtered at 0.2 Hz, in some experiments, the pushing lamellipodia exerted an increasing force maintaining a constant velocity (Fig.1h). In the great majority of the experiments performed, however, force generation was characterized by large fluctuations of the velocity. This shows that force generation in lamellipodia is probabilistic in nature and only *average* $\langle Fv \rangle$ relationships (Fig.2b) exhibit a flat shape, during which the mean velocity remains constant while the force can increase. Therefore, autocatalytic models correctly describe force generation in a mean approximation. In individual experiments, the velocity does not remain constant but oscillates and can become negative. In these experiments, the same force can be exerted with a positive and negative velocity, a characteristic feature of systems exhibiting hysteresis (20). The time duration of periods with a negative velocity has a power law distribution reminiscent of self-organized systems near criticality (28). During these events, the force exerted on the bead by the lamellipodium acts in the opposite direction of its velocity, indicating that the bead is not simply sliding on the membrane, but that the actin filaments network is retracting, possibly due to local catastrophe or organized depolymerization controlled by cofilin and other severing proteins (10). Therefore, force generation is not a smooth process but it is characterized by a random alternation of fast growths and retractions of the lamellipodia leading edges.

Possible mechanisms underlying local retractions. What could be the mechanism underlying the unstable dynamics responsible for the frequent occurrence of negative velocities of lamellipodia leading edge? Proteins controlling the network of actin filaments, such as cofilin, could randomly

sever a large branch of actin filaments leading to a local catastrophe causing a transient retraction of lamellipodium leading edge. Although the occurrence of local catastrophes seems the most likely biological mechanisms underlying local transient retractions, it is possible that instability could originate also from interactions with the cellular membrane. Growing and branching of the actin filaments can also be instable because of the action of membrane tension. Indeed, the maximum measured force F_{\max} is approximately $20\text{-}100\text{ pN}/\mu\text{m}^2$, of the same order of the force exerted by a membrane with a surface tension γ equal to $0.005\text{ k}_B\text{T}/\text{nm}^2$ axially deformed by $1\text{ }\mu\text{m}$ (33). The actin filament network is confronted with a membrane exerting a force similar to F_{\max} , so that the actin filament network is only marginally stable and its propulsive force is almost counterbalanced by the membrane tension. Growing and retracting in conditions of marginal stability allows fast reactions and could provide lamellipodia the flexibility necessary for its physiological functions.

In conclusion, autocatalytic models (15, 17, 18) capture basic molecular mechanisms underlying force generation in a mean approximation. The network of actin filaments underlying force generation in lamellipodia, besides giving origin almost continuously to new branches of actin filaments, grows in a probabilistic way with fast forward motions consuming up to 10^4 molecules of ATP $\text{s}^{-1}\text{ }\mu\text{m}^{-2}$ alternating with local catastrophes, whose duration have a power law distribution.

Methods

Neuron preparation. Wistar rats (P10–12) were anesthetized with CO_2 and sacrificed by decapitation in accordance with the Italian Animal Welfare Act. DRGs were incubated with trypsin (0.5 mg/ml), collagenase (1 mg/ml), and DNase (0.1 mg/ml) in 5 ml Neurobasal medium in a shaking bath (37°C , 35–40 min). They were mechanically dissociated, centrifuged at 300 rpm, resuspended in culture medium, and plated on poly-L-lysine (PLL)-coated ($0.5\text{ }\mu\text{g}/\text{ml}$) 30 mm coverslips in Neurobasal medium containing 10% fetal bovine serum (FBS) at a density of 2.5×10^5 cells/ cm^2 . Cells were incubated for 24 to 48 hours before the measurements. At this stage, filopodia tips and lamellipodia leading edge could move at a speed of $1\text{ }\mu\text{m}\text{ s}^{-1}$. After 2–3 days plated neurons formed a dense network and the growth cones motion was drastically reduced.

AFM imaging. We determined the three dimensional (3D) structure of DRG lamellipodia and filopodia using Atomic Force Microscopy (AFM). Before imaging with AFM, DRG neurons were fixed with Glutaraldehyde. DRG growth cones were imaged using a commercial AFM (Nanowizard II, JPK Berlin) combined with an inverted optical microscope (Zeiss Axiovert 200), and a fluorescence set-up (Zeiss X-cite). Soft tips from VEECO with low force constant (OBL, $0.03\text{N}/\text{m}$) were utilized and forces were kept between 100 pN and 1 nN during scanning.

Optical tweezers set-up. The optical tweezers set-up was built as described in (24). The dish containing the differentiating neurons and the beads (PSI-1.0NH2, G.Kisker GbR, Steinfurt Germany) was placed on the microscope stage which could be moved by a 3 axes piezoelectric nanocube (17 MAX 301, Melles Griot Inc., USA). The temperature of the dish was kept at 37°C by a Peltier device. The dish was maintained in an environment providing a controlled level of CO_2 (5%) and moisture (95%). The bead position $\mathbf{x} = (x, y, z)$ was determined along all the axes (x , y and z) with an accuracy of 10 nm using back focal plane (BFP) detection, which relies on the interference between forward scattered light from the bead and unscattered light (23, 27, 34). The BFP of the condenser was imaged onto a quadrant position detector (QPD; Hamamatsu C5460SPL 6041) and the light intensity was converted to differential outputs digitized at 20 kHz and low pass filtered at 5 kHz. Bead z position

was determined using the Gouy phase shift effect (23). The trap stiffness $\mathbf{K}_{x,y,z}=(k_x,k_y,k_z)$ and the detector sensitivity were calibrated using the power spectrum method (23). Detector sensitivity was also checked by measuring voltage signals originating from displacements of a bead stuck to the coverslip obtained with the 3 axis piezoelectric nanocube. The force exerted by the lamellipodium \mathbf{F} was taken as equal to $-\mathbf{F}_{\text{trap}}$. When the displacement of the bead from its equilibrium position inside the trap $\mathbf{d}=(d_x,d_y,d_z)$ was less than 400 nm, $\mathbf{F}_{\text{trap}} = (F_x, F_y, F_z)$ was calculated as $F_x= d_x k_x$, $F_y=d_y k_y$ and $F_z=d_z k_z$ (23). All experiments of force recordings were monitored by video imaging with a CCD camera at a frame rate of 20 Hz. Visual inspection of recorded images allowed to discard from the analysis all force recordings during which visible debris interfered with the optical determination of the bead position \mathbf{x} .

Data Analysis. The velocity $\mathbf{v}=(v_x,v_y,v_z)$ of the bead was obtained by numerical differentiation of its sampled position $\mathbf{x}=(x(n),y(n),z(n))$ $n=1,\dots,N$. Numerical differentiation was computed either by convolution of the position components $x(n),y(n)$ and $z(n)$ with the derivative of a Gaussian filter $1/(\sigma\sqrt{2\pi}) \exp(-t^2/\sigma^2)$ (Gaussian filtering) or by Linear regression. In the Linear regression method, the components $v_x(n)$, $v_y(n)$ and $v_z(n)$ of velocity \mathbf{v} were calculated by a linear least square fit of the equations $x(n)= a_x + v_x(n) (i - n)\Delta t$, $y(n)= a_y + v_y(n) (i - n)\Delta t$ and $z(n)= a_z + v_z(n) (i - n)\Delta t$ with $i = -W, \dots, W$ where Δt was the sampling interval. The two parameters a_x and $v_x(n)$ were determined by minimizing the cost function:

$$[v_x, a] = \arg \min_{[v, a]} \left[\sum_{i=-W}^{n+W} (a + v_x (i - n)\Delta t - y(i))^2 \right]$$

and similarly for a_y and $v_y(n)$ and for a_z and $v_z(n)$. The computation of derivatives with the Linear regression method depended on the number of samples W . Fv relationships obtained from the same force measurement sampled at 10 kHz with the Linear regression method with $W=2200$ (black trace in Fig.3c) and obtained by using a Gaussian filter with a cut-off frequency of 1 Hz (red trace in Fig.3c) had the same number of knots. Similarly, Fv relationships obtained with the Linear regression method with $W = 10,000$ and by using a Gaussian filter with a cut-off frequency of 0.2 Hz had the same shape. When the number of points, W , considered for Linear regression was increased, it was equivalent to decreasing the band width of the Gaussian filter.

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Figure Legends

Fig. 1. (a) AFM image of a lamellipodium. The height is coded as in the coloured scale bar and horizontal scale bar (white), 2 μm . (b) Occurrence histogram of measured height of lamellipodium leading edges from 7 growth cones. (c -d) A 1 μm bead in front of a thick and a thin lamellipodium respectively. (e) Low resolution image of a lamellipodium in front of a bead trapped with an infrared laser. Scale bar, 2 μm . (f) Successive frames showing the lamellipodium (55 s) growing towards the bead (64 s) and lifting it up (68.2 s). Subsequently, the lamellipodium retracted (94 s) and grew under the bead pulling it out of the trap during retraction (100-123 s). Cross indicates the centre of the optical trap. Scale bar, 2 μm . (g) F_z (grey trace) used for computing the Fv relationship. The dotted box indicates the section of force measurement used to compute the Fv relationship after Gaussian filtering at 0.2 Hz (green trace). (h-i) Fv relationships obtained after smoothing at 0.2 Hz (green trace in h) at 1 Hz (pink trace in h) and at 10 Hz (i).

Fig. 2. (a) Power spectrum density of forces measured far from the lamellipodium (red trace) and when the lamellipodium pushed the bead (blue trace), computed from the red and blue traces respectively shown in the inset. Green, pink and, black arrows indicate 0.2, 1 and 10 Hz respectively. (b) Average $\langle Fv \rangle_x$ relationships from data filtered up to a bandwidth of X Hz. $\langle Fv \rangle_{0.2}$, (green trace), $\langle Fv \rangle_1$ (pink trace), and $\langle Fv \rangle_{10}$ (black trace). (c) Probability distribution $p(v < -3\sigma_v | F/F_{\text{max}})$ of velocities being less than $-3\sigma_v$ at fixed forces (F/F_{max}). Average Fv relationships from 95 experiments obtained from data filtered with a Gaussian filter at a cut-off frequency of 0.2 (green), 1 (red) and, 10 (blue) normalized to F_{max} . (d-e) Distribution of ON and OFF events, respectively. Black straight lines have a slope of -0.6 and -0.78 in (d) and (e) respectively.

Fig. 3. (a) Low resolution image of a lamellipodium near the trapped bead. Scale bar, 2 μm . (b) Micrographs of the lamellipodium pushing the bead laterally during its protrusion. Images taken at different times during force generation as shown in panels c-e. Cross indicates the centre of the optical trap. Scale bar, 2 μm . (c-e) The three components F_x , F_y and F_z of force recordings used to compute the Fv relationships (grey trace) and after Gaussian filtering at 0.2 and 1 Hz (green and pink traces). The dotted box indicates the section of the recording used to compute Fv relationships in f-h. (f) Green and black Fv relationships computed with Gaussian filtering at 0.2 Hz and Linear regression, respectively. (g-h) Fv relationships computed with Gaussian filtering at 0.2 and 1 Hz (green and red traces) from the F_x and F_y component of the force. Dotted red and black lines represent $-3\sigma_v$ at the 0.2 and 1 Hz bandwidths, respectively. During the push F_y becomes negative and therefore, in panel h, transient retractions are associated to positive velocities. (i) Relationship between standard deviation of velocity distribution as a function of smoothing and for two trap stiffnesses of 0.005 pN/nm (squares) and 0.045 pN/nm (circles).

Fig. 4. (a) Low resolution image of a lamellipodium pushing a trapped bead. Scale bar, 2 μm . (b) Successive frames taken at different times during the push. Cross indicates the centre of the optical trap. Scale bar, 2 μm . (c) Three components of the force F_x (blue), F_y (green) and F_z (red) exerted by a lamellipodium during the push smoothed at 10 Hz. (d) Instantaneous power $\mathbf{F} \cdot \mathbf{v}$ acting on the bead. (e) Time evolution of $\text{Arcos}(\mathbf{F} \cdot \mathbf{v} / |\mathbf{F}| |\mathbf{v}|)$ during the push. Data obtained after smoothing at 0.2 Hz. (f) The trajectory of the bead in a 3D space. The black arrow indicates the direction of the trajectory. Red and blue arrows on A and B indicate the instantaneous \mathbf{F} and \mathbf{v} respectively at the two times

corresponding to 54 and 58 s in panels b-e. When \mathbf{F} and \mathbf{v} are parallel $\text{Arcos}(\mathbf{F} \cdot \mathbf{v} / |\mathbf{F}| |\mathbf{v}|)$ is close to 0 and when \mathbf{F} and \mathbf{v} are antiparallel $\text{Arcos}(\mathbf{F} \cdot \mathbf{v} / |\mathbf{F}| |\mathbf{v}|)$ is close to π . (g) Histogram of the $\text{Arcos}(\mathbf{F} \cdot \mathbf{v} / |\mathbf{F}| |\mathbf{v}|)$ when $|\mathbf{F}|$ was smaller than 2 pN. (h) Histogram of the $\text{Arcos}(\mathbf{F} \cdot \mathbf{v} / |\mathbf{F}| |\mathbf{v}|)$ when $|\mathbf{F}|$ was larger than 2 pN.

Fig. 5. (a-c) Micrographs of a lamellipodium pushing the bead at different times (see time scale in d). Scale bar, 2 μm . Red angles drawn by eye. (d) Evolution of estimated contact area S_c during the push. (e) Histograms of the value of S_c obtained from 4 experiments during which lamellipodia pushed the bead.









