Growth Cone Behavior and Production of Traction Force

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Abstract. The growth cone must push its substrate rearward via some traction force in order to propel itself forward. To determine which growth cone behaviors produce traction force, we observed chick sensory growth cones under conditions in which force production was accommodated by movement of obstacles in the environment, namely, neurites of other sensory neurons or glass fibers. The movements of these obstacles occurred via three, different, stereotyped growth cone behaviors: (*a*) filopodial contractions, (*b*) smooth rearward movement on the dorsal surface of the growth cone, and (*c*) interactions with ruffling lamellipodia. More than 70% of the obstacle movements were caused by filopodial contractions in which the obstacle attached at the extreme distal end of a filopodium and moved only as the filopodium changed its extension. Filopodial contractions were characterized by frequent changes of obstacle velocity and direction. Contraction of a single filopodium is estimated to exert 50–90 μdyn of force, which can account for the pull exerted by chick sensory growth cones. Importantly, all five cases of growth cones growing over the top of obstacle neurites (i.e., geometry that mimics the usual growth cone/substrate interaction), were of the filopodial contraction type. Some 25% of obstacle movements occurred by a smooth backward movement along the top surface of growth cones. Both the appearance and rate of movements were similar to that reported for retrograde flow of cortical actin near the dorsal growth cone surface. Although these retrograde flow movements also exerted enough force to account for growth cone pulling, we did not observe such movements on ventral growth cone surfaces. Occasionally obstacles were moved by interaction with ruffling lamellipodia. However, we obtained no evidence for attachment of the obstacles to ruffling lamellipodia or for directed obstacle movements by this mechanism. These data suggest that chick sensory growth cones move forward by contractile activity of filopodia, i.e., isometric contraction on a rigid substrate. Our data argue against retrograde flow of actin producing traction force.

The growth and guidance of neurons depends on the growth cone, a highly motile cellular compartment at the distal end of axons and dendrites (Lockerbie, 1987; Smith, 1988; Bray and Hollenbeck, 1988). This motility is believed to reflect the general mechanisms of amoeboid-like, "crawling" by metazoan cells (Wessells, 1982; Trinkaus, 1985; Bray and White, 1988). However, the connection between growth cone locomotion and its complex behavior is poorly understood and somewhat controversial. Direct cytomechanical measurements on growing, cultured neurons show that growth cones pull as they move forward (Lamoureux et al., 1989), confirming a long standing hypothesis (Bray, 1982; Wessells, 1982; Purves and Lichtman, 1985; Lockerbie, 1987). However, careful observation of growth cone behavior failed to suggest a pulling mechanism (Goldberg and Burmeister, 1986; Aletta and Greene, 1988). Rather, these studies suggested that lamellipodial protrusion played a principal role in forward motility and specifically argued against the involvement of filopodial contraction. Support for the importance of lamellipodial function comes from studies in intact organisms where growth cone advance rate is strongly correlated with lamellipodial presence and activity (Argiro et al., 1984; Bovolenta and Mason, 1987). However, it is not yet clear that protrusion or retraction of filopodia or lamellipodia produce traction force. An attractive, recent model for the mechanism of growth cone motility is independent of these behaviors: force is exerted by a retrograde flow of cortical actin in the growth cone (Bray, 1970; Forscher and Smith, 1988). If such a cortical flow occurred on the ventral, substrate-apposed surface of the growth cone, a "caterpillar tractor" mechanism for motility is possible (Bray and White, 1988; Smith, 1988). Retrograde flow of the lipid bilayer membrane per se has also been proposed as a mechanism of locomotory force production (Bretscher, 1984).

Newton's third law requires that growth cones exert a rearward force in order to advance. We follow Harris (1982) in calling this "traction force." A key question then is which growth cone behavior(s) produces traction force. This is difficult to discern from typical observations because localized traction forces are difficult to measure or correlate with complex growth cone behavior. We report here observations of growth cone behavior under conditions in which the production of force was accommodated instead by move-

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ments of environmental mass. That is, cultured chick sensory growth cones interacted with and moved two kinds of obstacles, neurites of other cells and short, glass fibers. The majority of obstacle movements were initiated by attachment to the extreme distal ends of filopodia followed by subsequent shortening of the filopodia. Although we also observed obstacle movements consistent with retrograde actin flow, these were a minority and were observed only on the top surface of the growth cone, not on the substrate surface. Our observations support a model for growth cone advance in which filopodia contract isometrically against the substrate to produce traction force.

Materials and Methods

Sensory neurons were isolated from the lumbosacral dorsal root ganglia of 10-12-d-old chicken embryos and cultured as previously described (Baas et al., 1987) except that the medium contained streptomycin at 136 mg/liter and methylcellulose was omitted. Neurons were cultured on glass slides treated with 0.1% polylysine for 30 min, rinsed three times with water, then immersed in 10 μg/ml laminin (Collaborative Research, Lexington, MA) for 60 min and placed into culture medium. Before observation, narrow, rectangular glass pieces cut from coverslips were placed at both ends of the slide to serve as supports for an overlying coverslip, this "sandwich" being sealed with molten wax (50% paraffin, 50% lanolin). In several experiments, short glass fibers were applied to the slide prior to coverslip placement. The glass fibers were prepared by grinding glass fiber filters (GF/A; Whatman Inc., Clifton, NJ) in a mortar and pestle. We selected for fibers <5 μm long by subjecting the ground glass to several 1 *g* sedimentations in water. The course of the selection was monitored by light microscopy and the conditions altered empirically until a suitable sample was obtained. An aliquot from this sample was prepared each week by treating with 0.1% polylysine for 20 min, rinsing four times with water, and suspending the fibers in tissue culture medium.

Specimens were observed by differential interference contrast optics on a Leitz Ortholux Pol I microscope equipped with a Calflex heat filter, a television camera (series 67; Newvicon tube; Dage-MTI Inc., Michigan City, IN), and a time-lapse videotape recorder (RCA model TC 3920). Interactions of growth cones and obstacles were recorded at 12 times normal speed with a clock display on screen. The videotape was analyzed for amounts, rates, and time courses of obstacle movements from videoscreen measurements taken with the aid of a 1-mm gridwork screen overlay, the grid being calibrated from an image of a stage micrometer.

Results

We have analyzed 136 obstacle movements with 33 neurons (16 interacting with glass fibers and 17 with obstacle neurites). Given the complexity of growth cone behavior, we were surprised that obstacle movements fell into three very distinct categories: (*a*) filopodial retractions, (*b*) smooth rearward movements on the dorsal growth cone surface and, occasionally, (*c*) movements due to interactions with ruffling lammelipodia. Generally, the most informative interactions were those with neurites because their tethered state and elasticity allowed for continuous gradations of deflection that were easily interpreted in terms of force increases and decreases. Further, by using previous measurements of neurite tensions and elasticities (Dennerll et al., 1989) it was possible to estimate local force production by the growth cone. Movements of glass primarily confirmed that the growth cone activity seen with neurites was general and not specific to neurite/growth cone interactions. However, in the case of movements caused by lamellipodial ruffling, described later, movements of glass fibers were more informative because they occurred over reasonable periods and distances without attachment to the growth cone.

The majority of interactions (100/136) involved a filopodium attaching by its extreme distal end to an obstacle and moving it with a concomitant change in filopodial extension. We observed obstacle movement both toward the neurite shaft (retrograde movement) and in the direction of growth cone advance (anterograde movements). Anterograde and retrograde obstacle movements were accompanied by equal filopodial elongation or shortening, respectively. Fig. 1 is an example of this type of filopodial interaction, which we call "grappling hook." The ability of filopodia to retract and deflect obstacle neurites has been observed previously (Nakai, 1960; Wessells et al., 1980; Kapfhammer and Raper, 1987). As shown in Fig. 1, obstacle movements caused by advancing growth cones were predominantly retrograde and were accompanied by a thickening of the retracting filopodium. All grappling hook retractions longer than 5 μm were accompanied by visible filopodial thickening. Retractions without obvious filopodial thickening were short, averaging 1.2 μm, suggesting that thickening was too slight to see. Fig. 2 *a* shows the pattern of "instantaneous" obstacle velocities and direction (actually measured over 30-s intervals) for a typical grappling hook sequence from first contact between the growth cone and obstacle neurite until the obstacle neurite reached the central region of the growth cone. The velocity changes and directional reversals shown in Fig. 2 *a* were characteristic of grappling hook interactions and made them look rather inefficient. For example, it took between 7 and 30 min for obstacles to travel from the filopodial tip to the central regions of growth cones, the average was 14.9 min from measurements of 10 neurons (2 with glass, 8 with neurites) comprising more than two dozen interactions. Anterograde movements generally occurred as brief episodes among retrograde movements. Concerted anterograde movements occurred only with fortuitous neurite retractions while growth cones were attached to obstacle neurites. As shown in Fig. 3, such anterograde movements were accompanied by some relaxation of force on the obstacle and an elongation of the attached filopodium. Although the vast majority of grappling hook interactions (94/100) were initiated by attachment of the obstacle at the extreme distal end of filopodia, infrequent alternative modes of attachment provided the only qualitative variations in this filopodial contraction process. We observed three instances of obstacle attachment along the length of a filopodium, rather than at its distal end. We also observed three clear instances of filopodia wrapping around a neurite, the appearance in time-lapse being similar to a chain thrown around a pipe.

As described by Nakai and Kawasuki (1959) and Nakai (1960), growth cones colliding with another neurite typically continue along the neurite. However, we observed 5 instances of growth cones advancing over the top of an obstacle neurite and continuing past it (Fig. 4). (Growth cones were never observed to advance over glass fibers possibly because of geometric constraints [Dunn and Heath, 1976]). All these obstacle neurites were pulled retrograde by the concomitant shortening and thickening of filopodia (Fig. 4, *a–c*). Fig. 4, *c–e* shows that substantial protrusion of growth cones' cytoplasm over obstacles occurred only after filopodia retracted a considerable distance. That is, growth cone cytoplasm ap-

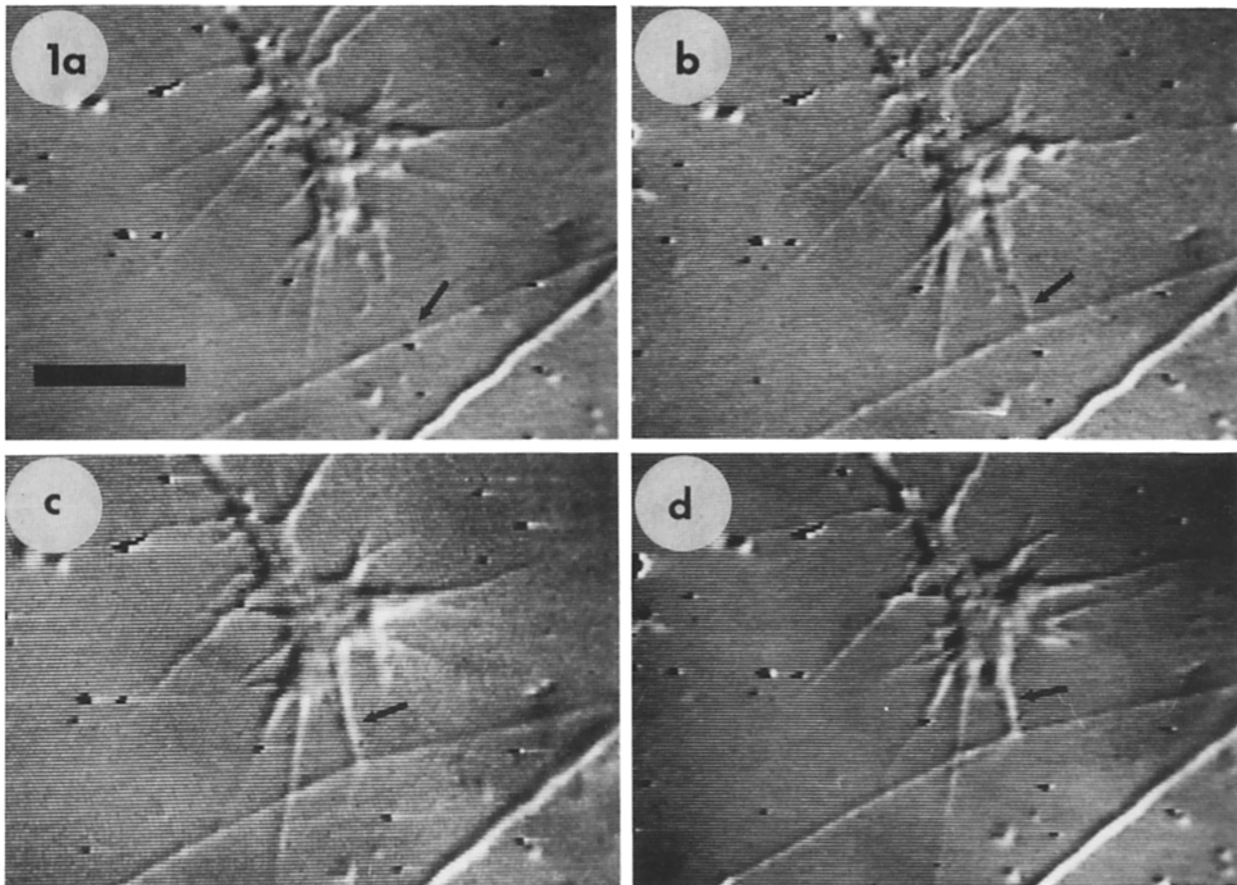

Figure 1. Videotape frames of retrograde obstacle neurite movement by grappling hook interaction with growth cone. (*a*) Very thin filopodium attaches to obstacle neurite (*arrow*) (*b*) 10 s later, filopodial tip has attached (*arrow*) and neurite deflection begins. (*c*) 30 s later, the filopodium has shortened and thickened (*arrow*) as neurite deflection increases. (*d*) 15 s later, filopodial shortening and thickening continue as does neurite deflection. Bar, 10 μm.

peared to flow forward only after the filopodia had exerted considerable force. Fig. 2 *b* shows that obstacle movements beneath growth cones were characterized by frequent pauses, directional reversals, and changes of velocity, entirely similar to those of grappling hook movements generally (Fig. 2 *a*). Fig. 5 plots the magnitude of obstacle neurite deflection over time for two ventral interactions. Figs. 4 and 5 also show that that the advance of the growth cone over the obstacle was slow, requiring 20 min or more for the obstacle neurite to move from the periphery to the central region. Significantly, in all ventral interactions, as shown in Fig. 5 and Fig. 4, *e–f*, obstacle neurites relaxed gradually as they passed beneath an area somewhat distal to the central region of the overlying growth cone. Both the periods of increasing and decreasing force on the obstacle took place while the growth cone advanced forward. While the retrograde deflections increased despite the anterograde movements of growth cones, the relaxations were closely correlated with the forward advance of the growth cones.

Fig. 6 shows another class of obstacle movement we called "escalator." In these movements, obstacles moved smoothly over the top surfaces of the growth cones without accompanying changes in the extension of filopodia or lamellipodia until the obstacles reached the central regions. Escalator movements were substantially less frequent than grappling hook movements, only 31/136 interactions were escalators. Escalator movements were observed along the length of filopodia per se, as well as on the lamellipodial, palmate region of the growth cone. Fig. 2 *c* shows that changes of direction essentially did not occur during escalator movements. The few observed instances of directional reversal occurred only along the length of filopodia. These clearly involved momentary loss of adhesion by obstacle neurites rather than reversal of the motive force; the neurite suddenly relaxed like a stretched rubber band upon release. Two other aspects of Fig. 2 *c* are noteworthy. First, the long lag time between first contact with the obstacle neurite and extensive obstacle movement reflects the observation that filopodia or lamellipodia advanced beneath obstacles without attachment until the obstacle came into contact with the dorsal growth cone surface and was "loaded" onto the escalator. Second, the smooth pattern of obstacle acceleration and deceleration beginning at ~15 min confirms the visual impression of escalator movements; after loading, the obstacles moved rapidly and gracefully to the central regions of their growth cone. The absence of pauses or directional changes made obstacle movements on the escalator appear significantly more efficient than grappling hook movements. For example, despite

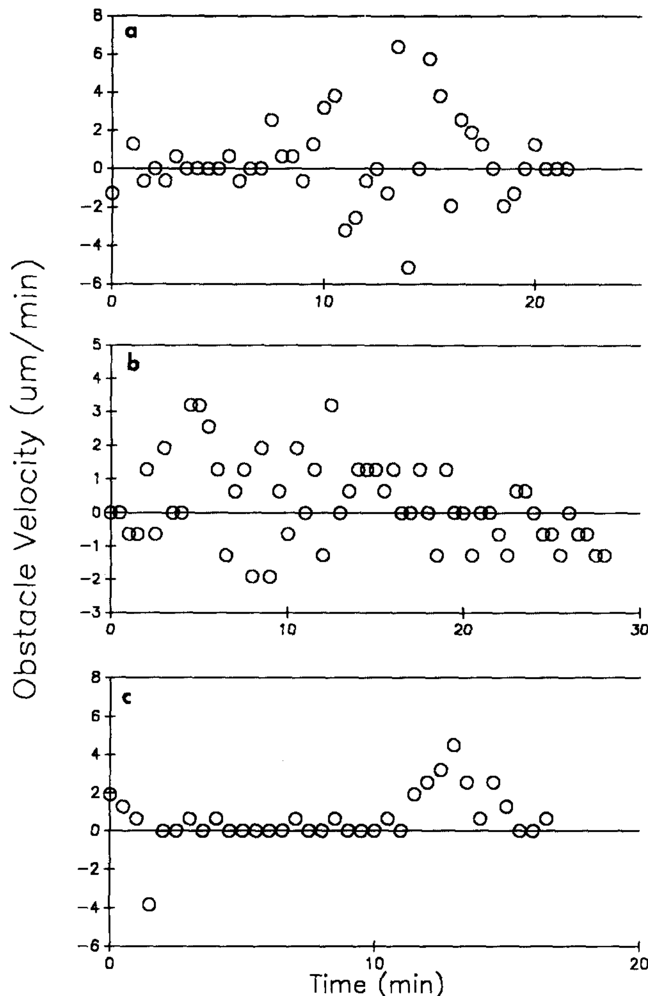

Figure 2. Direction and velocities of obstacle neurite movement caused by three different interactions with growth cones. Each data point is a measurement of the direction and velocity of an obstacle movement during a 30-s interval. These are plotted as a function of the elapsed time of the interaction of the growth cone with the obstacle. Each record begins with the first contact between a growth cone and an obstacle and ends when the obstacle reached the central region of the growth cone. Retrograde movements are shown as positive values and anterograde movements are shown as negative values. (*a*) A grappling hook interaction ending on the dorsal surface of the growth cone showing the irregular pattern of obstacle velocities, pauses, and directional reversals typical for this type of interaction. (*b*) An interaction in which the obstacle neurite moved beneath the ventral surface of the growth cone, i.e., the obstacle moved between the culture surface and growth cone. The pattern of velocities, pauses and directional reversals is similar to that shown in *a*. (*c*) An escalator obstacle movement over the dorsal surface of the growth cone. After first contact between growth cone and obstacle, some time was required for obstacles to load onto the escalator and begin significant movement. Once concerted movement began pauses and directional reversals were essentially absent and the obstacle moved smoothly to the central region.

similar maximum velocities of grappling hook and escalator movements (Fig. 2), it required only 2–9 min for an obstacle to travel by escalator from the growth cone periphery to the central region (not including the lag time for loading). The average of 10 measurements (5 glass, 5 neurites) was 5.9 min. All escalator movements invariably ceased at the central regions of the growth cones, obstacles remained firmly attached there without relaxation for the duration of our observations (Fig. 5).

In our conditions, lamellipodia typically extend and retract in a planar fashion, along the dish surface. However, those few growth cones with actively ruffling lamellipodia, i.e., moving up and retrograde, occasionally produced a third type of obstacle movement caused by the interaction of ruffling lamellipodia and an obstacle. In contrast to escalator and grappling hook movements, we could not discern any attachment of the obstacle to the interacting growth cone during these events. Rather, it appeared that the obstacle moved only incidentally, having gotten in the way of a lamellipodium. Obstacle neurites were occasionally "plucked" as they were struck by moving lamellipodia. In no case did obstacle neurites remain distended for measurable times as for the other interactions. Use of glass particles were more informative for these interactions and confirmed the lack of adhesive interaction with lamellipodia. That is, glass fibers failed to attach to ruffling lamellipodia, despite treatment with polylysine to aid attachment, as shown by their strong tendency to drop away from the growth cone onto the dish during lamellipodial interactions. Despite this lack of attachment lamellipodial ruffling occasionally caused complex movements of glass fibers. Watching such interactions at time-lapse speed (12 times) independently reminded two of the authors of a magician manipulating playing cards with one hand. In one instance, a short glass fiber was kept in continuous motion for 11 min on the top surface of a ruffling growth cone undergoing 15 360° rotations before falling to the side of the growth cone. Regrettably, still photographs of such interactions are not informative, showing merely a glass fiber on a growth cone, and are not shown here. Glass fibers interacting with lamellipodia never "settled down" to attach to the growth cone or to undergo one of the other types of obstacle motion.

Discussion

We found that two growth cone behaviors, grappling hook and escalator, are candidates to advance the growth cone. Either behavior exerts sufficient force on obstacle neurites to account roughly for the tension magnitudes of advancing growth cones in chick sensory neurons (Lamoureux et al., 1989). The force on an obstacle neurite deflected perpendicular to its axis by a filopodium or escalator is twice the obstacle neurite tension times the sine of the deflection angle, as shown by Dennerll et al. (1988). The angle of obstacle neurites at their maximum deflection was typically 12–15°. A conservative estimate for obstacle neurite tensions is 125–175 μdyn based on previous cytomechanical measurements of chick sensory neurons' rest tensions (typically ~125 μdyn), spring constants (Dennerll et al., 1989), and the small elastic increases in length (2–4 μm) caused by deflection. Thus, we estimate that single filopodial retraction and escalator interactions exerted 50–90 μdyn of force. Given the number of filopodia on growth cones and the much larger area of growth cone–substrate attachment than of growth cone–obstacle attachment, this force can account for

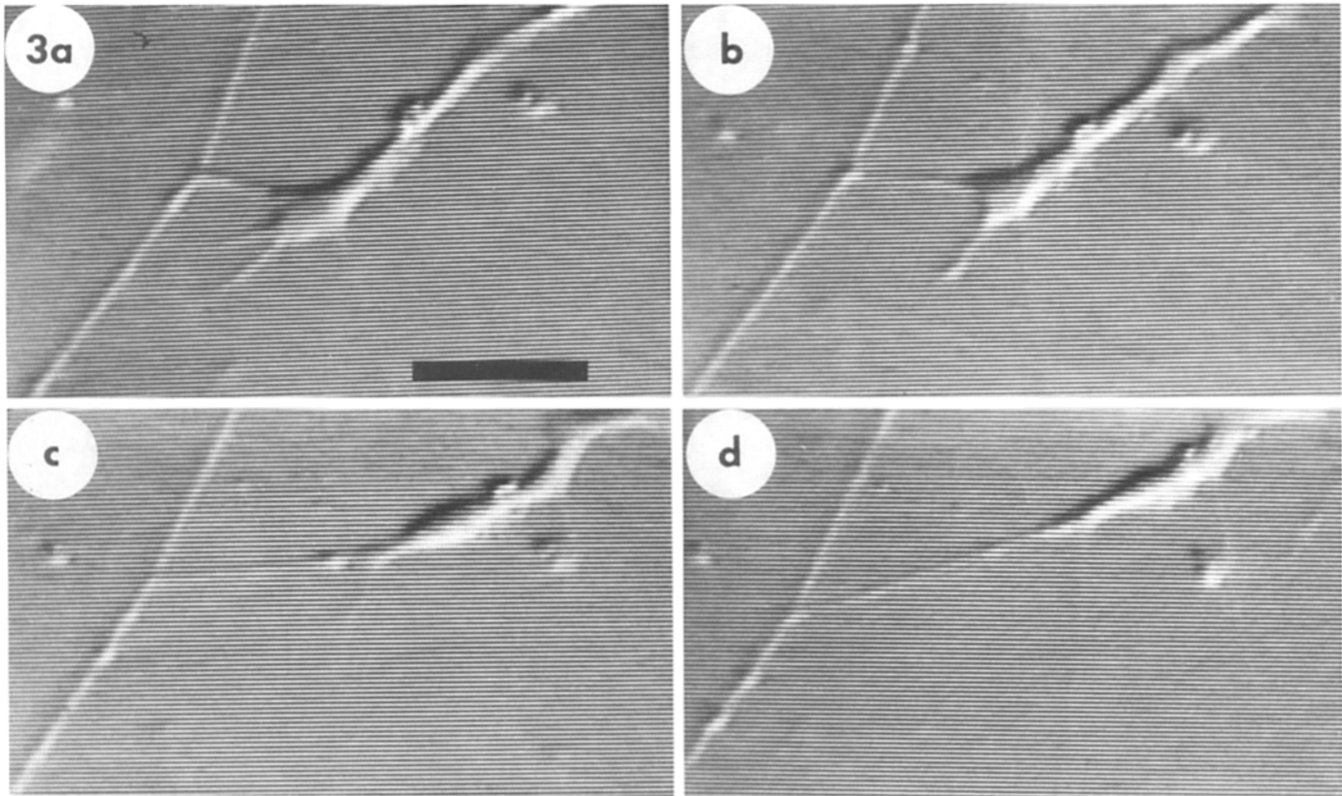

Figure 3. Videotape frames of extensive anterograde obstacle neurite movement via grappling hook behavior accompanying growth cone/neurite retraction. (*a*) Obstacle neurite deflected by attachment to growth cone filopodium at its extreme distal end. The small bit of detritus beneath the figure number can be used to ascertain degree of neurite deflection. (*b*) 41 s later, neurite has relaxed (moved anterograde) as attached filopodium lengthens and thins. (*c*) 1 min 8 s later, neurite continues to retract, filopodium continues elongating and thinning as obstacle neurite continues to move anterograde. (*d*) 52 s later, neurite retracts faster than filopodial elongation/thinning causing small retrograde movement of obstacle neurite. Bar, 10 μm.

the 100–500 μdyn of force exerted by advancing growth cones (Lamoureux et al., 1989). The sustained deflections imposed on obstacle neurites also indicate at least equivalent attachment forces, i.e., ∿50–90 μdyn to break attachment of obstacles to the growth cone. These attachments were generally initiated at the extreme distal ends of filopodia, suggesting a specialization for attachment at filopodial tips like that observed by Tsui et al. (1985).

Grappling hook and escalator movements clearly reflect two of the proposed mechanisms for growth cone advance. Grappling hook movements are filopodial contractions in which the obstacle moves only through changes in length of the filopodium and all retractions of any distance were accompanied by thickening of the filopodium. Contractile filopodia have long been a favored mechanism for growth cone locomotion (Bray, 1982; Bray and Chapman, 1985; Wessells, 1982; Trinkaus, 1985). We found no evidence that lateral, "sweeping," movements of filopodia produce force as suggested by Bray and Chapman (1985). Our observations of escalator movements are consistent with those postulated by models of retrograde flow of cortical actin (Bray and White, 1988; Smith, 1988; Mitchison and Kirschner, 1988): The obstacle moves smoothly over the dorsal growth cone surface without change in growth cone extension. Further, the range of escalator velocities, as shown in Fig. 2 *c*, matches those reported by Forscher and Smith (1988) for retrograde movement of cortical actin. The magnitude of force exerted by escalator movements and the low viscosity of the lipid bilayer argues against these being caused by lipid bilayer flow per se (Bretscher 1984), supporting the recent conclusion of Sheetz et al. (1989). Assume a lipid bilayer 5 nm thick moving around an obstacle 10 μm in circumference (an estimate of the attachment region size) between two still water layers creating a boundary layer of 1 μm around the obstacle. Fluid velocity is force × boundary layer divided by the product of the area exposed to flow and the fluid's viscosity. Using the maximum measured membrane surface shear viscosity value of 10^{-3} dyn-s/cm (Evans and Hochmuth, 1978), the lipid bilayer would need to flow at ∿10^4 cm/s in order to exert 50 μdyn of force. It therefore seems likely that escalator movements are the result of cytoskeletal function.

Although either filopodial contraction or retrograde actin flow exerts sufficient tension, our observations suggest that filopodial contraction is primarily responsible for growth cone traction, at least in our conditions. This conclusion is moderately surprising to us. Our own time-lapse observations of growth cone behavior in the absence of obstacles (Lamoureux et al., 1989) supported the conclusion that filopodia are not pulling (Goldberg and Burmeister, 1986; Aletta and Greene, 1988). Several aspects of our observa-

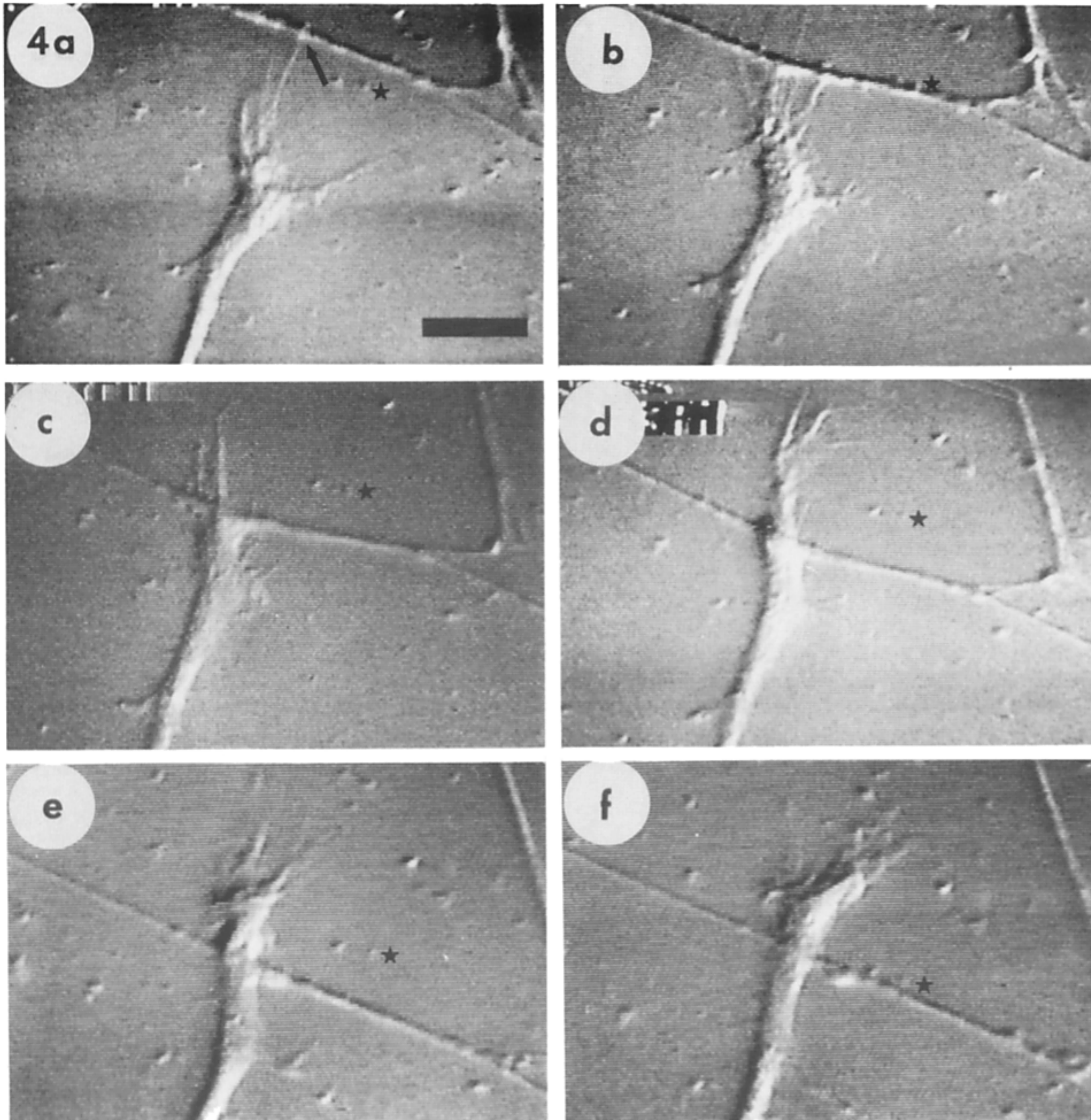

Figure 4. Videotape frames from one of five instances in which an obstacle neurite moved beneath the ventral growth cone surface. (*a*) Filopodium (*arrow*) attaches to obstacle neurite. Two punctate bits of detritus on plate (*star*) serve as markers for the degree of neurite deflection. (*b*) 4 min 36 s later, filopodium retracts and thickens as obstacle neurite deflects. (*c*) 8 min 9 s later, filopodial retraction and obstacle neurite deflection continues. Note that only a small region of the growth cone has advanced beyond the growth cone. (*d*) 2 min 36 s later, growth cone moves over the obstacle neurite as deflection of obstacle reaches its maximum. (*e*) 7 min 10 s later, more growth cone material moves over the obstacle as the obstacle begins relaxing. (*f*) 6 min 16 s later, obstacle neurite is almost entirely relaxed as space formerly occupied by the growth cone has consolidated into neurite shaft (see Discussion). Bar, 10 μm.

tions changed this view. First, filopodia were clearly observed to contract and to produce force thereby. Obstacle movements were caused by contractions three times more frequently than by escalator movements. Also, the time course and very variable pattern of obstacle velocity and direction caused by filopodial contractions (Fig. 2, *a* and *b*) are more consistent with the temporally and directionally intermittent advance of growth cones (Katz et al., 1984) than the more efficient cortical escalator. Last and most persuasive were the movements of obstacles beneath ventral growth cone surfaces, i.e., obstacle interactions that most nearly mimic the usual growth cone–culture surface interaction. These were exclusively grappling hook interactions based on (*a*) their involvement of contracting filopodia as shown in Fig. 4; (*b*) the similar pattern of velocity changes and directional reversals in ventral obstacle movements (Fig. 2 *b*) and grappling hook interactions generally (Fig. 2 *a*); and (*c*) the absence of any observable escalator movements on the ventral surface. With respect to the last point, our observations suggest that retrograde actin flow occurs mainly, if not only,

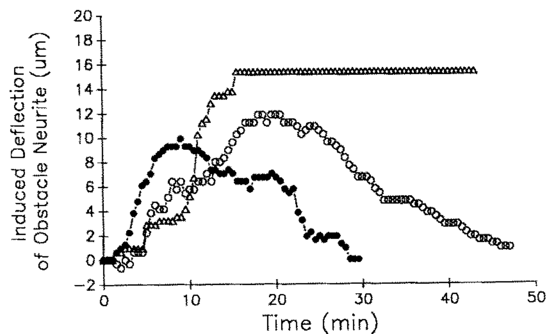

Figure 5. Neurite deflection as a function of elapsed time of growth cone–obstacle interaction. Neurite deflections were measured relative to a culture surface reference. Circles are examples of two growth cones that moved over obstacle neurites as in Fig. 4. Note the gradual relaxation after maximum deflection. Open triangles record the deflection caused by an escalator interaction as shown in Fig. 6. Once the obstacle neurite reached the central region of the growth cone by escalator it remained attached and deflected.

on the dorsal surface of growth cones. Dorsal restriction of retrograde actin flow would be consistent with data from fibroblasts in which smooth rearward flow of particles (Abercrombie et al., 1970) and rearward flow of actin bundles (Heath, 1983) occur on dorsal surfaces. A particularly noticeable difference between dorsal and ventral growth cone surfaces was the dissimilarity in obstacle movement near the central regions. Whereas we observed no obstacle relaxations during escalator movements, obstacle relaxations invariably occurred beneath the ventral, palmate region of the growth cones. Because obstacle neurite deflection is a direct measure of force exerted on the obstacle, the data on ventral interactions of Figs. 4 and 5 suggest that traction force is primarily exerted beneath the distal one-third of the growth cone. The loss of traction force more proximally on the ventrum might reflect the termination of force-producing, filopodial actin filaments in this region (Letourneau, 1979; Bridgman and Dailey, 1989) or it may reflect a loss of obstacle adhesion in this region since relaxation was correlated with growth cone advance. On typical immovable culture substrates, filopodial contractions would exert isometric tension. Bray (1987) pointed out that this mechanism would not be obviously recognizable as pulling. We believe this explains the disparity between observations of growth cone behavior with and without obstacles.

In view of the evidence that lamellipodial extrusion is a key event in growth cone advance (see Introduction) the question arises how filopodial tension achieves lamellipodial protrusion. An attractive possibility is suggested by our observations that most cytoplasmic flow occurred only after considerable filopodial contraction (Fig. 4, *c* and *d*) and by information on the gel → sol transitions of actin suspensions and cytoplasm (Kerst et al., 1990). Isometric tension of filopodia could create a sufficient shearing force in the palmate regions of the growth cone to cause a solid, "gel" to fluid, "sol" transition of the axoplasm. Such gel–sol transitions are characterized by a shear force threshold, thus at some shearing threshold the cytoplasm would flow forward. This model is consistent with: (*a*) the extension of filopodial actin filaments back into the cytoplasm of the palmate growth cone region (Letourneau, 1979; Bridgman and Dailey, 1989); (*b*) the frequently observed, rapid advance of lamellipodia between filopodia (Bray and Chapman, 1985; Goldberg and Burmeister, 1986; Aletta and Greene 1988); (*c*) the shear predicted by virtually all models for filopodial force production (e.g., Fig. 3 in Huxley, 1973; Fig. 12 in Mitchison and Kirschner, 1988); and (*d*) the long standing insight that amoeboid movement involves thixotropy (gel–sol transitions) of the cytoplasm (Siefriz, 1942; Taylor and Condeelis, 1979). This model also provides a mechanistic interpretation for the "protrusion" and "engorgement" events of growth cone advance (Goldberg and Burmeister, 1989). The final, "consolidation" step of growth cone advance, in which microtubules assemble into the space formerly occupied by the growth cone, may be due to a shift in compression force on the axonal microtubules postulated to accompany traction force, as previously detailed (Buxbaum and Heidemann 1988; Dennerll et al., 1988).

Interactions of obstacles with ruffling lamellipodia did not suggest a role in developing rearward traction force; ruffling lamellipodia produced neither obstacle attachment nor sustained rearward deflection of neurites. A similar conclusion was reached in studies of fibroblast locomotion despite the close correlation of lamellipodial activity with advance rate (Abercrombie et al., 1970). Abercrombie et al. (1970) argued that this correlation reflects rapid assembly/recycling of new surface material, which would be highest in cells rapidly adding new surface by growth. We wish to suggest another possibility, that dorsal lamellipodial ruffling derives directly from ventral traction force via conservation of angular momentum. Traction force, from any mechanism, creates an unresolved moment of force at the dorsal surface. This is because the forward force exerted by traction occurs at the dish surface. This is balanced by tension in the rearward neurite (Lamoureux et al., 1989), which, as shown by manipulations with needles (Dennerll et al., 1988, 1989), is suspended some small distance above the dish surface. This displacement of forces creates a torque (moment couple) that causes thin, low mass, lamellipodial regions to move up and back.

Although we find chick sensory growth cones exert traction force by filopodial contraction, we urge caution in generalizing this finding too broadly. Chick sensory neurons are characterized by highly filopodial growth cones. Our observations here would appear to be inapplicable to neurons whose growth cones lack filopodia yet advance rapidly (Argiro et al., 1984; Bridgman and Daily, 1989). Also, we previously argued that under appropriate conditions axonal elongation could occur via net tension or net compression (Dennerll et al., 1988; Buxbaum and Heidemann, 1988; Lamoureux et al., 1990). Given the complexity and variability of growth cone morphology and behavior, we suspect that growth cones have more than one mechanism for moving forward.

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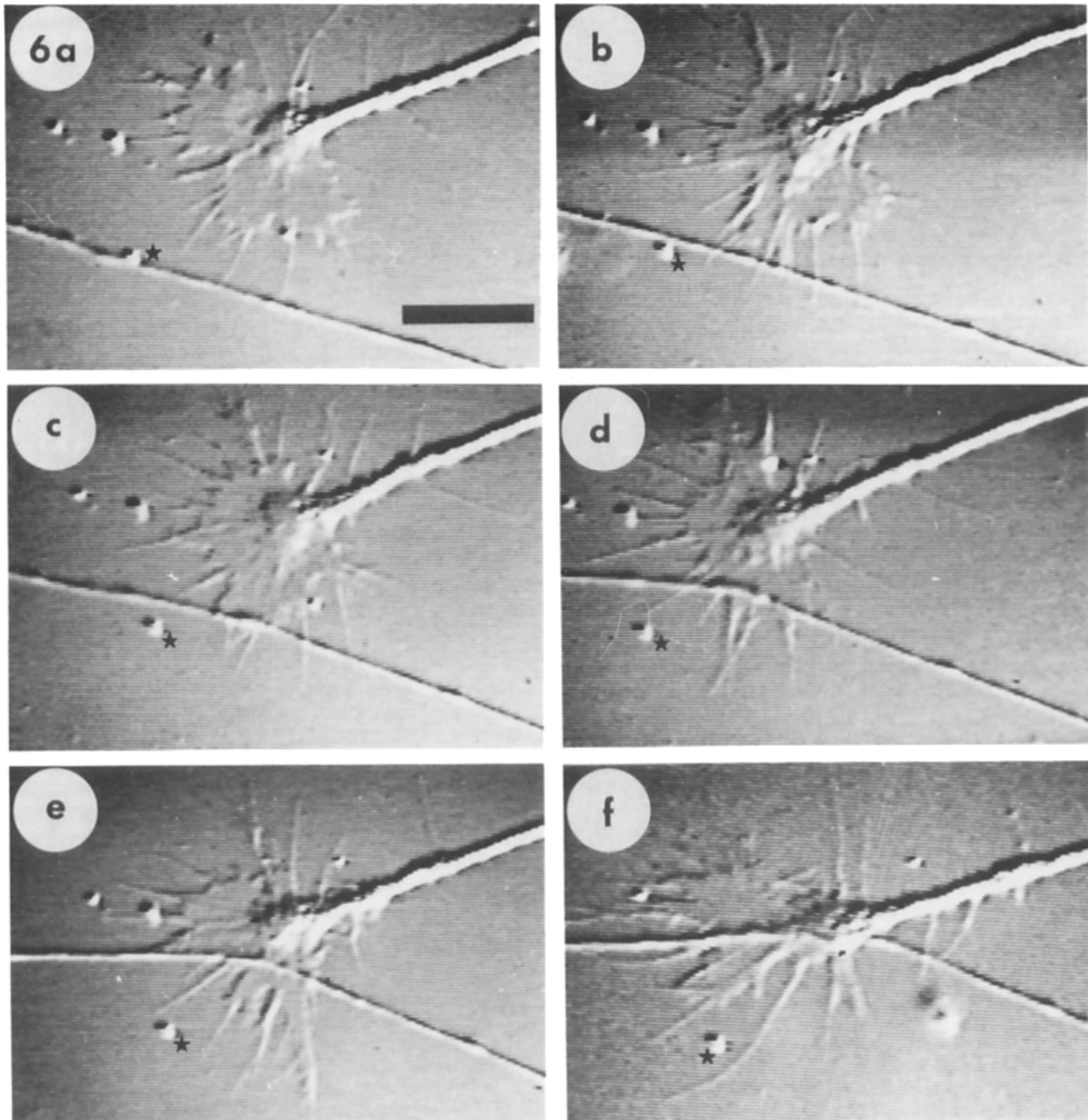

Figure 6. Videotape frames from an example of an escalator interaction with obstacle neurite. (*a*) Growth cone reaches obstacle neurite but does not attach for some period of time. Bit of detritus on dish marked with star serves as a marker for neurite deflection. (*b*) 2 min 35 s later, filopodia moves beneath the obstacle neurite and initiates attachment leading to a very small amount of obstacle deflection. (*c*) 40 s later, once loaded onto the escalator, the obstacle neurite moves smoothly retrograde on extended filopodia beneath. (*d*) 59 s later, continuing retrograde obstacle movement on dorsal lamellipodial surface. (*e*) 1 min 31 s later, retrograde movement of obstacle neurite continues until obstacle reaches the central region of the growth cone. (*f*) 4 min 36 s later, obstacle neurite remains deflected and attached to growth cone central region. Bar, 10 μm.

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