Short-term interactions between microtubules and actin filaments underlie long-term behaviour in neuronal growth cones

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We present two new computational models of microtubule dynamics in the neuronal growth cone. These extend previous models of microtubule dynamics, which have neglected the effect of microtubule interactions with one another and with F-actin in the growth cone. Ultimately, these interactions determine whether the nerve cell makes the right target connections. In the first model, analysis of the effect of microtubule bundling on axonal elongation shows that small interaction effects between individual microtubules can be amplified within the microtubule bundle to significantly alter the rate of axonal growth. The second model concerns the effect of interactions between microtubules and F-actin on growth-cone turning. The model simulates microtubule invasion into the growth cone after contact with a target cell. Results suggest that microtubules do not randomly invade the growth cone, which supports the recent view that microtubules play a more active role in pathfinding than previously expected. Our results indicate that microtubule interactions with F-actin and with other microtubules play a fundamental role in axonal elongation and growth-cone turning.

Keywords: neural development; growth cone; axon branching; microtubule dynamics; F-actin; computational model

1. INTRODUCTION

The growth cone is a structure at the tip of the developing axon which guides the axon to its target cells. It is involved in decision making, turning, and elongation of the axon (see Tanaka & Sabry (1995) for a review). When a growth cone reaches its correct target, it halts, and remodels itself to form a synapse. Figure 1 shows the main components of the growth cone. The central zone includes the end of the neurite shaft and is dominated by microtubules. These are polymerized proteins made up of tubulin monomers which are important in giving structural support to the neurite shaft and growth cone. The peripheral zone has few microtubules present and a high concentration of F-actin polymer. Microtubules and F-actin interact to enable the growth cone to combine the extracellular signals it receives into an internal signal that can be used to direct its movement (Joshi *et al.* 1985). Understanding the cytoskeletal processes that enable the growth cone to carry out the complicated wiring of the nervous system is a major goal of developmental biology. We introduce two new computer models of microtubule dynamics in the growth cone. In the first model, the effects of stabilizing interactions between bundled microtubules are analysed. We show that small, local interactions between microtubules have a large effect on the rate of axonal growth. The second model simulates interactions between microtubules and F-actin during microtubule invasion of the growth cone after contact

with a target cell (Lin & Forscher 1995). Analysis of this model indicates that microtubule invasion of the growth cone is not random, and suggests that these interactions play a key role in determining the future direction of axonal growth.

2. BACKGROUND GROWTH-CONE BIOLOGY

In the central zone of the growth cone (see figure 1), a population of dynamic microtubules undergoes rapid periods of growth and shrinkage known as dynamic instability (Mitchison & Kirschner 1984). In its simplest form, dynamic instability can be characterized by four parameters (Hill 1987): growth speed, shrinkage speed, rescue frequency (switching from shrinking to growing) and catastrophe frequency (switching from growing to shrinking). The average biological values for these parameters are shown in table 1.

As a result of dynamic instability, microtubules frequently extend into the growth cone and then retract back into the axon (figure 1). Microtubule extension into the growth cone may be characterized by 'looped', 'bundled' and 'splayed' behaviour (Tanaka & Kirschner 1991). Microtubules can become looped in the growth cone owing to the high compressive forces exerted on them by the rearward F-actin flow. In bundled behaviour, microtubules extend into the growth cone as a bundle with little deviation away from the central axis. In splayed behaviour, the microtubules appear randomly extended throughout the growth cone.

Figure 1. The diagram shows bundled, splayed and looped microtubules extending from the tip of the neurite shaft into the growth cone. At the transition zone they encounter F-actin flowing rearward from the peripheral zone where it is assembled. Dynamically unstable microtubules alternately grow out from the neurite shaft into the growth cone before experiencing a catastrophe (a switch from growth to shrinkage) and then shrink back into the neurite shaft where they are rescued (shrinkage to growth).

Table 1. Values of parameters characterizing microtubule dynamics (mean $\pm$ s.d.)

		<i>Xenopus</i> neurons ^a	<i>Xenopus</i> extract ^b
growth ($\mu\text{m min}^{-1}$)	V_g	10.5 ± 1.9	9.3 ± 4
shrinkage ($\mu\text{m min}^{-1}$)	V_s	9.7 ± 2.2	12.8 ± 6
rescue frequency (min^{-1})	F_{res}	1.74	$0.6 \rightarrow 1.86$
catastrophe frequency (min^{-1})	F_{cat}	0.72	$0.54 \rightarrow 0.96$

^a From Tanaka & Kirschner (1991).
^b From Belmont *et al.* (1990).

F-actin polymerizes at the leading edge of the growth cone and then flows rearwards towards the central zone at a rate of $3\text{--}6\text{ }\mu\text{m min}^{-1}$ (Forscher & Smith 1988). Lin & Forscher (1995) showed that contact of the growth cone with a target cell can result in a reduction in the rearward flow of F-actin, which is rapidly followed by microtubule invasion of the branch that contacted the target. The advance of the axon is inversely proportional to retrograde F-actin flow. The slower the rearward F-actin flow, the faster the microtubule invasion of the target segment. To account for this behaviour, they suggested that a ‘clutch mechanism’ may be involved. Interaction with growth-promoting substances may modify the strength of clutch complexes that bind F-actin to the substrate. The rate of rearward flow is then inversely proportional to the strength and number of clutch complexes formed. An increase in the amount of clutch could then change the default retrograde movement of F-actin into a forward movement of the growth cone (Lin & Forscher 1995).

Previous biophysical models of microtubule dynamics (e.g. Hill & Chen 1984; Bayley *et al.* 1990) have concentrated on reaction mechanisms occurring at the tip of the individual microtubule polymer lattice, which result in periods of growth and shrinkage characteristic of dynamic-instability behaviour. However, they have not modelled how microtubule interactions will affect their dynamic behaviour. In this paper, we extend the dynamic-instability model to include interactions between microtubules themselves, and with F-actin.

3. THE MODELS

(a) *Simulating bundled microtubule behaviour*

There are between *ca.* 10 and 40 microtubules in the average neurite shaft (Tanaka & Kirschner 1991). Interactions between microtubules mean that the dynamic properties of, for example, 20 tightly bundled microtubules (figure 1) may be very different from the dynamics of a single microtubule. Figure 2*a,b* illustrates two reasons why this is so.

1. As the tips of leading microtubules share the compressive F-actin load (Buxbaum & Heidemann 1992), leading microtubules will shield all rearward microtubules by fractionally reducing the F-actin flow against them (figure 2*a*).
2. Bundled microtubules are stabilized against shrinkage owing to cross-links formed by microtubule-associated proteins (MAPs) (Takemura *et al.* 1990). MAPs modulate dynamic instability by increasing the rescue frequency, and by decreasing the catastrophe frequency of microtubules (Pryer *et al.* 1992). As a result, microtubules in close proximity are stabilized from shrinking further back into the bundle (figure 2*b*).

Both interactions generate a successive shielding effect on rearward microtubules in the bundle. The strength of this shielding effect will be changing constantly as microtubule dynamic instability results in a terminal tip position that fluctuates rapidly over a period of seconds to minutes. Under these conditions, the growth dynamics of the microtubule bundle, and consequently the elongation of the axon, are not equivalent to the average behaviour of the same number of individual microtubules. The average velocity V of growth or shrinkage of a microtubule can be calculated using the following equation:

$$V = \frac{V_g T_{\text{cat}} - V_s T_{\text{res}}}{T_{\text{cat}} + T_{\text{res}}}, \tag{1}$$

where V_g and V_s are the growth and shrinkage speeds of the microtubule. $T_{\text{cat}} = 1/F_{\text{cat}}$, and $T_{\text{res}} = 1/F_{\text{res}}$ are the average lengths of time that a microtubule grows or shrinks before it undergoes a catastrophe or rescue. Using the biological values of microtubule dynamics (column 2

Figure 2. Growth-cone interactions that generate microtubule shield effects. (a) Leading microtubules successively reduce the strength of the opposing flow of F-actin polymer on rearward microtubules. (b) Microtubule-associated proteins (MAPs) stabilize rearward microtubules in the bundle from shrinkage by providing cross-links between

of table 1) gives an elongation rate of $4.66 \mu\text{m min}^{-1}$. Our model extends the standard four-parameter dynamic-instability model to include the favourable dynamics of rearward microtubules due to reductions in F-actin compression, and MAP interactions. Starting from the front of the microtubule bundle, the frequency of catastrophe/rescue is successively reduced/increased at each rearward microtubule by a shielding interaction S . This generates a progressive shield effect from the front to the back of the microtubule bundle. In the simulations the shielding interaction was varied from 0% to 20%. Figure 2c shows how the shield effect varies for shielding interaction values of 1, 5 and 10% for a bundle of 20 microtubules. The effect of a linear (step) increase of 5% at each microtubule is also shown. Figure 2c shows that the dynamics of the leading microtubule are unchanged, whereas rearward microtubules receive the largest shield effect. The average velocity V_{bundle} of a bundle of N microtubules with shielding interaction S is then given by

$$V_{\text{bundle}} = \sum_{i=1}^N \frac{V_g T_{\text{cat}}^{(i)} - V_s T_{\text{res}}^{(i)}}{T_{\text{cat}}^{(i)} + T_{\text{res}}^{(i)}}, \quad (2)$$

where $T_{\text{cat}}^{(i)} = T_{\text{cat}} / (1 - S)^{i-1}$, $T_{\text{res}}^{(i)} = T_{\text{res}} (1 - S)^{i-1}$.

The effect of including these shielding interactions on the average growth velocity of the microtubule bundle is given in § 4.

(b) Simulating microtubule branch invasion and growth-cone turning

This section analyses the effect of interactions between microtubules and F-actin on growth-cone turning. We model microtubule invasion of a growth-cone segment after contact with a target cell (Lin & Forscher 1995).

The sequence of events simulated by the model is shown in figure 3a–c. The growth-cone model consists of a one-dimensional axon of length $50 \mu\text{m}$, which ends in a $10 \mu\text{m}$ growth cone of angular width 100° . At the start of the simulation, 20 microtubules (each $50 \mu\text{m}$ long) grow from the base of the growth cone. Initially, filopodia undergo random excursions, with microtubules displaying random ‘played’ behaviour (figure 3a). The filopodia projections are not modelled explicitly in the simulation. The rearward F-actin flow has a uniform value throughout the growth cone (Lin & Forscher 1995), which affects the microtubule dynamics. At each time-step of 1 s, the microtubule may undergo a random transition based on its current catastrophe and rescue frequencies. All microtubules grow straight forward in the axon and are given a fixed random direction each time they enter the growth cone. Shrinking microtubules are rescued if their length falls below $1 \mu\text{m}$. Initially the simulation runs for 10 min to eliminate start-up effects.

After 10 min the model simulates a filopodium encounter with a target cell (figure 3b). In the model, the value of the rearward F-actin flow in a 5° target segment is reduced by

(Continued) neighbouring microtubules. (c) The shield effect for any given microtubule plotted against its relative position in a bundle of 20 microtubules. Values for model shield interactions of 1–5 and 10% are shown alongside a 5% linear shielding interaction.

Figure 3. The growth-cone model. (a) Random filopodial and microtubule behaviour (time: 0–10 min). (b) Contact with target cell leads to reduced F-actin flow in target segment (time: 10 min). (c) Rapid microtubule invasion of target segment (time: 10–20 min).

30% of its original value (giving a microtubule a 5% chance of entering the segment randomly). This was the smallest reduction in value seen to occur in encounters between growth-cone and target cells (Lin & Forscher 1995), and provides the most difficult conditions for microtubules to enter the segment. The reduction in F-actin flow reduces the compressive load at the tip of a growing or shrinking microtubule in this region. As a result, microtubules already present in this segment, or which subsequently enter it, have dynamic properties enhanced by 30% from the rest of the growth cone. After 20 min, the number of microtubules further than 5 μm into the target segment (the target zone) are counted (figure 3c).

In the first set of simulations, microtubules entered the growth cone from the neurite shaft in a random direction. This modelled splayed microtubule behaviour where the microtubules appear randomly spread throughout the growth cone (Tanaka & Kirschner 1991). The aim of these simulations was to test whether the random exploration of microtubules into the growth cone was sufficient to allow large numbers of microtubules to invade the target zone.

In the second set of simulations, we investigated how the number of microtubules invading the target zone is altered when the microtubules enter the growth cone

specifically in the direction of the least rearward F-actin flow (in this case into the target segment itself). This modelled the behaviour seen after contact with a target cell (Lin & Forscher 1995). This behaviour may also occur during growth-cone turning, as microtubules have been seen to invade the growth-cone branch that later becomes the axon before any visible signs of turning (Tanaka & Kirschner 1991).

In both sets of simulations we investigated how the numbers of microtubules invading the target zone changed under the following conditions.

- 1 The probability of microtubule rescue in the neurite shaft was double the value in the growth cone and the catastrophe rescue was halved. This simulated the lack of rearward F-actin flow in the neurite shaft.
- 2 The rearward flow of F-actin in the target segment was reduced by 90% instead of 30%. In some encounters between growth cones and target cells the rearward F-actin flow is almost stationary (Lin & Forscher 1995).
- 3 Shielding interactions of 1 and 10% were applied (the default was 0%). This simulates the stabilizing effects of MAP cross-linking between microtubules, as discussed above. As the cross-links are short-range, shielding was limited to the shorter of two microtubules that grew

Figure 4. Effects of microtubule-shielding interactions on growth. Filled arrows: a single microtubule with the dynamic-instability parameters given in table 1 moves forward at the average rate of $4.66 \mu\text{m min}^{-1}$ (equation (1)). With a shield interaction of only 1%, the growth of a bundle of 20 of these microtubules would be increased by over 16% from 4.66 to $5.41 \mu\text{m min}^{-1}$ (equation (2)). The unfilled arrows show what shield interaction is required for the bundle to grow forward at $4.66 \mu\text{m min}^{-1}$: e.g. with a 10% shield interaction a bundle of 20 microtubules moves forward at the rate of $4.66 \mu\text{m min}^{-1}$, although the microtubules would individually shrink at a rate of $3.55 \mu\text{m min}^{-1}$.

with less than 1° separating their directions. This included all microtubules in the neurite shaft.

With these three conditions applied, the microtubules are more likely to invade the target segment and remain in the target zone.

4. RESULTS

(a) Effect of microtubule interactions on axonal growth

Figure 4 shows that for a bundle of 20 microtubules, a shielding interaction of only 1% at each microtubule leads to an increase of 16% in the forward movement of the bundle compared with the rate of growth of individual microtubules (from 4.66 to $5.41 \mu\text{m min}^{-1}$). With a 10% shielding interaction, 20 bundled microtubules nearly double their forward rate of movement (from 4.66 to $8.81 \mu\text{m min}^{-1}$). Consequently, the forward movement of an axon could result from the cooperative interaction of microtubules which, individually, have a much lower growth rate. A microtubule with growth characteristics $T_{\text{cat}}=1.0$, $T_{\text{res}}=2.3$ shrinks at the rate of $-3.55 \mu\text{m min}^{-1}$ (equation (1)). An axon containing a bundle of 20 such microtubules with a 10% shield interaction would move forward at the biological average rate of $4.66 \mu\text{m min}^{-1}$ (equation (2)). It should be noted that the validity of these results is not dependent on the precise form of the shielding model presented here. For small interaction values, the shield effect increases linearly from the front to the rear of the microtubule bundle (see the 1% interaction curve in figure 2c). For larger interaction values, a linear increase generates a greater shield effect (and thus a higher growth rate) than the curve generated by the model (e.g. the 5% linear plot in

figure 2c). The results seen in figure 4 would be qualitatively similar for any mechanism that results in increased shielding for rearward microtubules. Thus, small changes in the strength of interactions between individual microtubules may have a large effect on the behaviour of the growth cone as a whole. The shielding mechanism effectively increases the forward rate of microtubule and axonal growth. It also results in energy-efficiency savings for the cell as a lower concentration of non-polymerized tubulin is able to produce the same overall growth rate. A further advantage of the microtubule-shielding effect on growth-cone motility is discussed below.

(b) Effect of interactions on microtubule branch invasion

Figure 5 shows the number of microtubules in the target zone 10 min after the target was encountered. In each simulation the catastrophe and rescue frequencies are varied from 0.1 to 5.0 transitions per minute in steps of 0.1. Each point is the average of 50 trials. Unless otherwise specified, the default settings in each simulation are 20 microtubules with normal axon recovery, and a 30% reduction in F-actin flow in the target zone. In the graphs, the cross shows the average microtubule catastrophe/rescue frequency in the growth cone (0.72 and 1.74 transitions per minute) (Tanaka & Kirschner 1991). The rectangle includes the range of microtubule rates obtained from cell extracts during interphase (Belmont *et al.* 1990).

(i) Random microtubule invasion of the growth cone

In the simulations where microtubules invaded the growth cone randomly, very few microtubules were able to reach the target zone (figure 5a). This result was unaffected by doubling the axon recovery (figure 5b), slowing the target flow by 90% (figure 5c) or applying a

Figure 5. The graphs show how many of the 20 microtubules reach the target zone 10 min after simulated growth-cone contact with a target cell. Each graph point represents a single pair of catastrophe/rescue frequencies averaged over 50 trials. Experimental values of microtubule catastrophe/rescue frequency transitions are plotted from Tanaka & Kirschner (1991) (+) and Belmont *et al.* (1990) (□). The curve shows the maximum number of microtubules reaching the target zone at each catastrophe frequency. Types of simulation: (a) random: default; (b) random: axon recovery ON; (c) random: reduced target flow; (d) flow-directed: default; (e) flow-directed: axon recovery ON; (f) flow-directed: reduced target flow; (g) random: 10% shielding; (h) flow-directed: 1% shielding; (i) flow-directed: 10% shielding. See text for further details.

shield interaction of 10% (figure 5g). In all of these situations the maximum number of microtubules found in the target zone was only 2.9/20. When the axon recovery was doubled and the F-actin flow was reduced simultaneously by 90%, a maximum of 6.4/20 microtubules reached the target zone (not shown).

(ii) Flow directed microtubule invasion of the growth cone

In contrast, flow-directed microtubules invaded the target segment in large numbers with a maximum of 12.2/20 microtubules reaching the target zone 10 min after the target was encountered (figure 5d). Reducing the F-actin flow in the target segment by 90% or doubling the axon recovery increased the maximum number of microtubules reaching the target zone to over 15/20 (figure 5e,f). Simultaneously reducing the target F-actin flow and increasing axon recovery allowed all 20 microtubules to reach the target zone for certain pairings of catastrophe/rescue parameter values (not shown). Using the default parameters with a 1% shield interaction increased the maximum number of microtubules in the target zone by over 24% (to 15.2/20) (figure 5h). Finally, with a 10% shield interaction all 20 microtubules reached the target zone for some values of the catastrophe/rescue pairings (figure 5i).

Holy (1994) showed that dynamic instability provides a better mechanism for searching the environment than other polymer kinetics. For flow-directed microtubules, shielding interaction effects improve the efficiency of this search by increasing the frequency of microtubule invasions into the growth cone. This reduces the numbers

of very short and very long microtubules that are unable to reach the target zone.

The flow-directed graphs show that a wide range of pairs of catastrophe/rescue values allow a high number of microtubules to reach the target zone. In addition, the curve showing the maximum number of microtubules found in the target zone for each value of the catastrophe frequency does not vary greatly throughout the tests. The important characteristic is the ratio of the catastrophe/rescue frequencies and not their absolute values.

6. DISCUSSION

Both models confirm that interactions among microtubules and F-actin in the growth cone play a more important role in neuronal pathfinding than previously expected (Yamada *et al.* 1970). The bundled-microtubule model shows that interactions between microtubules may allow forward growth of the axon when individual microtubules would shrink. Here the interactions may be either mechanical, as a result of a decrease in the compressive rearward F-actin flow, or chemical as a result of MAP interactions between nearby microtubules, or both. Such interactions may ensure that an axon containing bundled microtubules continues to elongate through regions which the growth cone would be unable to cross if the microtubules did not act cooperatively. Individual microtubules would only be able to elongate in off-axis directions if they were dynamically favourable, for example owing to reduced F-actin flow in that region. The model predicts that bundled microtubules in the central zone of the

growth cone will have more favourable dynamic properties (faster recovery and longer catastrophe times) than in the peripheral zone. Owing to the imaging difficulties of tracking microtubules within the bundle, this has not yet been verified.

The branch-invasion model shows that microtubule invasion into a growth cone after contact with a target cell cannot occur unless microtubules interact actively with F-actin to extend in the direction of least-rearward F-actin flow. A 90% reduction in the F-actin flow in the target did not increase axon recovery, and shielding interactions did not enable most microtubules to reach the target zone if they invaded the growth cone randomly. Intuitively, it can be seen why this will be the case for dynamic microtubules in the biological growth cone. The position of the microtubule tip fluctuates around the central zone with zero net growth or shrinkage. Microtubules with these characteristics are unlikely to reach the target zone before they undergo a catastrophe. Combined with the small probability of growing in the target direction, microtubules randomly sampling the growth cone are unable to reach the target zone. The model predicts that in order to maximize growth-cone motility, microtubules do not explore the growth cone randomly. Instead, as a result of interactions with F-actin, microtubules invade the growth cone in the direction of least F-actin flow. To test this prediction, the dynamics of individual microtubules would need to be monitored during growth-cone encounters with a target cell. Microtubules may simply extend in the direction of least F-actin flow owing to physical constraints imposed at the microtubule tip when it extends into the growth cone. Alternatively, some as yet unknown microtubule/F-actin interaction mediated by MAPs may guide the microtubules in the direction of least F-actin flow.

The combined results of both models suggest that short-term microtubule-shielding interactions between neighbouring microtubules and with F-actin will have a significant long-term effect on growth-cone behaviour. Although the individual shielding interactions may exist for only a few seconds, they result in both energy-efficient axonal elongation and an increased growth-cone motility. These models show that computer simulations of the short-term dynamics of the growth cone can provide an increased understanding of the long-term pathfinding ability of the neuron.

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