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# Competition for neurotrophic factor in the development of nerve connections

**Arjen van Ooyen*† and David J. Willshaw**

*Institute for Adaptive and Neural Computation, Division of Informatics, University of Edinburgh, 2 Buccleuch Place, Edinburgh EH8 9LW, UK*

The development of nerve connections is thought to involve competition among axons for survival promoting factors, or neurotrophins, which are released by the cells that are innervated by the axons. Although the notion of competition is widely used within neurobiology, there is little understanding of the nature of the competitive process and the underlying mechanisms. We present a new theoretical model to analyse competition in the development of nerve connections. According to the model, the precise manner in which neurotrophins regulate the growth of axons, in particular the growth of the amount of neurotrophin receptor, determines what patterns of target innervation can develop. The regulation of neurotrophin receptors is also involved in the degeneration and regeneration of connections. Competition in our model can be influenced by factors dependent on and independent of neuronal electrical activity. Our results point to the need to measure directly the specific form of the regulation by neurotrophins of their receptors.

**Keywords:** competition; neurotrophic factors; neurotrophin receptors; development of nerve connections; regeneration; neurodegeneration

## 1. INTRODUCTION

The development of connections between neurons and their target cells often involves an initial stage of superinnervation followed by elimination of axons (Purves & Lichtman 1980). In some cases, elimination continues until the target is innervated by just a single axon (Hume & Purves 1981; Crepel 1982; Jansen & Fladby 1990), whereas in many other cases several innervating axons remain (Purves & Lichtman 1985).

The cells that act as targets for the innervating axons release limited amounts of so-called neurotrophic factors, which are taken up by the axons via specific receptors at their terminals (Bothwell 1995; Lewin & Barde 1996) and which affect the growth and branching of the axons (see § 2b). An important class of neurotrophic factors are neurotrophins, with nerve growth factor (NGF) as its best-characterized member (Bothwell 1995; Lewin & Barde 1996).

Competition among innervating axons for neurotrophic factors is thought to be involved in the elimination of axons and the generation of different patterns of innervation (Grinnell *et al.* 1979; Purves & Lichtman 1985; Purves 1988). There is, however, little understanding of the nature of the competitive process and the underlying mechanisms. In this paper we introduce a new theoretical model to analyse competition in the development of nerve connections.

* Author for correspondence.
† Present address: Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands (a.van.ooyen@nih.knaw.nl).

## 2. THE MODEL

### (a) *Release and removal of neurotrophin*

A single target cell (e.g. a neuron) is considered, at which there are $n$ innervating axons each from a different neuron (figure 1). A single axon is defined as the largest branching structure in which all terminals contact the target under consideration. We calculate the time-dependent changes of the extracellular concentration of neurotrophin ($L$), released by the target at rate $\sigma$, and removed by degradation with rate constant $\delta$. In addition, neurotrophin is removed by the innervating axons. The capacity of an axon to remove neurotrophin depends on the total amount of neurotrophin receptor it has over all its terminals. For each axon $i$, we therefore calculate the time-dependent changes of the total amounts of unoccupied receptor ($R_i$), inserted at rate $\phi_i$, and neurotrophin–receptor complex ($C_i$), formed by a reversible binding of neurotrophin to receptor, with association and dissociation constants $k_{a,i}$ and $k_{d,i}$, respectively. Complex is taken up and degrades with rate constant $\rho_i$, while unoccupied receptor degrades with rate constant $\gamma_i$. Assuming standard reaction dynamics, the rates of change of $C_i, R_i$ and $L$ are

$$\frac{dC_i}{dt} = (k_{a,i}LR_i - k_{d,i}C_i) - \rho_i C_i, \tag{1}$$

$$\frac{dR_i}{dt} = \phi_i - \gamma_i R_i - (k_{a,i}LR_i - k_{d,i}C_i), \tag{2}$$

$$\frac{dL}{dt} = \sigma - \delta L - \sum_{i=1}^{n} (k_{a,i}LR_i - k_{d,i}C_i)/v. \tag{3}$$

The term $(k_{a,i}LR_i - k_{d,i}C_i)$ represents the net amounts of receptor and neurotrophin being converted into complex; $v$ is the volume of the extracellular space. Equations (1) and (2) are


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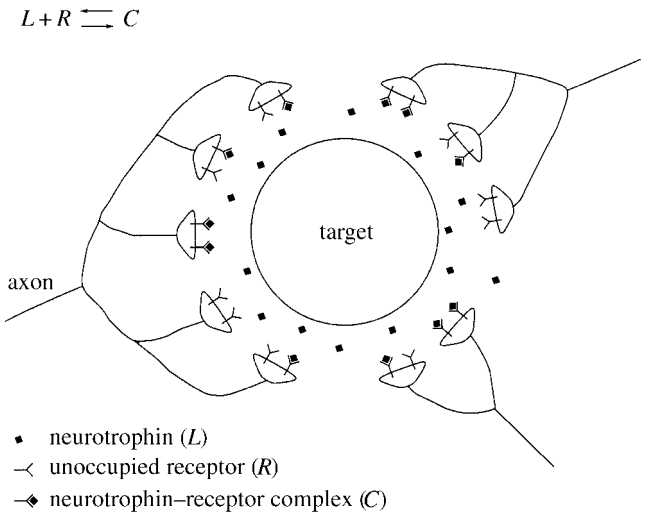


Figure 1. Single target with three innervating axons. The target releases neurotrophin that is bound by neurotrophin receptors at the axon terminals

similar to the ones used in experimental studies for analysing the cellular binding, internalization, and degradation of polypeptide ligands such as neurotrophins (Wiley & Cunningham 1981; Bernd & Greene 1984).

### (b) *Axonal growth*

The binding of neurotrophin to receptor triggers the biological response (Bothwell 1995). Many studies have shown that neurotrophins locally enhance axonal growth and branching, as well as synaptogenesis. This has been observed for NGF (Edwards *et al.* 1989; Yasuda *et al.* 1990; Garofalo *et al.* 1992; Yunshao *et al.* 1992; Diamond *et al.* 1992; Miller *et al.* 1994; Burgos *et al.* 1995) and for the neurotrophins BDNF (Cohen-Cory & Fraser 1995; Causing *et al.* 1997), NT-3 (Schnell *et al.* 1994), and NT-4/5 (Funakoshi *et al.* 1995). In addition to increasing the number of synapses, NGF can enlarge the size of the presynaptic elements (Garofalo *et al.* 1992). Neurotrophins may also be able to upregulate the density of their own receptors (Bernd & Greene 1984; Lindsay *et al.* 1990; Holtzman *et al.* 1992; Li *et al.* 1995; ElShamy *et al.* 1996; Ninkina *et al.* 1996).

In all the above mentioned effects of neurotrophins (enhancing the arborization of an axon near its target, the number and size of its synapses, and the density of its receptors) the capacity of an axon to bind neurotrophin will become larger because in all cases the axon's total amount of receptor will increase. For the total amount of receptor to increase in response to neurotrophin, the total amount of unoccupied receptor that is inserted into the axon per unit time, $\phi_i$, must increase in response to neurotrophin. We therefore assume $\phi_i$ to be an increasing function, $f_i(C_i)$, of the amount of bound neurotrophin, $C_i$. Function $f_i(C_i)$ is called the growth function. To account for the fact that axonal growth takes place on a relatively slow time-scale (e.g. Campenot 1982), $\phi_i$ lags behind $f_i(C_i)$ with a lag given by

$$\tau \frac{\mathrm{d}\phi_i}{\mathrm{d}t} = f_i(C_i) - \phi_i, \tag{4}$$

where the time constant $\tau$ of growth is of the order of days. The value of $\phi_i$ will change until, at steady state, $\phi_i = f_i(C_i)$.

The precise form of $f_i(C_i)$ is not known, and therefore we examine different forms of this function, using the general growth function

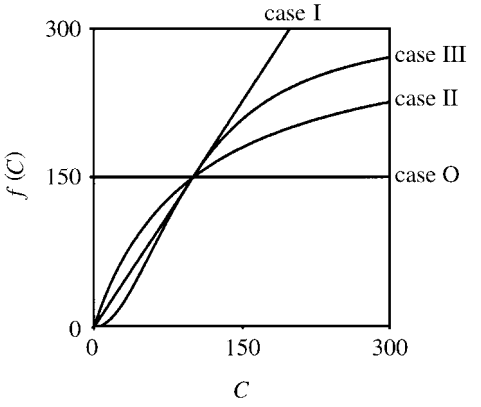


Figure 2. Growth function $f(C) = \alpha C^m/(K^m + C^m)$ for the different cases described in the text. For case O, $\alpha = 300$; for case I, $\alpha/K = 1.5$; for cases II and III, $\alpha = 300$ and $K = 100$.

$$f_i(C_i) = \frac{\alpha_i C_i^m}{K_i^m + C_i^m}. \tag{5}$$

This is an increasing function that saturates towards a maximum, $\alpha_i$. Parameter $K_i$ is the value of $C_i$ at which the response is half its maximum. Setting $m = 1$ yields the Michaelis–Menten function; setting $m = 2$ yields the Hill function, which is sigmoidal (figure 2). By means of numerical simulations and mathematical analysis (see Appendix B), we examine the patterns of innervation that result for four specific forms of this general growth function. Axons that at the end of the competitive process have no neurotrophin ($C_i = 0$; equivalent to $\phi_i = 0$) are assumed to have withdrawn or died, while axons that do have neurotrophin ($C_i > 0$; equivalent to $\phi_i > 0$) have survived.

## 3. UNITS AND PARAMETER VALUES

For the numerical simulations, the parameter values were taken from the data available for NGF: $k_a = 4.8 \times 10^7$ $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$, $k_d = 1.0 \times 10^{-3}\,\mathrm{s}^{-1}$ (Sutter *et al.* 1979). These are the values for the high affinity binding site, which mediates the biological response (Bothwell 1995); $\gamma = 2.7 \times 10^{-5}\,\mathrm{s}^{-1}$ (Zupan & Johnson 1991); $\rho \approx 2.0 \times 10^{-5}\,\mathrm{s}^{-1}$ (Layer & Shooter 1983); $\delta \approx 1.0 \times 10^{-5}\,\mathrm{s}^{-1}$ (Jeanprêtre *et al.* 1996); $\sigma \approx 2.0 \times 10^{-16}\,\mathrm{M\,s}^{-1}$ (Blöchel & Thoenen 1995; Jeanprêtre *et al.* 1996). To show the effects of changing $\sigma$, in some of the simulations a higher or lower value was used; $\tau = 2$ days, based on growth of the amount of receptor (Bernd & Greene 1984). The value of $v$ is $1.7 \times 10^{-11}$ l.

The values of $R_i$, $C_i$ and $K_i$ are in number of molecules, the value of $L$ is in M (i.e. mol $\mathrm{l}^{-1}$). The values of $\alpha_i$ and $\phi_i$ are in number of molecules $\mathrm{h}^{-1}$. Time is in hours (h). Only the value of $\alpha_i$ varies among axons. Unless otherwise indicated, the initial value of all $\phi_i$ is 10.0 molecules $\mathrm{h}^{-1}$. The initial values of $R_i$, $C_i$ and $L$ are such that, when keeping all $\phi_i$ at their initial value, the system is in equilibrium.

## 4. RESULTS

We study four qualitatively different cases of the general growth function $f_i(C_i)$, equation (5).

### (a) *Case O*

For $m = 0$, $f_i(C_i)$ is a constant ($f_i(C_i) = \alpha_i/2$) and independent of the level of bound neurotrophin, $C_i$. There is

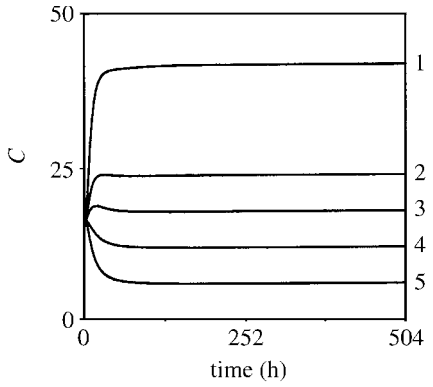


Figure 3. Case O. All the initial five axons survive. $\alpha_1 = 700$, $\alpha_2 = 400$, $\alpha_3 = 300$, $\alpha_4 = 200$ and $\alpha_5 = 100$.

no elimination of axons, and so all axons that were initially present survive (figure 3).

**(b) *Case I***

For $m = 1$ and large $K_i$ ($K_i \gg C_i$, $f_i(C_i) \approx \alpha_i C_i/K_i$), growth is linear over a large range of $C_i$. Elimination of axons takes place until one axon remains (single innervation) (figure 4). No more than one axon can survive, regardless of the rate of release of neurotrophin, $\sigma$. The axon that will outcompete all the others is the one with the highest value of the quantity $\beta_i \equiv (k_{a,i}(\alpha_i/K_i - \rho_i))/(\gamma_i(k_{d,i} + \rho_i))$, which we interpret as the axon's competitive strength.

**(c) *Case II***

For $m = 1$ and smaller values of $K_i$ ($K_i \not\gg C_i$, $f_i(C_i) = \alpha_i C_i/(K_i + C_i)$), elimination of axons will occur, and either one or several axons will survive (single versus multiple innervation), depending on the parameters of the growth function (figure 5). The lower the amount $C_i$ of bound neurotrophin at which the growth function saturates (i.e. the smaller the value of $K_i$), the more axons survive. Again, axons with higher competitive strength $\beta_i$ have an advantage in survival. In this case there is a dependence on the rate of release of neurotrophin, $\sigma$: the larger $\sigma$, the more axons survive.

**(d) *Case III***

For $m = 2$ ($f_i(C_i) = \alpha_i C_i^2/(K_i^2 + C_i^2)$), single and multiple innervation are possible, as for case II, and there is a similar dependence on the competitive strength $\beta_i$, the rate of release of neurotrophin $\sigma$, and the parameters of the growth function (figure 6). Unlike cases O, I and II, where there is just one stable equilibrium of innervation for any set of parameter values, here several stable equilibria can coexist. Apart from extreme cases, there always coexist at least $n$ stable equilibria: one for each axon where it is stable and all the others have died or withdrawn. In addition, there can be stable equilibria of multiple innervation. Which equilibrium will be reached in any specific situation will depend on the initial values of $\phi_i$, and the sizes of the basins of attraction of the equilibria, which are sensitive to the values of $\beta_i$.

**(e) *Competitive strength***

The competitive strength, $\beta_i$ for axon $i$, depends on the parameters of growth ($\alpha_i$ and $K_i$) and neurotrophic signalling ($k_{a,i}$, $k_{d,i}$, $\rho_i$ and $\gamma_i$). Various factors in the innervating axon, some dependent on and some independent of its electrical activity, may influence the values of these parameters and hence $\beta_i$. For example, the finding that increased presynaptic electrical activity increases the amount of neurotrophin receptor (Birren *et al.* 1992; Cohen-Cory *et al.* 1993) implies that increased electrical activity affects growth (i.e. higher $\alpha_i$ or lower $K_i$) or neurotrophic signalling (e.g. lower $\gamma_i$) or both. As the level of

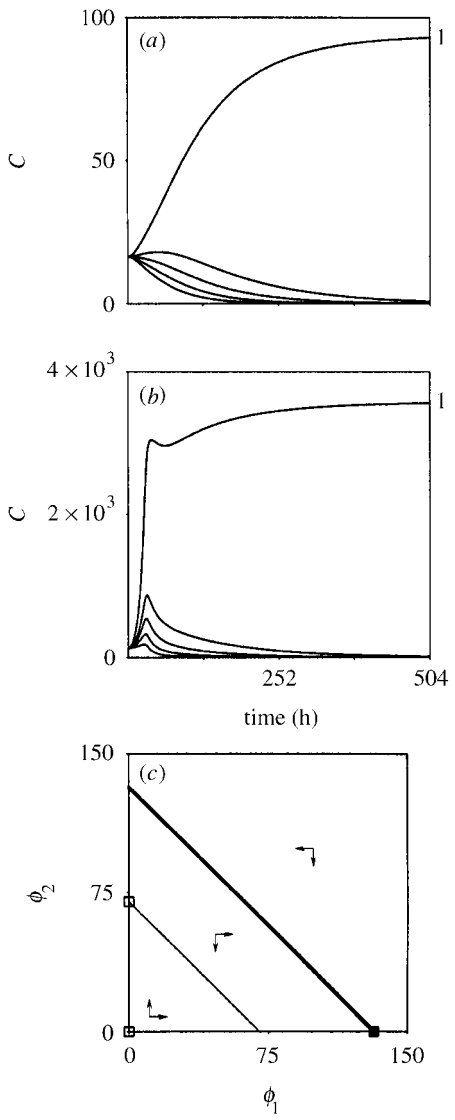


Figure 4. Case I: single innervation. (*a*) The axon with the highest value of $\alpha_i/K_i$ among the initial five axons survives. $\alpha_1/K_1 = 1.4$, $\alpha_2/K_2 = 0.8$, $\alpha_3/K_3 = 0.6$, $\alpha_4/K_4 = 0.4$ and $\alpha_5/K_5 = 0.2$. (*b*) Rate of release of neurotrophin, $\sigma$, is 35 times higher than the standard value. Other parameter values as in (*a*). (*c*) A system of two innervating axons ($n = 2$). The variables $\{R_i, C_i, i = 1,2\}$ and $L$ are at quasi steady state. The bold line depicts the solutions of the equation $d\phi_1/dt = 0$ and the light line those of $d\phi_2/dt = 0$ (the lines $\phi_1 = 0$ and $\phi_2 = 0$ are also solutions of $d\phi_1/dt = 0$ and $d\phi_2/dt = 0$, respectively, but are not drawn). These two nullclines do not intersect and, consequently, there is no equilibrium point where both axons can coexist. Recall (see Appendix B) that $\phi_i > 0 \Leftrightarrow C_i > 0$ and $\phi_i = 0 \Leftrightarrow C_i = 0$. Vectors indicate direction of change. Filled square indicates stable equilibrium point, and open square unstable equilibrium point. $\alpha_1/K_1 = 1.4$ and $\alpha_2/K_2 = 0.8$.

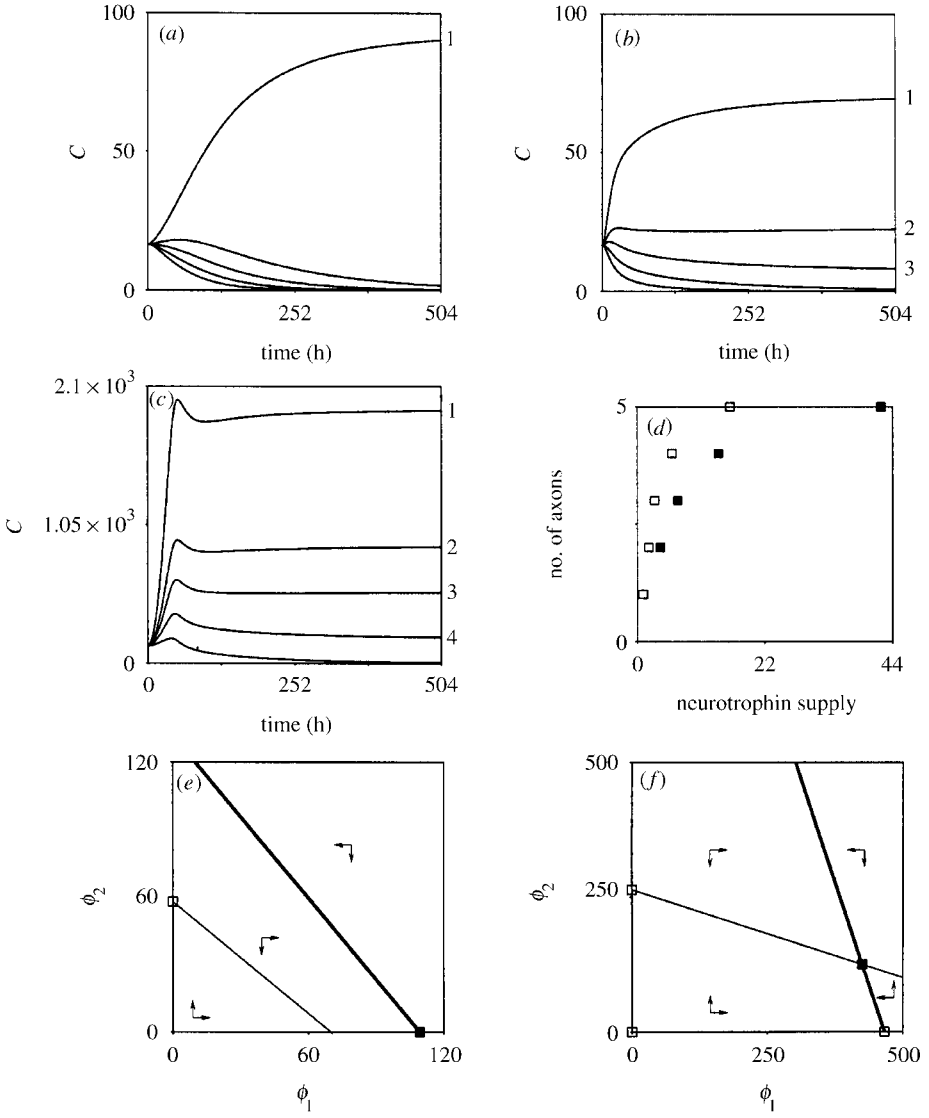


Figure 5. Case II: single and multiple innervation. (*a*) Single innervation. The axon with the highest value of $\alpha_i$ among the initial five axons survives. $\alpha_1 = 700$, $\alpha_2 = 400$, $\alpha_3 = 300$, $\alpha_4 = 200$, $\alpha_5 = 100$ and $K = 500$. (*b*) Multiple innervation with $K = 40$. Other parameter values as in (*a*). (*c*) Multiple innervation with a rate of release of neurotrophin, $\sigma$, that is 35 times higher than the standard value. Other parameter values as in (*a*). (*d*) Relationship between the rate of release of neurotrophin (in units of the standard value) and the number of axons with $C_i > 10$ at $t = 504$, for $K = 500$ (filled squares) and $K = 150$ (open squares). Other parameter values as in (*a*). (*e*) A system of two innervating axons ($n = 2$). For explanation lines and symbols, see figure 4*c*. The two nullclines do not intersect and, consequently, there is no equilibrium point where both axons can coexist. $\alpha_1 = 700$, $\alpha_2 = 400$ and $K = 500$. (*f*) As (*d*) but the rate of release of neurotrophin, $\sigma$, is ten times higher than the standard value. The two nullclines intersect in the stable equilibrium point of the system, where both axons coexist.

electrical activity and other factors may vary among innervating axons, there will be variation in $\beta_i$. The competitive strength $\beta_i$ is crucial in interpreting several important phenomena.

(i) *Survival*

Axons with high $\beta_i$ survive, and the activity dependence of $\beta_i$ means that these are the most active ones, provided that the variation due to other factors does not predominate.

(ii) *Displacement*

An axon can displace existing axons if its value of $\beta_i$ is high enough (in addition, for case III, its initial value of $\phi_i$ must be high enough) (figure 7). This selects axons with high $\beta_i$ when axons arrive at their target at different times. The average value of $\beta_i$ of the surviving axons will increase over time.

(iii) *Regeneration*

The coexistence of several stable equilibria in case III implies that an axon that is removed from a multiply innervated target may not necessarily survive ('regenerate') when replaced (figure 8). The higher its value of $\beta_i$, the more likely it is to regenerate.

(iv) *Degeneration*

For cases I, II and III, an axon will regress if $\beta_i$ is too small. For cases I and II analysis shows that an axon will always regress if $\beta_i \leqslant \delta/\sigma$. This will occur for reduced electrical activity, reduced growth of receptors (both affect the value of $\beta_i$), a low rate of release of

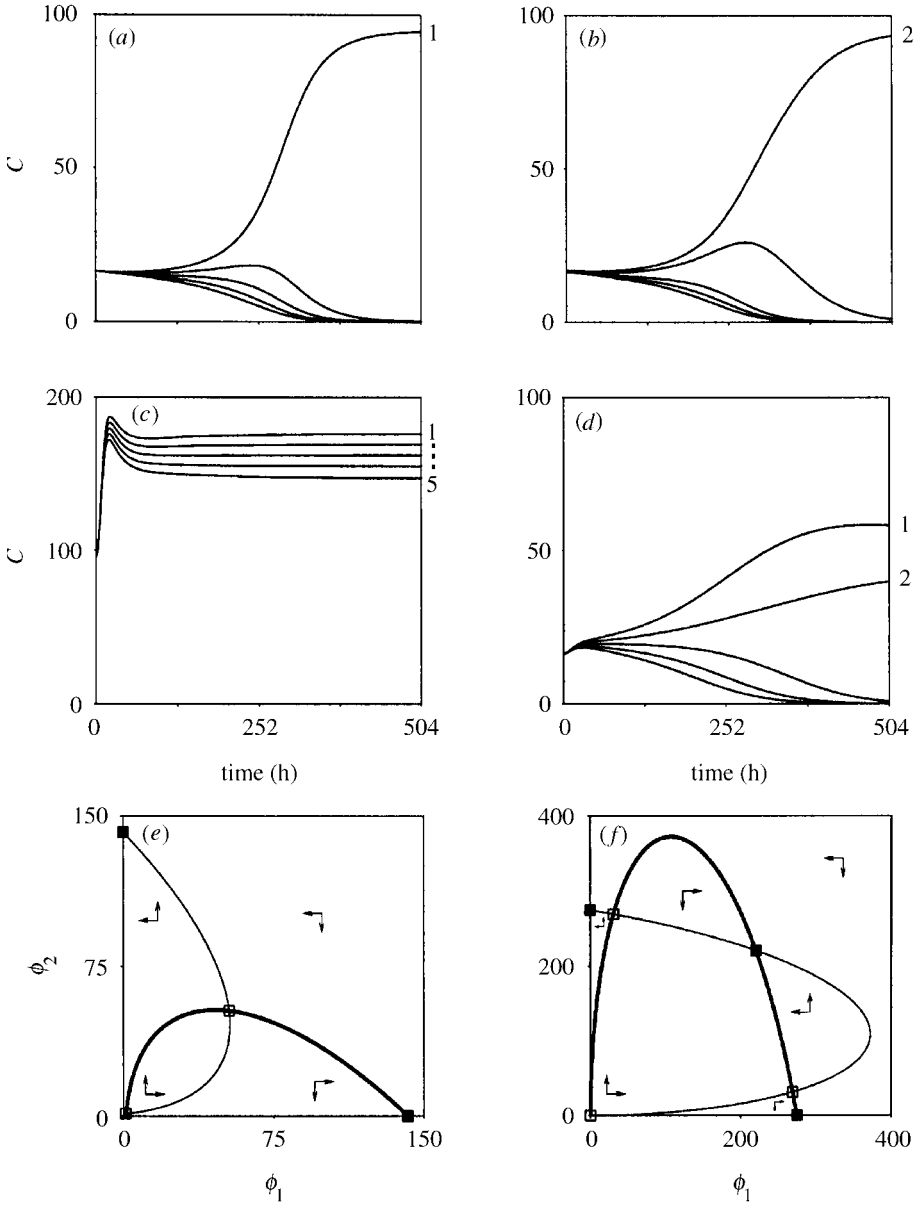


Figure 6. Case III: single and multiple innervation and dependence on initial conditions. (*a*) The axon with the highest value of $\alpha_i$ among the initial five axons survives. $\alpha_1 = 312$, $\alpha_2 = 306$, $\alpha_3 = 300$, $\alpha_4 = 294$, $\alpha_5 = 288$ and $K = 100$. (*b*) When the initial value of $\phi_2$ is high enough, axon 2 wins rather than axon 1, although axon one has a higher value of $\alpha$. $\phi_2 = 10.3$, all other $\phi_i = 10.0$. Other parameter values as in (*a*). (*c*) Multiple innervation with a rate of release of neurotrophin, $\sigma$, that is eight times higher than the standard value. Other parameter values as in (*a*). (*d*) Multiple innervation with $K = 40$. Other parameter values as in (*a*). (*e*) A system of two innervating axons ($n = 2$). For explanation lines and symbols, see figure 4*c*. There are stable equilibrium points where either axon is present but not both. The stable equilibrium point at ($\phi_1 = 0, \phi_2 = 0$) is not indicated because it is too close to another, unstable point. $\alpha_1 = 300$, $\alpha_2 = 300$ and $K = 100$. (*f*) As (*e*) but with $K = 30$. There is a stable equilibrium point where both axons coexist.

neurotrophin, $\sigma$ (figure 9), or a high rate of degradation of neurotrophin, $\delta$. These conditions could occur in ageing or in disease-related neurodegeneration, such as Alzheimer's disease (Rylett & Williams 1994; Salehi *et al.* 1996).

## 5. COMPARISON WITH EMPIRICAL DATA

The model can account for the development of both single and multiple innervation. Examples of single innervation are the innervation of skeletal muscle fibres (Jansen & Fladby 1990), autonomic ganglion cells with few dendrites (Hume & Purves 1981), and the climbing fibre innervation of cerebellar Purkinje cells (Crepel 1982), whereas many kinds of neurons are multiply innervated (Purves & Lichtman 1985).

In agreement with the model, in skeletal muscle stable states of single and multiple innervation can coexist, as in case III. Persistent multiple innervation is found in partial denervation experiments after reinnervation and recovery from prolonged nerve conduction block (Barry & Ribchester 1995). In terms of the model, the conduction block changes the competitive strengths of the axons and hence the sizes of the basins of attractions of the equilibria. This can cause the system to go to an equilibrium of multiple innervation, while under normal conditions

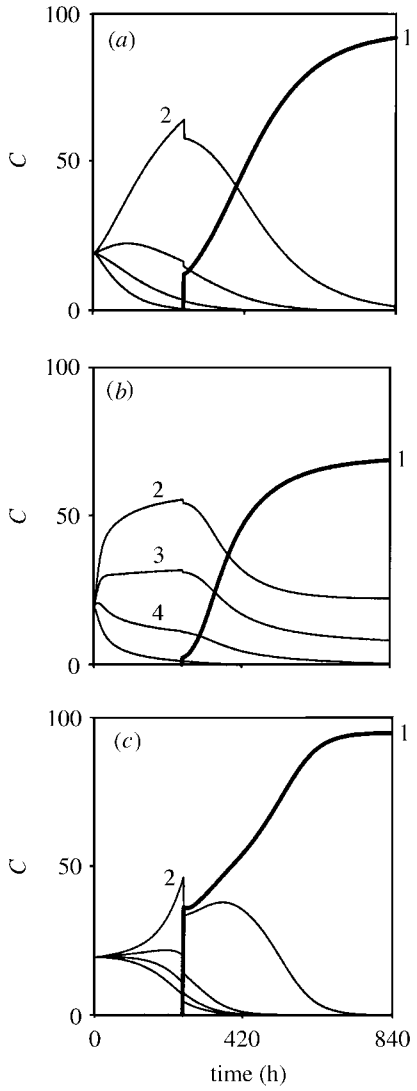


Figure 7. Innervating axons displace existing axons. (*a*) Case I. Initially there are four axons: numbers 2, 3, 4 and 5 (used in figure 4*a*); $\alpha_2/K_2 = 0.8$, $\alpha_3/K_3 = 0.6$, $\alpha_4/K_4 = 0.4$ and $\alpha_5/K_5 = 0.2$. At $t = 252$ h we introduce axon 1 (bold line) with $\alpha_1/K_1 = 1.4$ and initial conditions $\phi_1 = 10.0$, $R_1 = \phi_1/\gamma$ and $C_1 = 0$. Axon 1 replaces axon 2, which otherwise would have survived. (*b*) Case II. Initially there are four axons: numbers 2, 3, 4 and 5 (used in figure 5*b*); $\alpha_2 = 400$, $\alpha_3 = 300$, $\alpha_4 = 200$, $\alpha_5 = 100$ and $K = 40$. At $t = 252$ h we introduce axon 1 (bold line) with $\alpha_1 = 700$ and initial conditions $\phi_1 = 10.0$, $R_1 = \phi_1/\gamma$ and $C_1 = 0$. Axons 1, 2 and 3 survive instead of 2, 3 and 4, which otherwise would have survived. (*c*) Case III. Initially there are four axons: numbers 2, 3, 4 and 5 (as used in figure 6*a*); $\alpha_2 = 306$, $\alpha_3 = 300$, $\alpha_4 = 294$, $\alpha_5 = 288$ and $K = 100$. At $t = 252$ h we introduce axon 1 (bold line) with $\alpha_1 = 312$ and initial conditions $\phi_1 = 35.0$, $R_1 = \phi_1/\gamma$ and $C_1 = 0$. Axon 1 replaces axon 2, which otherwise would have survived. To do this, the initial value of $\phi_1$ must be high enough, unlike in cases I and II.

single innervation develops. Once the conduction block is removed, the system will remain in the basin of attraction of the multiple-innervation equilibrium.

Axonal competition involves both activity-dependent and activity-independent mechanisms (Ribchester 1988). This is consistent with the model in that the competitive strength of an axon can be influenced by factors dependent on and independent of neuronal electrical activity. Elimination of axons in the model also occurs on the same time-scale as observed in various biological systems (Purves & Lichtman 1985).

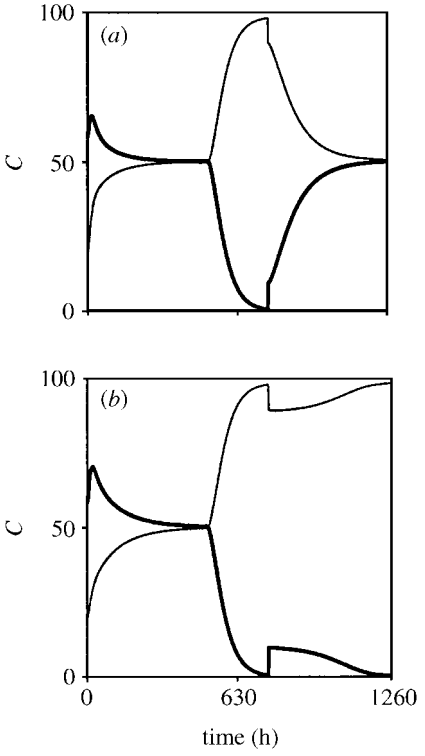


Figure 8. Removal of an axon from a multiply innervated target and subsequent replacement. (*a*) Case II. $\alpha_1 = \alpha_2 = 400$ and $K = 40$. Initial conditions $\phi_1 = 30$ and $\phi_2 = 10$. At $t = 504$ h we remove axon 1 (bold line) by setting $\alpha_1 = 0$. At $t = 756$ h we replace axon 1 by setting $\alpha_1$ to its original value, with initial conditions $\phi_1 = 30$, $R_1 = \phi_1/\gamma$ and $C_1 = 0$. Axon 1 survives and multiple innervation is restored. (*b*) Case III. Parameter values as in figure 6*f*. Initial conditions $\phi_1 = 30$ and $\phi_2 = 10$. At $t = 504$ h we remove axon 1 (bold line) by setting $\alpha_1 = 0$. At $t = 756$ h we replace axon 1 by setting $\alpha_1$ to its original value, with initial conditions $\phi_1 = 30$, $R_1 = \phi_1/\gamma$ and $C_1 = 0$. Axon 1 does not survive.

The model (cases II and III) accounts for the experimental finding that increasing the amount of target-derived neurotrophin increases the number of axons innervating that target (e.g. in the peripheral nervous system: Albers *et al.* 1994). Similarly, excess neurotrophin prevents the formation of ocular dominance columns (Cabelli *et al.* 1995).

An essential feature of the model is the growth function, which can be determined experimentally *in vitro* by measuring the total number of terminals of an axon or, more specifically, the axon's total amount of neurotrophin receptor for different concentrations of neurotrophin in the medium (see Appendix B for details). The model predicts that (i) for axonal elimination to occur, increasing the concentration from a low level should increase the axon's total amount of neurotrophin receptor; (ii) the relationship between the concentration of neurotrophin applied and the number of surviving axons depends on the specific form of the growth function. For example, the smaller the value of $K_i$, the lower the concentration of neurotrophin needed to rescue more axons (figure 5*d*); and (iii) the degree of multiple innervation in various cell types will be negatively correlated

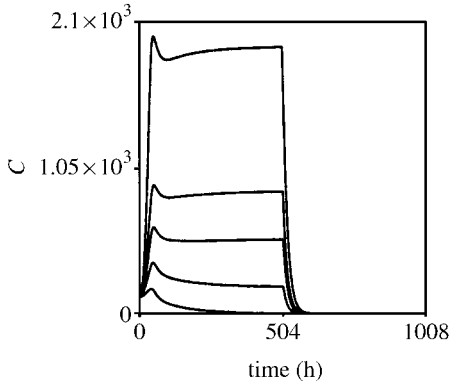


Figure 9. Complete loss of innervation when the rate of release of neurotrophin is below a certain critical level. At $t = 504$ h the value of $\sigma$ is lowered from $2.0 \times 10^{-16}$ M s$^{-1}$ to $1.587 \times 10^{-17}$ M s$^{-1}$. This makes the largest value of $\beta_i$, $\beta_1$, to be just smaller than $\delta/\sigma$. Case II, parameter values as in figure 5*c*.

with the value of $K_i$ (provided the supply of neurotrophin is the same).

The model further predicts what factors should be changed to change the competitive strength of an axon.

## 6. CONCLUSIONS AND DISCUSSION

We have formulated a new model to analyse competition in the development of nerve connections. The model links the formation of nerve connections with the underlying actions and biochemistry of neurotrophins. The model accounts for the development of single and multiple innervation together with several other experimental findings, and makes testable predictions.

Our analysis suggests that the regulation of axonal growth by neurotrophins is crucial to the competitive process in the development, maintenance and regeneration of nerve connections. Of the many axonal features that change during growth in response to neurotrophin (number of terminal branches, number and size of synapses), the consequent change in the axon's total amount of neurotrophin receptor, changing its capacity to remove neurotrophin, is what drives the competition. The form of the dose–response relationship between neurotrophin and total amount of neurotrophin receptor determines what patterns of innervation can develop and what the capacity for regeneration will be.

Although the parameter values were taken from the data available for NGF, the mathematical analysis shows that our results are general and do not depend on specific parameter values. The results are therefore relevant also for other neurotrophins.

If the axons in the model were to have more than one target, the rate of insertion of receptor could be different in branches innervating different targets. The essential results of our study will not change, but in addition to competition among different axons there will be competition among branches of the same axon (as in Rasmussen & Willshaw 1993; Van Ooyen & Willshaw 1999).

In most existing models of the development of nerve connections, competition is based on fixed amounts of resources that become partitioned among the individual competitors (e.g. Gouzé *et al.* 1983; Bennett & Robinson 1989; Rasmussen & Willshaw 1993; Elliott & Shadbolt 1996), i.e. there is no production and no decay or consumption of resources, which is biologically less plausible. Our model, like that by Jeanprêtre *et al.* (1996) for the development of single innervation, incorporates the production of resource (neurotrophin) and its consumption by innervating axons. Effects similar to those exhibited by our model are seen in consumer-resource systems in population biology, e.g. single innervation versus 'competitive exclusion' (Yodzis 1989; Ribchester & Barry 1994; Van Ooyen & Willshaw 1999).

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## APPENDIX A. LIST OF SYMBOLS

| | |
|---|---|
| $i$ | index of axon |
| $L$ | concentration of neurotrophin |
| $R_i$ | amount of unoccupied receptor |
| $C_i$ | amount of neurotrophin–receptor complex |
| $\sigma$ | rate of release of neurotrophin |
| $\delta$ | rate constant of degradation of neurotrophin |
| $\phi_i$ | rate of insertion of unoccupied receptor |
| $\gamma_i$ | rate constant of degradation of unoccupied receptor |
| $k_{a,i}$ | association constant of binding of neurotrophin to receptor |
| $k_{d,i}$ | dissociation constant of binding of neurotrophin to receptor |
| $\rho_i$ | rate constant of degradation of neurotrophin–receptor complex |
| $v$ | volume of extracellular space |
| $f_i$ | growth function |
| $m,\alpha_i,K_i$ | parameters of growth function |
| $\tau$ | time constant of growth |
| $\beta_i$ | competitive strength $\equiv (k_{a,i}(\alpha_i/K_i - \rho_i))/(\gamma_i(k_{d,i} + \rho_i))$ |

## APPENDIX B. MATHEMATICAL ANALYSIS

The rate of change of $\phi_i$ is of the order of days, so that we can make quasi-steady-state approximations for the other variables on the time-scale of $\phi_i$. From equations (1) and (2), the quasi-steady-state approximations for $R_i$ and $C_i$ (i.e. $\mathrm{d}R_i/\mathrm{d}t = \mathrm{d}C_i/\mathrm{d}t = 0$) give

$$C_i = \frac{\phi_i L}{b_i + \rho_i L}, \tag{A1}$$

where $b_i = \gamma_i(k_{d,i} + \rho_i)/k_{a,i}$.

From equation (3) the quasi-steady-state approximation for $L$ gives

$$L = \frac{\sigma - a}{\delta}, \tag{A2}$$

where $a = \sum_{i=1}^{n} \rho_i C_i/v$. Because $L$ must be positive and $\delta$ is a positive constant, $\sigma - a > 0$. Combining equations (A1) and (A2) gives the following expression for $\phi_i$:

$$\phi_i = C_i\left(\rho_i + \frac{b_i\delta}{\sigma - a}\right). \tag{A3}$$

Because all constants are positive, it follows that $\phi_i = 0 \Leftrightarrow C_i = 0$ and $\phi_i > 0 \Leftrightarrow C_i > 0$. The rate of change of $\phi_i$ is given by

$$\tau \frac{\mathrm{d}\phi_i}{\mathrm{d}t} = f_i(C_i) - \phi_i. \tag{A4}$$

For $f_i(C_i)$ we use the general function

$$f_i(C_i) = \frac{\alpha_i C_i^m}{K_i^m + C_i^m}. \tag{A5}$$

Inserting equation (A5) and the expression for $\phi_i$ obtained in (A3) into equation (A4) yields

$$\tau \frac{\mathrm{d}\phi_i}{\mathrm{d}t} = f_i(C_i) - \phi_i = b_i C_i \left( \frac{1}{b_i} \left( \frac{\alpha_i C_i^{m-1}}{K_i^m + C_i^m} - \rho_i \right) - \frac{\delta}{\sigma - a} \right). \tag{A6}$$

At equilibrium $\mathrm{d}\phi_i/\mathrm{d}t = 0$, i.e. for $m \geqslant 1$,

$$C_i = 0 \text{ or } \frac{1}{b_i} \left( \frac{\alpha_i C_i^{m-1}}{K_i^m + C_i^m} - \rho_i \right) - \frac{\delta}{\sigma - a} = 0. \tag{A7}$$

For each axon $i$, we define the constant $\beta_i \equiv (\alpha_i/K_i - \rho_i)/b_i$. As $\beta_i$ is activity dependent and 'real-valued', all $\beta_i$ will be distinct, i.e. all axons will be different.

We now consider specific cases for the form of $f_i(C_i)$.

### (a) *Case O: no growth*

For $m = 0$, $f_i(C_i) = \alpha_i/2$. At equilibrium $\mathrm{d}\phi_i/\mathrm{d}t = 0$, and all axons survive with $\phi_i = \alpha_i/2$.

### (b) *Case I: linear function*

For $m = 1$ and $K_i \gg C_i$, $f_i(C_i) \approx \alpha_i C_i/K_i$. At most one axon can survive, as will now be shown. Introducing the constant $\beta_i$, the solutions in equation (A7) are $C_i = 0$ or $a = \sigma - \delta/\beta_i$. Taking the second solution for all $n$ axons defines a system of $n$ equations with only one free variable, $a$. Because all the $\beta_i$ are distinct, at most one equation can be satisfied. Hence no more than one $C_i$ can be non-zero in equilibrium.

The axon that can be in a stable equilibrium in which $C_i > 0$ will be the one with the highest value of $\beta_i$. Let this be axon 1. At equilibrium $\mathrm{d}\phi_1/\mathrm{d}t = 0$ and so $\beta_1 = \delta/(\sigma - a)$. For any other axon $i$, $\beta_i < \beta_1 = \delta/(\sigma - a)$, and so $\mathrm{d}\phi_i/\mathrm{d}t < 0$ for $C_i > 0$. For these axons, $\phi_i$ will decrease until $\phi_i = 0$ and $C_i = 0$.

This is the only possible stable equilibrium in which not all axons have disappeared. If axon 2, for example, had reached equilibrium with $C_2 > 0$, $\beta_2$ would have been equal to $\delta/(\sigma - a)$. However, because $\beta_1 > \beta_2$, $\mathrm{d}\phi_1/\mathrm{d}t > 0$ for $C_1 > 0$, and $\phi_1$ would continuously increase, contradicting the assumption that this was a stable equilibrium. As $\beta_1 \neq \beta_2$ both axons cannot be in stable equilibrium.

An axon can never be maintained if $\beta_i \leqslant \delta/\sigma$. For any $a > 0$, $\beta_i < \delta/(\sigma - a)$. Therefore $\mathrm{d}\phi_i/\mathrm{d}t < 0$ for $C_i > 0$, and so the axon will disappear. Choosing, arbitrarily, axon 1 to have the largest value of $\beta_i$, if $\beta_1 \leqslant \delta/\sigma$, all axons will be eliminated. In contrast, if $\beta_1 > \delta/\sigma$ there is always an $a > 0$ such that $\beta_1 = \delta/(\sigma - a)$, and the axon survives.

### (c) *Case II: Michaelis–Menten function*

For $m = 1$ and $K_i \gg C_i$, $f_i(C_i) = \alpha_i C_i/(K_i + C_i)$. Taking the second solution in equation (A7) for all $n$ axons defines a system of $n$ equations with $n$ unknowns, $C_i$. At most all equations can be satisfied simultaneously; i.e. coexistence of any number of the $n$ axons is possible.

The greater the value of $\sigma$, the greater the number of surviving axons. In the limiting case $\sigma - a \gg \delta$, from the second solution in equation (A7), maximally all axons can survive with $C_i = \alpha_i/\rho_i - K_i$. The smaller the value of $\sigma$, the smaller the number of surviving axons. Because $a$ is bounded from above by $\sigma$ as $\sigma - a > 0$, the smaller the value of $\sigma$, the smaller $a$, and hence the smaller the individual values of $C_i$. If these values are small enough, $K_i \gg C_i$ and we obtain case I, where maximally one axon survives.

Also if we directly change $K_i$, the greater the value of $K_i$, the smaller the number of surviving axons. In the limiting case $K_i \gg C_i$ we obtain again case I. The smaller the value of $K_i$, the greater the number of surviving axons. In the limiting case $K_i \ll C_i$, and $f_i(C_i) \approx \alpha_i$. This is similar to case O, and all axons survive with $\phi_i = \alpha_i$.

As in case I, an axon can never be maintained if $\beta_i \leqslant \delta/\sigma$. For any $C_i > 0$ and $a > 0$, $(\alpha_i/(K_i + C_i) - \rho_i)/b_i < \delta/(\sigma - a)$ and therefore $\mathrm{d}\phi_i/\mathrm{d}t < 0$. Choosing, arbitrarily, axon 1 to have the largest value of $\beta_i$, if $\beta_1 \leqslant \delta/\sigma$, all axons will be eliminated. In contrast, if $\beta_i > \delta/\sigma$, there is always a combination of values of $C_i$ such that $(\alpha_i/(K_i + C_i) - \rho_i)/b_i = \delta/(\sigma - a)$, and one or more axons survive.

### (d) *Case III: Hill function*

For $m = 2$, $f_i(C_i) = \alpha_i C_i^2/(K_i^2 + C_i^2)$. Taking the second solution in equation (A7) for all $n$ axons defines a system of $n$ equations with $n$ unknowns, $C_i$. At most, all equations can be satisfied simultaneously; i.e. coexistence of any number of the $n$ axons is possible. The analysis for this case is less tractable. The rest of our findings rely on the results of our simulation experiments, which reveal that for one and the same set of parameter values there exist stable equilibria of single and multiple innervation. Which equilibrium will be reached depends on the initial conditions.

### (e) *Experimental determination of the growth function $f_i(C_i)$*

Consider a medium with an approximately constant concentration of neurotrophin, $L$. When axonal growth has reached equilibrium, $\phi_i = f_i(C_i)$. From equation (A1), $\phi_i$ can be obtained if the amount of bound neurotrophin, $C_i$, is known. The value of $C_i$ can be either measured directly or, if the total amount of receptors, $R_i + C_i$, is measured, calculated from $C_i = (R_i + C_i)L/(L + (k_{\mathrm{d},i} + \rho_i)/k_{\mathrm{a},i})$, from setting $\mathrm{d}C_i/\mathrm{d}t = 0$ in equation (1). Repeating the whole procedure for different concentrations of neurotrophin in the medium will give $\phi_i$ for different values of $C_i$, and thus $f_i$. Even if the exact values of $k_{\mathrm{a},i}$, $k_{\mathrm{d},i}$, $\rho_i$ and $\gamma_i$ are not known, the shape of the growth function can still be determined.

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