A Dynamical System Model of Transport of Neurofilaments in Axons*

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Abstract

We develop a dynamical system model for the transport of neurofilaments in axons, inspired by Brown's "stop-and-go" model for slow axonal transport. We use fast/slow time scale arguments to lower the number of relevant parameters in our model. Then, we use experimental data of Wang and Brown to estimate all but one parameter. We show that we can choose this last remaining parameter such that the results of our model agree with pulse-labeling experiments of Xu and Tung and with stochastic simulation results of Brown and Jung.

Key words: intracellular transport, nerve cell, neurofilaments, motor proteins, dynamical system.

1 Introduction

Axonal proteins are transported along axons by the mechanisms of axonal transport in association with discrete cargo structures that move at different rates (see Brown [3]). One type of cargo structure are neurofilaments, which are macromolecular protein polymers that function as space-filling structures in axons (see Williamson et al. [12]). Studies on populations of neurofilaments *in vivo* on a time scale of weeks and months using radio-isotopic pulse-labeling have demonstrated that these cytoskeletal polymers move along axons in the slowest component of axonal transport at average rates of about 0.01 μm/s (about 1 mm/day; see Lasek et al. [7]). The radiolabeled proteins in these experiments distribute along the axon in the form of a bell-shaped wave that spreads as it moves distally.

A mathematical model for slow axonal transport has been proposed by Blum and Reed [1]. The model assumed the existence of a hypothetical engine that

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moves at a constant velocity. Proteins were considered to move by reversible association with the engine, either by binding directly to the engine or by piggy-backing on other structures that bind directly to the engine. Microtubules were considered to interact directly with the engine and neurofilaments were considered to move by piggy-backing on the moving microtubules. According to this model, the average velocity of neurofilaments was dependent on the equilibrium constants of the interactions between neurofilaments and microtubules and between microtubules and the engine. By computer simulation of the equations of the model, the authors demonstrated that they could account for the shape of the bell-shaped waves of labeled proteins observed in the radio-isotopic pulse labeling experiments.

Since the development of the Blum-Reed model fifteen years ago, considerable progress has been made in the experimental study of neurofilament transport in axons. It is now known that the engines of axonal transport are motor proteins and that neurofilaments can interact directly with microtubule motor proteins and move along microtubule tracks (Shah et al. [9], Xia et al. [13], Helfand et al. [5]). Moreover, it is now possible to observe the movement of neurofilaments directly in cultured nerve cells, on a time scale of seconds or minutes, using fluorescence microscopy. These observations have demonstrated that the movement of neurofilaments is not slow, after all; neurofilaments actually move at fast rates (about 0.5 μm/s), and the movements are also infrequent, bidirectional and highly asynchronous (see Wang et al. [11], Roy et al. [8], Wang and Brown [10]). To explain these data, Brown proposed the "stop and go" model of slow axonal transport, in which the slow rate of movement is considered to be an average of rapid bidirectional movements interrupted by prolonged pauses (see Brown [2, 3]). In subsequent work, Brown and Jung [4] used a discrete model to show that the rapid intermittent movement of single neurofilaments observed by fluorescence microscopy in cultured nerve cells can account for the slow overall movement of populations of neurofilaments observed by radio-isotopic pulse labeling in vivo.

In the present paper we develop a new dynamical system model for the transport of neurofilaments in axons, based on the fast time scale observations in cultured nerve cells. Some parameters in the model are computed from the measurements in [10]. We then show, by simulation, that our dynamical system model can yield the same population profile of pulse-labeled neurofilaments as obtained experimentally in [14]. Thus, our model and the discrete stochastic model developed in [4], although they use completely different approaches, both rely on experimental measurements in cultured neurons [10], and both provide a very good agreement with the in vivo experimental results of [14]. Our model can be extended to other transport problems of proteins and vesicles in the axon, as was shown in [6].

2 The model

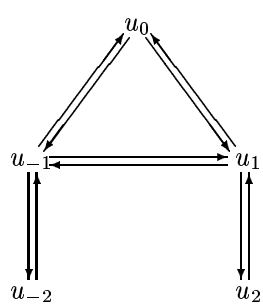
We consider that neurofilaments are cargo structures that move along microtubule tracks powered by microtubule motor proteins. Neurofilaments can interact with kinesin motor proteins, which move them towards the axon tip (anterograde direction) or with dynein motor proteins, which move them towards the cell body (retrograde direction). Our model does not require make any assumptions about the mechanism or kinetics of motor binding to neurofilaments. However, for the purposes of discussion we will consider that neurofilaments can bind only one type of motor at a time and that reversal occurs when one type of motor dissociates and another type binds.

As discussed by Brown and Jung [4], we assume that anterograde and retrograde neurofilaments can each exist in two states: those adjacent to a microtubule ("on track") and those further away ("off track"). At any point in time some neurofilaments are on track and others are off track. Neurofilaments are only capable of movement in the longitudinal dimension of the axon when they are on track but they can switch on and off track by lateral diffusion within the radial dimension of the axon. When off track, neurofilaments pause for long periods until they get back on track. When on track, neurofilaments alternate between short bouts of movement and short pauses. Thus we divide the on track population of neurofilaments into those that are moving and those that are making a short pause. Moreover, we designate separately the population of anterograde moving neurofilaments and the population of retrograde moving neurofilaments. In this manner, we divide the neurofilaments into five populations:

u_0: neurofilaments bound to kinesin or dynein, pausing, off track,
u_2: neurofilaments bound to kinesin, moving anterogradely, on track,
u_1: neurofilaments bound to kinesin, pausing, on track,
u_{-2}: neurofilaments bound to dynein, moving retrogradely, on track,
u_{-1}: neurofilaments bound to dynein, pausing, on track.

We also denote by $u_{-2}, u_{-1}, u_0, u_1, u_2$ the concentrations of these five populations of neurofilaments along the axon.

We assume that neurofilaments can reverse direction (i.e., switch motors) when they are pausing, either on track or off track. Thus we obtain the following diagram of possible transitions between the five neurofilament populations:



According to experimental results in [10], the rate of change from u_1 to u_{-1} should be much smaller than the rate of change from u_1 to u_2, and the population u_0 is much larger than u_1. Therefore the contribution of this rate in the numerical simulations will be negligible. Analogously, the contribution of the rate of change from u_{-1} to u_1 in the numerical simulations will be negligible. This leads us to the following diagram:

$$u_{-2} \rightleftarrows u_{-1} \rightleftarrows u_0 \rightleftarrows u_1 \rightleftarrows u_2$$

If we denote by $k_{i,j}$ the rate of change from u_i to u_j we have

$$u_{-2} \underset{k_{-2,-1}}{\overset{k_{-1,-2}}{\rightleftarrows}} u_{-1} \underset{k_{-1,0}}{\overset{k_{0,-1}}{\rightleftarrows}} u_0 \underset{k_{1,0}}{\overset{k_{0,1}}{\rightleftarrows}} u_1 \underset{k_{2,1}}{\overset{k_{1,2}}{\rightleftarrows}} u_2 \qquad (D)$$

These relations, together with mass conservation laws, yield the following dynamical system for $0 < x < L$, where L is the length of the axon:

$$\begin{aligned}
\frac{\partial u_2}{\partial t} &= -v_A \frac{\partial u_2}{\partial x} + k_{1,2}u_1 - k_{2,1}u_2, \\
\frac{\partial u_1}{\partial t} &= k_{2,1}u_2 + k_{0,1}u_0 - (k_{1,2} + k_{1,0})u_1, \\
\frac{\partial u_0}{\partial t} &= k_{1,0}u_1 + k_{-1,0}u_{-1} - (k_{0,1} + k_{0,-1})u_0, \\
\frac{\partial u_{-1}}{\partial t} &= k_{0,-1}u_0 + k_{-2,-1}u_{-2} - (k_{-1,0} + k_{-1,-2})u_{-1}, \\
\frac{\partial u_{-2}}{\partial t} &= v_R \frac{\partial u_{-2}}{\partial x} + k_{-1,-2}u_1 - k_{-2,-1}u_{-2},
\end{aligned} \qquad (T)$$

where v_A is the velocity of neurofilaments moving anterograde, and v_R is the velocity of neurofilaments moving retrograde. It is known [10] that the average velocities excluding pauses, v_A and v_R, are

$$v_A = 0.56\mu m/s, \qquad v_R = 0.62\mu m/s.$$

Strictly speaking, one needs to complement the system (T) with boundary conditions. However, we are modeling the distribution of labeled neurofilament proteins in pulse-labeling experiments, which decays to zero near the endpoints (see [4]). Consequently, our numerical results will deal with concentrations of neurofilaments which are negligible near $x = 0$ and $x = L$, so that the particular form of the boundary conditions will not enter into our simulation.

In order to be able to use the system (T) for computational purposes, we must identify the rate coefficients $k_{i,j}$. Before doing so, however, we need to make an important point:

Remark. We regard the transitions in (D) as happening on a "fast time scale", and the anterograde and retrograde transport as happening on a relatively "slow time scale", as suggested by experimental observations of individual

neurofilament movement made in [10]. We wish to use the dynamical system (T) to explain the results of radioisotopic pulse-labeling experiments in [14]. The experiments of Xu and Tung have been carried out on a time scale of several weeks, i.e., each experimental datum was taken after one or several weeks from the initial setup. By contrast, the experimental observations made in [10] were carried out on a much shorter time scale, i.e., over a period of a few minutes. During this short time interval, although some individual neurofilaments were in motion, the total neurofilament population of each u_j changed very little. In other words, both the dynamics and transport terms in the equations (T) could be neglected for the values u_j in [10]. Thus, if we equate the terms $\partial u_j/\partial t$, $v_A \partial u_2/\partial x$ and $v_R \partial u_{-2}/\partial x$ to zero in (T), and note that the last equation is then a consequence of the first four equations, we find that

$$\frac{u_{-1}}{u_{-2}} = \frac{k_{-2,-1}}{k_{-1,-2}}, \quad \frac{u_0}{u_{-1}} = \frac{k_{-1,0}}{k_{0,-1}}, \quad \frac{u_0}{u_1} = \frac{k_{1,0}}{k_{0,1}}, \quad \frac{u_1}{u_2} = \frac{k_{2,1}}{k_{1,2}}. \tag{$*$}$$

Furthermore we may replace the rates $k_{-1,-2}$ and $k_{-2,-1}$ in (D) by λ and $\lambda k_{-2,-1}/k_{-1,-2}$, respectively, where λ is a positive constant, provided that λ is large enough to keep the flow chart (D) within the fast time scale. Since the ratio of the new rates is the same, this replacement will not affect the fit with the experimental values of u_{-1} to u_{-2} in [10]. In the same way we may replace $k_{-1,0}$ and $k_{0,-1}$ in (D) by η and $\eta k_{0,-1}/k_{-1,0}$, where η is large enough, etc. As we shall see later on, our numerical results are unaffected by these rescalings of the rates $k_{i,j}$. Thus, we replace (D) with

$$u_{-2} \underset{\alpha\lambda}{\overset{\lambda}{\rightleftharpoons}} u_{-1} \underset{\beta\eta}{\overset{\eta}{\rightleftharpoons}} u_0 \underset{\gamma\sigma}{\overset{\sigma}{\rightleftharpoons}} u_1 \underset{\delta\mu}{\overset{\mu}{\rightleftharpoons}} u_2 \tag{D'}$$

where $\lambda, \eta, \sigma, \mu$ can be any numbers which are not too small.

Together with (D') we also have, as in $(*)$,

$$\frac{u_{-1}}{u_{-2}} = \alpha, \quad \frac{u_0}{u_{-1}} = \beta, \quad \frac{u_0}{u_1} = \gamma, \quad \frac{u_1}{u_2} = \delta. \tag{$**$}$$

We now need to determine the rates $\alpha, \beta, \gamma, \delta$ and, at this point, we use the experimental results from [10].

If we look at data in [10] we notice that, in most cases, individual neurofilaments moving anterogradely switch back and forth between populations u_1 (short pause) and u_2 (moving). If individual neurofilaments moving anterograde make more short pauses, then u_2 decreases and u_1 increases. The ratio $\frac{u_1}{u_2}$ depends on the time spent pausing and the time spent in processive motion. From the experimental measurements in [10] we have $\frac{u_1}{u_2} = \frac{u_{-1}}{u_{-2}} = \frac{67}{33}$. Hence

$$\alpha = \delta = \frac{67}{33}. \tag{1}$$

Wang and Brown [10] also established that the ratio between neurofilaments moving anterograde and neurofilaments moving retrograde is $69/31$, i.e.,

$$\frac{u_1 + u_2}{u_{-1} + u_{-2}} = \frac{69}{31}.$$

Using $(**)$ and the equality $\alpha = \delta$ we find that the left-hand side in the last equation is equal to β/γ. Hence

$$\beta = \frac{69}{31}\gamma. \tag{2}$$

From (1) and (2) we see that the flow chart (D') can be written in the form

$$u_{-2} \underset{\frac{67}{33}\lambda}{\overset{\lambda}{\rightleftarrows}} u_{-1} \underset{\frac{69}{31}\gamma\eta}{\overset{\eta}{\rightleftarrows}} u_0 \underset{\gamma\sigma}{\overset{\sigma}{\rightleftarrows}} u_1 \underset{\frac{67}{33}\mu}{\overset{\mu}{\rightleftarrows}} u_2 \qquad (D'')$$

As explained above, the choice of parameters $\lambda, \eta, \sigma, \mu$ should not affect the numerical results for the system (T), provided these parameters are not too small. But we still have the unresolved parameter γ. This parameter will be determined in a way that provides the best fit with experimental results in [14].

3 Numerical Results

To compare our model to the experimental radioisotopic pulse-labeling data, we selected the published data of Xu and Tung for the axonal transport of neurofilament protein L in motor axons of the mouse ventral root and sciatic nerve (see [14]). In this study, profiles of the neurofilament protein L distribution along the axon were obtained at one week intervals after injection of radioisotope. Interestingly, the average velocity was found to be approximately constant during the first three weeks, but then slowed at later times. These data indicate that the velocity of neurofilament transport can vary along the length of axons. For simplicity, we chose to focus on the initial three week period. Our strategy was to start with a distribution of neurofilaments that matches the experimental data at week one and then run the simulation and optimize the parameters to obtain the best possible match with the data at week three.

Given that the distribution of labeled neurofilament proteins in the pulse labeling experiments is approximately gaussian (see [14]), we take the initial condition to be such that the total population of neurofilaments has a concentration $Ae^{-(x-x_0)^2/M}$, where A, x_0, M were chosen such that the gaussian $Ae^{-(x-x_0)^2/M}$ best estimates the experimental measurements of Xu and Tung [14] for week one (see blue curve in Figure 1). Given our assumption that most neurofilaments are pausing off track, and since small changes in the initial conditions will not affect the population concentration after a relatively long time (for example, after one or two weeks), we take initial conditions $u_0(x, 0) = Ae^{-(x-x_0)^2/M}$, and

$u_j(x,0) = 0$ for all other j. We then solve the system (T) with the flow chart (D''), taking

$$\lambda = \eta = \sigma = \mu = 10,$$

and choose γ such that the profile of the total population of neurofilaments best fits the experimental results of [14] for week three. We find that $\gamma = 19.9$ gives an excellent fit, as shown in Figure 1. We have tried other choices of $\lambda, \eta, \sigma, \mu$ in the range 10-1000, and found hardly any change in the profile for the same value of γ.

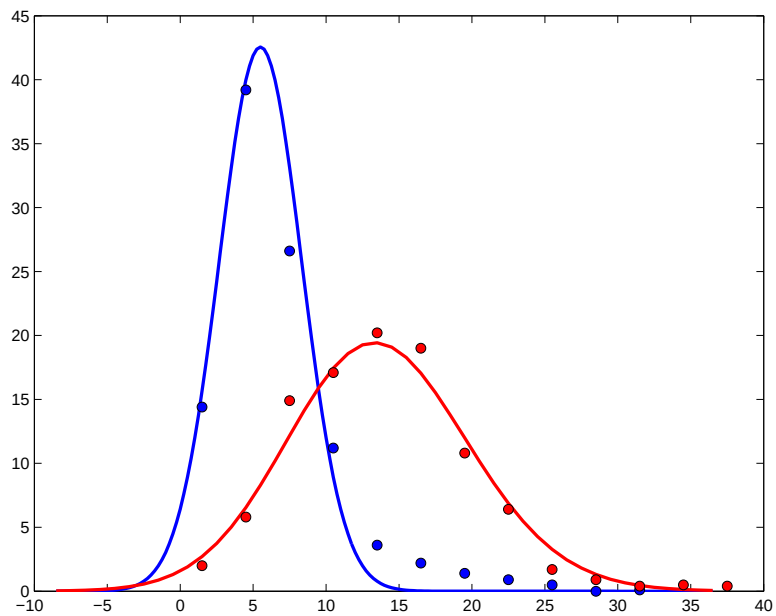


Figure 1: *Population profiles of neurofilaments along the axon: the dots represent experimental data of Xu and Tung [14]; blue is week one, and red is week three. The blue curve is the initial condition for our model; the red curve then follows from our dynamical system model.*

Our numerical results for week three can also be extended to the data of Xu and Tung for week two. What we find again is an excellent fit between our model and the profile in [14], but for a different value of the parameter, $\gamma = 39.2$. This difference could reflect subtle variations in the average rate in the second and third weeks, but it is more likely to be due to experimental error, as indicated by the standard error about the means in the original Xu and Tung data (not shown in Figure 1, see [14]).

Comparison with the Brown-Jung model. Brown and Jung [4] describe a discrete stochastic model for this problem. One of their model predictions is that, overall, neurofilaments spend 97% of the time pausing, i.e.,

$$\frac{u_{-2}+u_2}{u_{-1}+u_0+u_1}=\frac{3}{97}.$$

Using (**) and our choice of $\gamma = 19.9$, we can compute the left-hand side in terms of the parameters in our model, and we find that it is equal to 0.033, which is in good agreement with the value $\frac{3}{97}$.

Another model prediction in [4] is that, on average, only 8% of the neurofilaments are on track, i.e.,

$$\frac{u_{-2}+u_{-1}+u_1+u_2}{u_0}=\frac{8}{92}.$$

Again using (**) and the choice $\gamma = 19.9$, we can compute the left-hand side in terms of the parameters in our model, and we get the value 0.108, which is in good agreement with the value $\frac{8}{92}$.

4 Conclusions

In this paper we developed a dynamical system model of transport of neurofilaments in an axon. The model is based on mass conservation of concentrations of the various populations of neurofilaments. All but one of the parameters in our model, namely γ, are determined from experimental measurements by Wang and Brown [10]. We showed that we can then choose γ such that the model produces profiles that are in excellent fit with in vivo experiments of Xu and Tung [14]. A discrete stochastic model, entirely different from ours, was used in [4] to generate profiles which also fit the results in [14]. That model predicts some relations among the concentrations u_i. We have shown that our model makes the same predictions.

Our dynamical system approach can easily be refined to include diffusion, and can be extended to other transport problems, such as the transport of vesicles in an axon [6]. Our dynamical system approach should extend also to models of neurofilament concentration during axon growth, and hopefully also to models of neurofilament concentration at particular regions along the length of axons, such as at nodes of Ranvier.

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